ATTENUATION OF HYPOXIC PULMONARY VASOCONSTRICTION BY ACETAZOLAMIDE AND METHAZOLAMIDE: A RANDOMIZED CROSSOVER STUDY.

by

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Abstract

Context: Acetazolmaide (AZ) is used in the prophylactic treatment of altitude illnesses. AZ is also known to attenuate HPV and increase alveolar ventilation. Methazolamide (MZ) is an analog to AZ and its effects on ventilation and HPV are unknown. **Objective.** To determine if MZ can improve oxygenation and attenuate HPV to a similar extent as AZ in healthy humans exposed to poikilocapnic hypoxia. **Design, Setting, Participants and Interventions:** A randomized, placebo controlled, double-blinded trial was performed in healthy participants at the Cardiopulmonary Lab for Experimental and Applied Physiology. Prior to each of the three experimentation days, participants were administered one of three treatments (AZ, MZ, & placebo) at random for two days. Each treatment was separated by a 10-day washout period to avoid contamination from previous trials. During each trial, participants were exposed to poikilocapnic hypoxia ($F_IO_2 \approx 0.12$) for 60 minutes. **Primary Outcome Measures**: Partial pressure of alveolar O_2 (P_AO_2) represented oxygenation while pulmonary artery systolic pressure (PASP) and total pulmonary resistance (TPR) were chosen to represent the HPV response. **Results**: All participants (n = 11) completed all three trials. Change in $\dot{Q}$ from baseline to hypoxia was not different between treatments. Change in PASP was significantly lower with the AZ (8.0 ± 0.7 mmHg) and MZ (9.0 ± 0.9 mmHg) treatments compared to placebo (PASP: 14.1 ± 1.3 mmHg$P < 0.05$). Change in P_AO_2 was also decreased with both drug treatments (AZ: 54.8 ± 1.3 mmHg; MZ: 53.9 ± 1.3 mmHg) compared to placebo (48.5 ± 1.6 mmHg; $P < 0.05$). **Conclusion:** MZ attenuated HPV to the same degree as AZ. MZ also resulted in a similar improvement in P_AO_2 as AZ during hypoxia compared to placebo. **Trial Registration:** This study was registered with the U.S. National Institutes of Health: NCT02760121 **Funding:** This project was funded by the Canadian Foundation for Innovation and by the Natural Science and Engineering Council of Canada.

Preface

This thesis contains original data collected and analyzed for partial fulfillment of the author's Master of Science degree. All protocols were approved by the Clinical Research Ethics Board (UBC number: H16-00028) at the University of British Columbia and registered with the U.S. National Institutes of Health (NCT02760121). This thesis consists of a review of the literature (Chapter 1), and five additional chapters pertaining to the research questions, methodology, results, and discussion.

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List of Symbols, Abbreviations or Other

AMS	Acute mountain sickness
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AZ	Acetazolamide
BL	Baseline condition
BE	Base excess
CA	Carbonic anhydrase
Ca^{2+}	Calcium ion
CO_2	Carbon dioxide
DBP	Diastolic blood pressure
D_LCO	Diffusing capacity of the lung for carbon monoxide
FEV_1	Forced expiratory volume in 1 second
F_IO_2	Fraction of inspired O_2
FVC	Forced vital capacity
f_B	Breathing frequency
HACE	High altitude cerebral edema
HAPE	High altitude pulmonary edema
Hb	Hemoglobin
Hct	Hematocrit
HIF	Hypoxia inducible factor
HPV	Hypoxic pulmonary vasoconstriction
HR	Heart rate
HVR	Hypoxic ventilatory response
HX	Hypoxic condition
IVC	Inferior vena cava
LAP	Left atrial pressure
MAP	Mean arterial pressure
MZ	Methazolamide
NMA	N-methyl acetazolamide

NO	Nitric oxide
NOS	Nitric oxide synthase
Nrf-2	Nuclear factor (erythroid-derived 2)-related factor 2
O_2	Oxygen
PA	Pulmonary artery
PASMC	Pulmonary artery smooth muscle cells
PASP	Pulmonary artery systolic pressure
PBO	Placebo
P_ACO_2	Partial pressure of alveolar CO_2
P_AO_2	Partial pressure of alveolar O_2
PaCO2	Partial pressure of arterial CO_2
PaO2	Partial pressure of arterial O_2
P_CCO_2	Partial pressure of capillary CO_2
P_CO_2	Partial pressure of capillary O_2
PCO_2	Partial pressure of CO_2
$P_{ET}CO_2$	Partial pressure of end-tidal CO_2
$P_{ET}O_2$	Partial pressure of end-tidal O_2
PO_2	Partial pressure of O_2
PVR	Pulmonary vascular resistance
$\dot{Q}$	Cardiac output
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SaO_2	Arterial oxyhemoglobin saturation
ScO_2	Capillary oxyhemoglobin saturation
SpO_2	Peripheral oxyhemoglobin saturation
SEM	Standard error of the mean
SO_2	Oxyhemoglobin saturation
SV	Stroke volume
TPR	Total pulmonary resistance
TPR_{45}	Total pulmonary resistance corrected to a hematocrit of 45%

TR	Tricuspid regurgitation
TVS	Trigeminovascular system
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_A/\dot{Q}$	Ventilation-perfusion ratio
$\dot{V}CO_2$	Volume of CO_2 produced
V_D	Volume of dead space
$\dot{V}_E$	Minute ventilation
$\dot{V}_I$	Inspired ventilation
V_T	Tidal volume
$\dot{V}O_2$	Volume of O_2 consumed
ΔPmax	Maximum pressure gradient across tricuspid valve

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Chapter 1. Literature Review

The purpose of this chapter is to review (A) the pathology, epidemiology and treatment of acute high altitude illness (section 1.1, pg. 1), (B) describe the important pharmacological features of carbonic anhydrase (CA), its inhibitors, and its role in minimizing the risk of high altitude illness (section 1.2, pg. 6), and finally, (C) to characterize the normal pulmonary vascular response to high altitude exposure and the possible mechanisms and modulators of this response (section 1.3, pg. 9).

1.1 Acute High-Altitude Illness

Three major disorders are encompassed by the broad term high altitude illness including the cerebral syndromes associated with acute mountain sickness (AMS), high altitude cerebral edema (HACE) considered to be an exacerbated form of AMS, and high altitude pulmonary edema (HAPE). This section reviews their epidemiology, clinical presentation and underlying physiology, followed by an examination of their prophylactic treatment using CA inhibitors and a comparison of acetazolamide (AZ), the preferred treatment option, to methazolamide (MZ), a sulfonamide analog with slightly different pharmacokinetics and pharmacodynamics.

1.1.1 Clinical Presentation & Epidemiology

AMS is a broad term for a clinical syndrome manifested as cerebral symptoms that occur following a rapid ascent to high altitude and is diagnosed through reports of headache and a moderately severe secondary symptom including nausea, dizziness, fatigue or disturbed sleep (130). Symptoms occur in 10-25% of non-acclimatized people following an ascent to altitudes of 2500 m above sea level (65, 95) and incidence increases to 50-85% when the altitude is increased to above 4000 m (55). Rate of ascent is a major risk factor in the development of AMS, with a 24-33% reduction in incidence when altitudes are achieved in 3-6 days compared with one day (114, 140). Partial acclimatization, defined as spending a minimum of 5 out of the past 60 days at altitudes above 3000 m, reduces incidence by 10% and full acclimatization (>15 out of the past 60 days above 3000 m) reduces it to nearly nil (140). The elderly (>59 years) are less susceptible to AMS (65, 131) and one report suggests women are more susceptible to AMS (114) while another suggests no sex differences (55).

The level of physical fitness does not predict AMS incidence (16, 109). Regression analysis performed on data from a group of 3994 tourists on their first ever ascent to 4000 m above sea level suggest a low hypoxic ventilatory response (HVR) and a greater degree of arterial oxyhemoglobin desaturation as likely predictors of AMS (129). Typically, AMS is resolved within days at altitude, though in certain cases the symptoms progress into a lethargic state, which is believed to be caused by HACE. It begins with drowsiness and, if left untreated, HACE can progress to a state of confusion ultimately ending with a complete inability to satisfy one's own basic needs (56). Though the two have not been explicitly linked, HACE is often considered to be a progression of AMS, and for that reason, it carries the same risk factors as AMS though its incidence is significantly lower at <1-3.5% (55). However one report in a group of Nepalese found a 31% incidence of HACE following a rapid ascent to 4300 m; this unusually high incidence is likely related to the rapid ascent profile where pilgrims ascended from 2000 m - 4300 m in a single day (14).

Initially HAPE presents as dyspnea during exercise, with a lower tolerance for strenuous activity and an accompanying dry cough; the symptoms can progress to dyspnea at rest with sputum production (8). Unlike HACE, AMS is not a prerequisite for HAPE though many of the risk factors are the same. In addition to those identified above, cold and pulmonary hypertension are also believed to initiate or exacerbate HAPE (13). The incidence of HAPE ranges from <1 to 6% and 15-20% of those who develop HAPE, develop HACE simultaneously, in part due to worsening gas exchange in the fluid filled lungs (56). If left untreated, pharmacologically, with supplemental oxygen (O_2), or by descending from altitude, HAPE has a 50% mortality rate (163). HAPE is generally thought of as a condition affecting the un-acclimatized who undergo rapid ascent to altitude, though there are documented cases of high altitude natives experiencing HAPE upon return from a prolonged visit to low altitude, termed reentry HAPE (146). Studies suggest that HAPE susceptibility likely has some genetic basis and that children are far more afflicted than adults (58, 77, 112). Currently there is no wide spread consensus on the treatment of reentry HAPE, particularly for children (121).

1.1.2 Pathophysiology of AMS and Cerebral Edema

The exact etiology of AMS and HACE are poorly understood, though it is believed that HACE is a progressed form of AMS. An elevated intracranial pressure and cerebral edema

identified by magnetic resonance imaging are the hallmarks of disease progression though the origin of these features and how they are linked to the perceived symptoms is unknown (91). Studies show depressed arterial O_2 saturation (SaO_2) in those suffering from AMS (10), which may be the result of a lower HVR and elevated hypoxic pulmonary vasoconstriction (HPV) that often occur alongside AMS (9, 129). The cerebral hyperemic response that occurs with hypoxia increases intracranial blood volume and consequently pressure which is thought to drive some of the AMS symptoms. Blood entering the brain through a disruption of the blood brain barrier, known as vasogenic edema, during hypoxia is another possible source of AMS symptoms (54). Inhibition of venous outflow, through hypoxic venoconstriction, and an elevation in intracranial pressure have been considered as two possible contributors to the intracranial hypertension though the evidence appears to be inconsistent, possibly owing to large interindividual variability (91). More recent magnetic resonance imaging data suggests that while vasogenic edema often occurs concomitantly with AMS, it is not always present in those with AMS; the data identifies intracellular edema, a fluid shift into brain cells from the extracellular space without altering intracranial pressure, as a possible predictor of AMS (143).

The symptoms of pain associated with AMS are believed to originate from the trigeminovascular system (TVS); the perivascular trigeminal axons surrounding the intracranial vessels are activated by nitric oxide (NO), reactive oxygen species (ROS) or neurogenic inflammation caused by the hypoxic stimulus and result in a heightened sensitivity to pain (138). An emerging AMS theory implicating the TVS suggests that the intracellular edema causes astrocytic swelling and consequently the release of nitric oxide (5). Furthermore, this hypothesis suggests that circulating hypoxic-induced ROS plays an important role by not only disrupting the blood brain barrier but also sodium-potassium pumps throughout the brain leading to intracellular fluid imbalance and edema (5). One criticism of this theory is that hypoxic intracellular edema occurs predominantly in the white matter of the brain, relatively far from the TVS, which identifies a major gap in our understanding of how AMS symptoms arise and how the TVS is implicated (137). Though there are conflicting reports regarding the pathophysiology of AMS and HACE within the literature, most research agrees that the complexity of the topic owes to the large variability of the illnesses between individuals and the vast number of possible contributing factors.

1.1.3 Pathophysiology of High Altitude Pulmonary Edema

The pathophysiology of HAPE consists of an exaggerated pulmonary capillary pressure resulting in fluid leakage due to filtration or mechanical disruption of the alveolar-capillary barrier (163). The high pressure within the pulmonary capillaries is thought to be primarily due to a disproportionately large HPV response; following hypoxic exposure, individuals susceptible to HAPE typically present with significantly higher pulmonary artery (PA) pressures (~50-100 mmHg) compared to healthy controls (~30-50 mmHg) (51). There are believed to be two possible mechanisms for fluid entry into the alveolar space associated with HAPE: (A) an altered permeability or (B) a mechanical disruption of the alveolar-capillary barrier leading to the release of fluid and proteins into the alveolar space (163). Both mechanisms are thought to be driven by an abnormally high HPV response, a common attribute among HAPE-susceptible individuals. The cause of the exaggerated response is not clear, previous work points to hypoxic pulmonary venoconstriction causing congestion and increasing pulmonary vascular resistance (47). The particularly heterogenous pulmonary blood flow in HAPE-susceptible individuals exposed to hypoxia offers an explanation involving regional edemas (31, 66). Highly constricted regions of the lung could lead to other highly perfused regions and, when coupled with an increase in cardiac output due to physiological stressors including further hypoxia or exercise, the over perfusion could lead to damage and subsequent alveolar leakage (163).

Individuals who are HAPE susceptible have been shown to have a lower than average HVR in acute normobaric hypoxia (4); it is reasoned that depressed responsiveness of the peripheral chemoreceptors causes the individual to experience much lower arterial O_2 saturation for a given fraction of inspired O_2 (57, 61, 105). Considering O_2 as the primary stimulus for HPV, it follows that a low HVR is able to drive PA pressure through a greater degree of hypoxemia, though it is not understood how the two are linked. More recently it has been shown that with prolonged exposure at altitude, the HVR is not well correlated with the HPV response (64). Since prolonged exposure to hypobaric hypoxia alters the acid-base balance and the afferent input, central processing and efferent output of the peripheral chemoreflex arc, it was postulated that the HVR response magnitude loses its predictive value when combined with the complex mechanisms associated with high altitude acclimatization (64). Carbon dioxide (CO_2) also acts on the pulmonary vasculature,

particularly on individuals with a depressed HVR, who likely do not benefit from the blunting of PA pressure that results from the hypocapnia induced by hypoxic hyperventilation (7). The peripheral chemoreceptors exposed to hypoxia control not only the HVR response but also regulate hypoxic natriuresis through unknown mechanisms (164). An increase in muscle sympathetic nerve activity and sodium retention as well as a decrease in natriuresis and depressed HVR are all predictors of HAPE susceptibility (37, 164)

Bronchoalveolar lavage fluid collected from those suffering from HAPE was found to consist primarily of plasma proteins, erythrocytes and in certain cases neutrophils and proinflammatory cytokines (79, 141, 142). Treatment with dexamethasone, a common corticosteroid, prevents pulmonary edema in humans and rats exposed to hypoxia (94, 155). This finding led to the discovery that hypoxia induces leukocyte adhesion to pulmonary capillaries and the subsequent suggestion that this reaction compromises the permeability of the alveolar-capillary barrier and allows for fluid leakage (50). Given the evidence, it could be hypothesized that inflammation causes HAPE, though bronchoalveolar lavage data suggest that HAPE often occurs before inflammation (141, 142, 167). A disruption in alveolar fluid clearance has been identified as another potential mechanism contributing to HAPE, though unlikely to be a major factor due to the low fluid volume (116, 185).

1.1.4 Treatment with Acetazolamide

Among the many potential treatments for AMS, one widely used prophylactic treatment is AZ. A detailed review of CA distribution, function and inhibition can be found in section 1.2, pg. 6. Briefly, AZ is able to improve arterial oxygenation by renal CA inhibition causing systemic acidosis and an increase in circulating H^+ ions. Ventilation increases in an attempt to buffer the acidosis by expelling more CO_2, which is thought to mitigate symptoms of AMS (161). The influence of AZ on respiratory control can minimize periodic breathing typically observed during sleep at high altitude allowing for a more restful sleep (157, 179, 189). Intravenous administration of AZ also increases cerebral blood flow and oxygenation (38), which could account, in part, for the relief of the cerebral symptoms associated with AMS though more recent studies suggest oral administration does not result in the same effect (176). An effective AZ dosage schedule for the prophylactic treatment of AMS is every 8-12 hours with a dose that can range from 125-375 mg (158). The efficacy of AZ in preventing AMS is thought to have a small dose dependence; at doses of 125 mg it has been shown to be

approximately effective in 45% of the population, this efficacy increases to 55% when doses reach 375 mg (72, 90).

1.2 Carbonic Anhydrase

In this section, the structure, isoforms, distribution, and general function of human CA will be reviewed. In addition, the binding site for sulfonamides as well as the physiological implications of systemic inhibition will be described. Finally, the pharmacological properties of AZ and its methylated analog, MZ will be compared and contrasted.

1.2.1 Isoforms, Distribution and Function

CA is a widely distributed enzyme that catalyzes the interconversion of CO_2 and water with carbonic acid (H_2CO_3). This reaction plays an important role in many physiological processes including bone reabsorption, maintenance of cerebrospinal fluid, gas exchange, pH balance as well as ion balance, transport, secretion and reabsorption (150). Individuals with a mutated CA gene and a consequential CA deficiency are prone to developing osteoporosis, renal acidification and cerebral calcification. The reaction between CO_2 and H_2CO_3 can occur through two potential mechanisms:

1.2-1 $$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

1.2-2 $$CO_2 + OH^- \rightleftharpoons HCO_3^-$$

In the first reaction CO_2 is hydrated and forms carbonic acid, which is then rapidly hydrolyzed to form HCO_3^- (eqn 1.2-1). The second formation reaction involves a conversion between CO_2 and OH^- with HCO_3^- (eqn 1.2-2). Studies of CA structure suggest that the second reaction is favored by the enzyme given its increased likelihood of binding an OH^- over an H_2O, though this point is not physiologically relevant since the products and reactants are the same for both reactions (99). Fifteen isoenzymes of human CA have been identified to date, 12 of which have active binding sites containing a zinc ion bound to a water molecule (67, 99). Of particular interest for the treatment of altitude illnesses, are CA II, IV, XII and XIV which are found within the proximal tubules of the kidney (162). The majority of CA activity that contributes to gas exchange occurs within the cytosol of the circulating erythrocytes where CO_2 is converted to HCO_3^-, a process that facilitates the transport of CO_2 (59). It has been suggested that the CA found within skeletal muscle enables

the transport of CO_2 across cell membranes through bicarbonate specific transporters (59). Although CA present in lung tissue has been estimated to only account for approximately 5-10% of CO_2 excretion, largely due to spontaneous conversion and diffusion driven by the large CO_2 gradient that exists between the pulmonary arterial blood and the alveolar space (59). The CA enzymes present in the lungs are believed to play a larger role in buffering the pH of capillary blood and are thought to contribute to the matching of ventilation to perfusion, though it is unclear exactly how this occurs (59). Primarily found anchored in the apical epithelial cell of the proximal tubule, it is the isoenzyme CA IV that is involved in the dehydration of HCO_3^- to CO_2 (150). Once converted, the gaseous form of CO_2 is readily able to diffuse across the epithelial membrane to the cytosol, where it is effectively hydrated back to bicarbonate by cytosolic CA and then transported across the basolateral membrane and into the blood, completing the reabsorption process (150). Upon discovering the role of CA in the facilitation of CO_2 exchange and pH regulation, it was initially believed that CA was essential for life. Due to the relatively high reaction rate of the uncatalyzed CO_2 and HCO_3^- conversion, subsequent studies were able to show that though it is crucial for pH maintenance and gas exchange, it is not vital to sustain life (99).

1.2.2 The Inhibition of Carbonic Anhydrase and its Integrative Role in Physiology

The sulfonamide class of drugs includes a group of CA inhibitors that have been used in a number of clinical applications including as a diuretic, for the treatment of glaucoma and hypokalemic paralysis, to protect against epileptic episodes, to reduce cerebrospinal fluid production and reduce intracranial pressure. They are also commonly used for the for the prophylactic treatment of AMS. It is the renal CA inhibition that offers protective effects by arresting bicarbonate reabsorption and formation in the proximal tubule thereby leading to systemic acidosis when inhibitors are administered prophylactically (165). This systemic acidosis increases ventilatory drive and alveolar ventilation ($\dot{V}_A$), which ultimately results in a hypocapnic state (158). The hypocapnia caused by CA inhibition is believed to reset an individual's operating point on the metabolic hyperbola to a lower PCO_2 and therefore a steeper region of the curve, requiring larger changes in $\dot{V}_A$ to elicit any change in CO_2 (36). The individual's response to CO_2 is unaltered, though due to the steeper setpoint, larger increases in ventilation are required to reach a hypocapnic state and the apnic PCO_2 threshold which ultimately stabilizes breathing (178). Without CA inhibition, an altitude-induced

increase in ventilation causes hypocapnia and systemic alkalosis (161). This respiratory alkalosis suppresses chemoreceptor activity, attenuating the increase in ventilation and ultimately limiting arterial PO_2. Within a few days of exposure to altitude, the kidney begins to compensate for the respiratory alkalosis by reabsorbing less HCO_3^- and thereby restoring some of the hypoxic ventilatory drive (161). Renal CA inhibition caused by the prophylactic administration of AZ allows an individual to undergo renal acclimatization prior to a high altitude ascent effectively countering the hypoxia-induced respiratory alkalosis and maintaining hypoxic ventilatory drive (100, 158). Both the central and the peripheral chemoreceptors have been shown to express CA and they can both be inhibited *in vitro* by a direct application of CA inhibitor or *in vivo* by a sufficiently high dose of a CA inhibitor; in the carotid body CA inhibition manifests as a delay in the hypoxic response by reducing the chemoreceptor activity and sensitivity to CO_2 (69, 158). The effects of local CA inhibition on the central and peripheral chemoreceptors during hypoxia *in vivo* are not well understood due to changes in receptor sensitivity from both the acidosis and the hypoxic exposure (162). In summary, systemic CA inhibition effectively prevents altitude illness through a shift in acid-base status and an increase in ventilatory drive, effectively accelerating the acclimatization process.

1.2.2.1 Acetazolamide vs. Methazolamide

Both AZ and MZ have been used in the treatment of glaucoma, while only AZ is currently prescribed for preventative treatment of AMS. The molecular structures of AZ and MZ are very similar (Figure 1.2-1), MZ differing by a mere methyl group on the thiadiazole ring that alters both the kinetics and, to a lesser degree, the dynamics of the drug (100). AZ and MZ have similar CA inhibitor activity, but MZ has five fold more inhibiting activity on CA I and nearly three fold more inhibting activity on CA III; conversely AZ is 80 fold more effective at inhibiting CA IV (162). In addition, decreased plasma protein binding with MZ compared to AZ allows it to distribute more readily into the tissue including the cerebrospinal fluid, which results in a significantly longer half life (100). Both drugs are excreted into the urine; all of AZ is excreted unchanged whereas only 25% of MZ is excreted intact. The metabolism of the other 75% is unknown though it is suggested that its byproduct is readily reabsorbed in the kidney, which could account for the drastically lower renal clearance rate (100). In

summary, the increased tissue distribution and half-life of MZ could compensate for its depressed potency for CA IV (responsible for renal HCO_3^- reabsorption) inhibition, suggesting that MZ could prove to be as effective as AZ at similar doses (100).

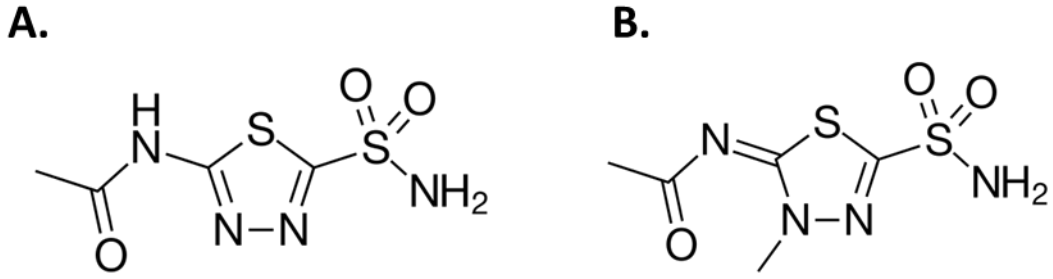

Figure 1.2-1 Molecular structure of (A) AZ and (B) MZ. The notable difference between the two structures is the methylation at the thiadiazole ring.

1.3 Hypoxic Pulmonary Vasoconstriction

In this section, an overview of the predominant stimuli as well as the physiological and temporal characteristics of HPV are reviewed. The potential mechanisms of O_2 sensing, and possible modulating influences from CO_2, hydrogen ions (H^+), sympathetic nerve activity, erythrocytes and endothelium will be reviewed. Finally, the influence of CA inhibition on HPV will be addressed

1.3.1 Characteristics of HPV

Also known as the Euler-Liljestrand mechanism, HPV was first accurately described in an *in vivo* cat model exposed to hypoxia (fraction of inspired O_2; $F_IO_2 \approx 0.11$-0.12) during a series of experiments designed to better understand the regulation of mammalian pulmonary blood pressure (40), though evidence of HPV can be found as far back as 1894. Shortly after von Euler and Liljestrand's characterization, HPV was reproduced in conscious humans breathing a similar hypoxic inspirate (113) and it is incredible to note that 70 years since its discovery, the underlying mechanism driving the phenomenon is still poorly understood despite being the subject of thousands of studies. This section navigates the predominate mechanism(s) behind HPV, beginning with the contributing stimuli and the dose-dependent relationship

with the HPV response, followed by the typical time course associated with the HPV response and finishing with the potential mechanisms and sites of O_2 sensing.

1.3.1.1 Stimulus-Response

The HPV response is considered to be an adaptation in the lung vasculature whereby the smooth muscle constricts in response to alveolar hypoxia thereby restricting regional perfusion (40). In a healthy lung, that is heterogeneously ventilated, the HPV response is considered to be an adaptive reflex that diverts blood away from regions of low ventilation towards regions of high ventilation, effectively maximizing O_2 exchange (3). The local HPV reflex is advantageous because it improves ventilation-perfusion ($\dot{V}_A/\dot{Q}$) matching but it becomes less desirable when alveolar hypoxia is global as is the case with high altitude exposure. In this instance, HPV leads to high pulmonary pressures and impaired $\dot{V}_A/\dot{Q}$ matching. In this review, HPV is in reference to pulmonary vasoconstriction resulting from global alveolar hypoxia, as is present in the high-altitude environment. An acute increase in PA pressure in response to a hypoxic challenge, such as an altitude above 2500 m or an F_IO_2 below 0.15 (85, 152, 169), is the hallmark characteristic of HPV. Intact animal studies have provided data demonstrating the HPV response as a linear function of the severity of hypoxia, up until extreme hypoxia is achieved ($F_IO_2 < 0.10$) at this point the effect is attenuated (19). There is a great deal of variability in the magnitude of HPV between species and this is believed to be partly due to interspecies differences in pulmonary vascular smooth muscle (42, 181). In healthy humans and those susceptible to HAPE, the HPV response can vary by approximately 4-5 times between individuals, within their respective groups (51). The accurate assessment of both the hypoxic stimulus and the HPV response is a challenge that has been tackled by a number of research groups, which has been reviewed extensively (172). It has been reasoned that since the volume of O_2 consumed by the lungs is nearly nil, the direct stimulus for HPV must be a combination of the alveolar partial pressure of O_2 (P_AO_2) and the PO_2 in the mixed venous blood. Studies show that manipulating the PO_2 of either mixed venous blood or alveolar gas, while clamping the other results in a proportional variation in the HPV response indicating both stimuli contribute to the response (101). Modeling data from the HPV response in anesthetized dogs determined P_AO_2 to be the dominant contributor and therefore it is often regarded as the primary stimulus for HPV (103). There is a considerable blood supply in the vaso vasorum of the pulmonary arteries

that has been shown to influence HPV, indicating that the PO_2 in the systemic circulation contributes to HPV (102). Administration of hyperoxia can completely reverse the HPV response and is considered to be both an assessment of the severity of HPV and a diagnostic tool for assessing pulmonary vascular remodeling (34, 78, 96). The HPV response is ultimately an increase in pulmonary vascular tone which is best approximated by pulmonary pressure-flow relationships, a measure that can only be accurately achieved in isolated lung models (172). Applying a vasoconstrictive stimulus decreases the slope of the pressure-flow relationship or pulmonary vascular conductance, suggesting that a given change in flow will result in a larger pressure change (120, 171). The pulmonary pressure-flow relationship is curvilinear owing to vascular distention and the recruitment of under-perfused regions of the lung with increasing flow. This direct assessment of pulmonary vascular conductance is achieved in isolated lung models by controlling the perfusion rate and in healthy humans by administering incremental exercise protocols (21, 88). Accurate indices of PA pressure, validated against PA catheterization, have been developed using a Doppler measure of TR velocity and a simplified Bernoulli equation (125). The Bernoulli equation estimates the pressure gradient between two vessels from the velocity of a fluid jet travelling down the gradient; it is simplified by assuming the gravitational acceleration, friction due to viscosity and the proximal velocity (within the left ventricle) are negligible (15). The increase in blood viscosity following prolonged hypoxic exposure could affect the estimation, though a right heart catheterization study in healthy and HAPE susceptible individuals acclimatized to 4559 m shows a strong correlation with PA pressure derived from echocardiography (20). Many studies use noninvasive pulmonary vascular resistance (PVR; PA pressure/cardiac output) as a surrogate of the actual pressure-flow relationship (43, 117, 180); the validity of this measure has been questioned due to the curvilinear pressure-flow relationship that demonstrates how increases in both pressure and flow results in a decrease in PVR without any alterations in vascular tone (172). Prominent researchers in the field argue that due to this nonlinear relationship that is nearly flat at normal physiological PA pressures normally seen in normoxia and hypoxia, tone is not necessarily associated to flow through Ohm's law and therefore estimations of PA pressure may provide a better index of tone than PVR (6, 33).

1.3.1.2 HPV Time Course As seen in Figure 1.3-1 the HPV response is biphasic with PA pressure rising rapidly within five seconds of hypoxic exposure; the rate of rise in PA

pressure tapers at approximately 45 minutes (29, 174). In phase II, HPV continues to develop reaching a peak response plateau after two hours in humans (33, 174). The distinct phases are likely due to two independent mechanisms with phase I possibly originating from O_2 sensing mechanisms in the mitochondria and phase II from an increase in hypoxia inducible factors (HIF) (*see* section 1.3.1.3, pg. 13) and modulated by iron status (*see* section 1.3.2.2, pg. 16; (44, 45, 172). Interestingly, in rabbits there is a third phase where PA pressures climb to nearly two fold what is seen in phase II over the course of the next six hours (182). Exposure to peak hypoxic pulmonary pressures can ultimately lead to vascular remodeling within the span of a few days manifesting as chronic pulmonary hypertension (153, 156). The temporal domains for isolated lung preparations are similar to what is observed in *in vivo* models during exposure to moderate hypoxia (PO_2 = 30-50 mmHg) though in cases of severe hypoxia (PO_2 < 30 mmHg) there is a steep fall in pressure, after 15 minutes of exposure, which is restored at about the 90 minute mark (172). The mechanism(s) accounting for the discrepancies seen between the temporal domains of the isolated lungs and *in vivo* models are not well understood though the likely factors include the lack of innervation or cardiac output or possibly the fact that more severe hypoxia can be applied to isolated lung models (160).

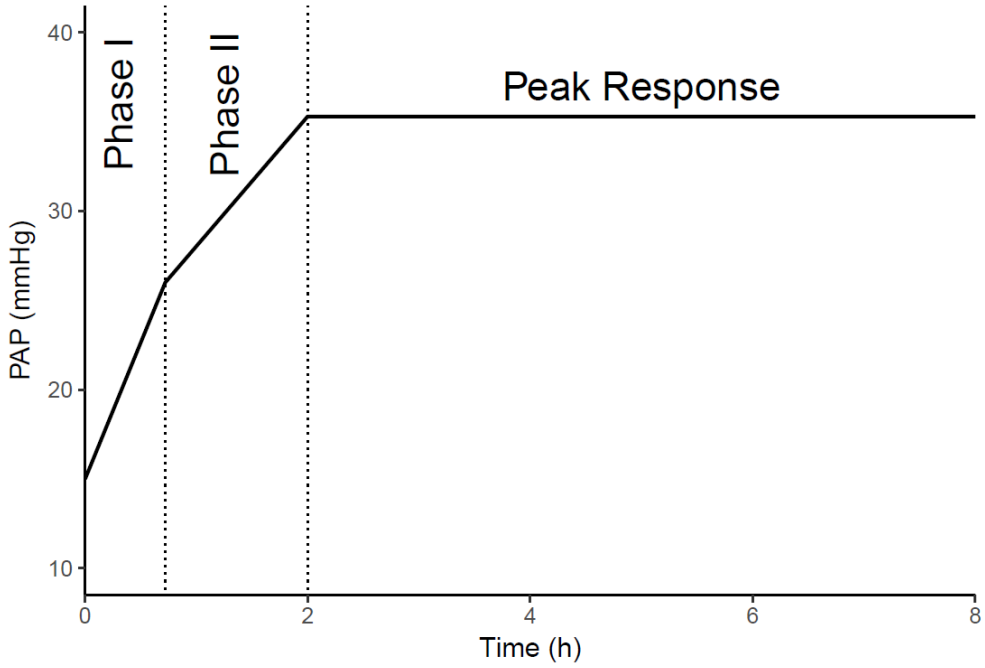

Figure 1.3-1 Time Course of Human HPV Response. Time zero represents onset of hypoxia (F_IO_2 ≈ 0.12). Phase I and II are characterized by the rate of increasing mean PA pressures (PAP).

1.3.1.3 Sensors within the Pulmonary Artery Smooth Muscle Cells

Broadly speaking, the HPV response is comprised of a mechanism that senses O_2, elicits a hypoxia-dependent signal, increases intracellular calcium $[Ca^{2+}]_i$ that causes constriction in pulmonary artery smooth muscle cells (PASMC). This process is not well understood but much progress has been made due to the advent of isolated PASMC cultures and the discovery that PASMC have the intrinsic ability to detect hypoxia (93, 115, 124). The PASMCs are cultivated from arterial vessels smaller than 500 μm; larger vessels show a diminished sensitivity to hypoxia with a complete loss of sensitivity at 800 μm (93). Since the PASMC is the site of O_2 sensing and both alveolar and mixed venous PO_2 has been identified as the predominant stimulus, there is a spatial disconnect between the two that could be accounted for by pre-capillary gas exchange (173) or through a membrane depolarization propagated from endothelium to smooth muscle via gap junctions (186).

The rise in $[Ca^{2+}]_i$ is thought to primarily occur through the activation of three Ca^{2+}channels: the store operated and voltage operated Ca^{2+}channels located on the plasma membrane and the ryanodine receptors located on the sarcoplasmic reticulum (172). Activation of these channels occurs via important O_2 sensors found in both the mitochondria and cytoplasm. A hypoxic induced-disruption of the mitochondrial electron transport chain gives rise to the three predominant hypotheses of mitochondrial O_2 sensing. The first and most likely is an increase in ROS generation at complex III of the mitochondrial electron transport chain during hypoxia (145). Acting as a signaling molecule, it is thought that ROS induces PASMC constriction by (A) directly activating the ryanodine receptors, releasing intracellular Ca^{2+} stores in the sarcoplasmic reticulum and (B) by activating RHO kinase that induces a smooth muscle Ca^{2+} sensitization (70). This hypothesis is supported by data that demonstrates HPV relief after the application of a superoxide scavenger or superoxide dismutase inhibitor in isolated rabbit lungs (188). A direct consequence of mitochondrial electron transport chain dysfunction is a decrease in adenosine triphosphate (ATP) production and a relative increase in intracellular adenosine monophosphate; this increase in adenosine monophosphate/ATP ratio is known to initiate a signaling cascade ending in the activation of ryanodine receptors that are likely to contribute to the HPV response (172). Finally, an mitochondrial electron transport chain disruption is thought to cause a reduced

state within the PASMC which closes plasma membrane potassium channels and induces a depolarization that opens voltage and subsequently store operated Ca^{2+} channels (172).

The regulatory mechanism of the hypoxia-inducible transcription factor (HIF) is also thought to act as an O_2 sensor in pulmonary vasculature; upon hypoxic activation, HIF upregulates a number of genes implicated in the progression of the HPV response (44). Under normoxic conditions the constitutively produced $HIF_{1\alpha}$ is hydroxylated by prolyl-hydroxylase domain enzyme using O_2 as a substrate and then tagged for proteosomic destruction by the von Hippel-Lindau protein (44). Hypoxic conditions reduce the available O_2 and consequently limit the prolyl-hydroxylase domain reaction, increasing cytoplasmic $HIF_{1\alpha}$, allowing it to bind to the readily available $HIF_{1\beta}$ and form a dimer that can bind to hypoxia response elements, ultimately upregulating transcription in the region (44). The mitochondria and cytoplasm of smooth muscle cells are likely the site of hypoxia detection, however there are a number of other important modulators of the HPV response that have been identified and will be discussed in the following sections.

1.3.2 Potential Modulators of HPV

Despite not knowing the precise mechanisms that underlie HPV, it is well accepted that the primary response occurs within the PASMCs. Much research has uncovered a number of important HPV modulators. The following section reviews these important modulators including the pulmonary vascular endothelium, erythrocytes, iron status, pH, CO_2 and sympathetic innervation.

1.3.2.1 Erythrocytes and Endothelium

It is hypothesized that secondary effectors modulate the HPV response through the release of local vasoconstrictors following hypoxic sensing; the two most likely candidates are the pulmonary endothelium and circulating erythrocytes. Studies of denuded isolated human small pulmonary arteries demonstrate an attenuation of phase I of the typical HPV response and a complete abolishment of phase II (134). The hypoxic signaling mechanisms in PA endothelial cells and their role in HPV is summarized in a comprehensive review; the central message of which suggests endothelial cells likely participate in the HPV response through the release of messenger molecules (2). The pulmonary endothelium constitutively produces nitric oxide synthase (NOS), an enzyme that synthesizes NO from O_2 and L-arginine (154).

Putative endothelial NO synthesis contributes to normal vascular tone by diffusing into smooth muscle cells and activating a second messenger system that ultimately lowers intracellular Ca^{2+} levels; an attenuation of NO synthesis due to a lack of O_2 substrate during hypoxia is thought to perhaps add to the HPV response (154). A study of healthy humans ascending to high altitude found no correlation between PA pressure and exhaled NO, suggesting the link is of only minor importance to the HPV response (32). Similar to NO, Prostacyclin is a common vasodilator that is constitutively produced by endothelial cells and acts through second messengers to reduce intracellular Ca^{2+} to maintain normal vascular tone. Inhaled prostacyclin has been shown to reduce pulmonary hypertension prior to cardiac surgery in patients undergoing valvular surgery (53), however in HPV exogenous prostacyclin appears to have no effect, despite its down regulation in hypoxic conditions (2). Endothelin is a small molecule released by the endothelium and acts as a potent vasoconstrictor by targeting endothelin G-coupled protein receptors on PASMCs (122). Activation of endothelin receptors initiates a secondary messenger system which results in the sensitization of Ca^{2+} channels and an amplification of all Ca^{2+}responses (122). The role of endothelin in HPV appears to be complex; ultimately it's thought to play a potentiating role in HPV as it is upregulated in hypoxia though the production of endothelin is not correlated with the HPV response itself (2). Hydrogen peroxide production is increased in endothelial cells during hypoxia, identifying a secondary source of ROS capable of acting on the smooth muscles (68). The hypoxic response of the pulmonary vascular endothelium likely plays an important role in modulating HPV.

Prolonged exposure to hypoxia leads to an increase in hematocrit (Hct) and hemodilution studies have shown that the resultant increase in blood viscosity contributes to an increase in PA pressure which can act as a confounder when assessing the contributions of erythrocytes to the HPV response (73, 98). Erythrocytes play an important role in NO scavenging through the binding of NO to deoxyhemoglobin and by oxidizing available NO and nitrite to nitrate (119). For this reason, elevated Hct could potentiate the HPV response simply due to the larger number of erythrocytes available (30). Through the process of oxidizing NO and nitrite, erythrocytes produce ROS that could enter PASMC and could also play a role in the HPV response (75, 119). Paradoxically erythrocytes have also been shown to exert a dilator effect; the hemoglobin (Hb) desaturation mechanism releases ATP and

directly drives endothelial NOS activity and S-nitrosothiol production, a potent vasodilator (26, 119). Taken together the contribution of erythrocytes to HPV could be nil, though it has been suggested that this balance of NO production and scavenging could be dysfunctional in certain disease states, perhaps this is also the case with hypoxic exposure (119).

1.3.2.2 Iron Status

Constitutively produced HIF has an incredibly short half-life (~ 5min), which is due in part to its constant proteosomic destruction (44). This destruction relies on the hydroxylation of HIF by the von Hippel-Lindau protein that depends on freely available iron to complete the reaction (44). When administered an iron scavenger, healthy participants experienced a pulmonary vasopressor response in normoxic conditions, which is similar to what is seen in hypoxia (6). Furthermore, iron infusions in healthy humans exposed to acute hypoxia significantly blunted the HPV response, particularly the phase II response (175). With the understanding of iron's integral role in the HIF pathway, hypotheses emerged suggesting that iron status could play a role in the augmented PA pressures seen in individuals susceptible to HAPE and could predict altitude illness, though this was shown not to be the case (44). Genetic analyses have identified two paralogues of the HIF_{α} subunit, most lowlanders express the $HIF_{1\alpha}$ whereas Tibetans express $HIF_{2\alpha}$ (45). The specific differences are not well understood though it is attributed to the Tibetan's diminished ventilatory response and decreased Hb concentration at altitude, compared to lowlanders and could interact with iron differently, adding a degree of complexity to the proposed mechanism (45). Regardless of the interaction between HIF expression and iron status, it is clear that they both could play a role in the variability of the HPV response seen between individuals.

1.3.2.3 Carbon Dioxide and Acid-Base Status

Carbon dioxide is involved in HPV modulation, particularly at altitude where a hypoxic-induced increase in resting ventilation results in an overall depression in partial pressure of alveolar CO_2 (P_ACO_2) and an increase in blood pH. In normoxic conditions both hypercapnia and hypocapnia show a respective pressor or dilator response that is fully developed after 1.5-2 h of exposure to stimulus (7). Most studies show that both hypercapnia and acidosis potentiate HPV while hypocapnia and alkalosis attenuate HPV in humans (39, 89), intact animals (17, 135, 149), and isolated lung models (127, 183). Studies in isolated rabbit lungs

indicate that hypercapnia can improve $\dot{V}_A/\dot{Q}$ matching during the HPV response compared to normocapnic hypoxia and consequently improve arterial oxygenation, despite the fact that CO_2 exacerbates HPV (74). Isolated lung models indicate that the potentiating effect of hypercapnia on HPV is lost when hypoxia is sufficiently severe ($PO_2 \approx$ 20-25 mmHg) whereas, hypocapnia continues to attenuate HPV in severe hypoxia (183). There is some data to suggest that CO_2 in the pulmonary circulation is a vasodilator (184), and that the constriction observed with hypercapnia is due to the accompanying acidosis as the effect is lost when pH is normalized following bicarbonate infusion (17). Considering hypoxic hyperventilation leads to hypocapnia, it is suggested that the lack of CO_2 and resultant alkalosis acts as an HPV modulator by attenuating vasoconstriction.

1.3.3 Influence of CA Inhibition on HPV

The attenuation of HPV by AZ is thought to occur through multiple mechanisms that are summarized in Figure 1.3-1. This section will address those mechanisms as well as consider whether MZ is able to affect HPV through similar actions.

1.3.3.1 Potential Mechanisms of Action

The attenuating effects of AZ on HPV were initially observed in anesthetized dogs (165). Further studies showed that AZ was capable of blunting the HPV response, which was thought to be partly due to the increased ventilation and arterial O_2 saturation associated with the drug-induced metabolic acidosis and partly due to an unknown mechanism that persists when arterial O_2 saturation is controlled (Figure 1.3-2) (62, 176). The contributions of metabolic acidosis are particularly evident in data that shows an abolishment of the AZ-attenuated HPV response following intravenous bicarbonate infusion (180). Administration of AZ to individuals that are partially or fully acclimatized to high altitude shows no effect on HPV, indicating that the attenuating effect of AZ is only effective against acute hypoxic exposure when administered prophylactically (12, 41). An infusion of AZ into isolated rabbit lungs shows an inhibition of the HPV response without inducing acidosis (29), suggesting that AZ may act through an alternative mechanism, though studies have shown no correlation between AZ and exhaled NO (1) or with potassium, endothelin or angiotensin (63). Intravenously administered AZ in conscious dogs also exhibits a depression of the HPV response without acidosis (62).

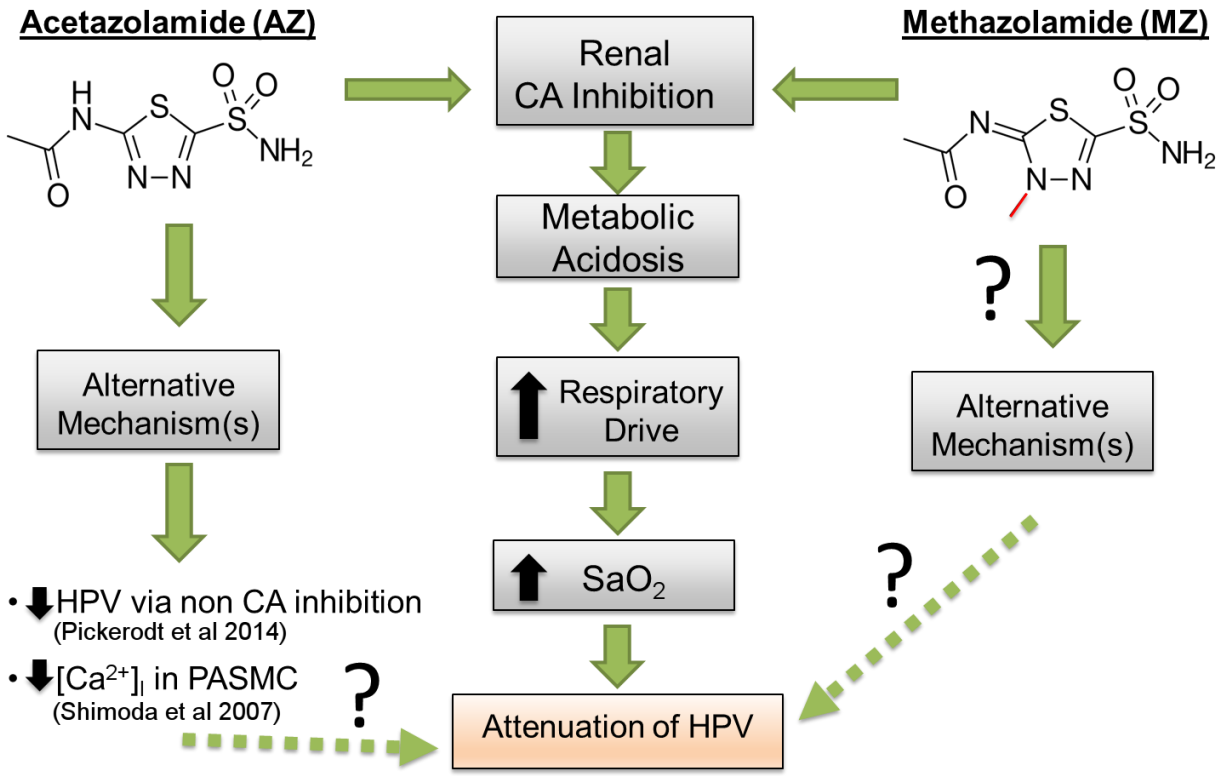

Figure 1.3-2 Summary of proposed mechanisms for the attenuation of HPV by AZ. Unique methyl group on thiadiazole ring of MZ highlighted in red. Question mark indicates a mechanistic pathway that is poorly understood or not known to exist.

Comparisons of AZ with other CA inhibitors administered intravenously demonstrate that AZ has a uniquely potent blunting effect on HPV that is not observed with other inhibitors suggesting the alternative mechanism is independent of CA inhibition (63). The most compelling evidence that AZ reduces HPV independent of CA inhibition is found in anesthetized dogs where intravenously administered N-methyl acetazolamide (NMA), an analog to AZ that is nearly identical in structure but is not able to inhibit CA, attenuates HPV to nearly half that of AZ (123). A similar result was found in PASMCs in that both AZ and NMA reduced intracellular Ca^{2+} in hypoxia by approximately 30% which occurred independent of changes in pH or membrane potential (148). An attenuation of erythropoiesis is an indirect effect of renal CA inhibition and AZ has been explored as a treatment for individuals with chronic mountain sickness experiencing polycythemia (128). Treatment with

AZ resulted in a significant increase in serum ferritin levels, a known index of iron status, though it was suggested to occur due to increased iron availability due to blunted erythropoiesis. Finally, there is evidence to suggest that molecules containing a thiadiazole ring have the potential to act as a ROS scavenger which could be a potential mechanism whereby both AZ and MZ could act to attenuate HPV (126). Furthermore MZ has been shown to upregulate nuclear factor (erythroid-derived 2)-related factor 2 (Nrf-2) which acts as a transcription factor that regulates over 90% of human antioxidant genes and could offer a potential pathway for attenuating HPV (87).

1.4 Summary

In summary HPV is a mechanism that is believed to improve $\dot{V}_A/\dot{Q}$ matching on a regional level. Global hypoxia, for example at high altitude, initiates global HPV response that raises PA pressure. An exaggerated HPV response is a characteristic of lowlanders who develop HAPE. It is thought that AZ attenuates HPV through initiating metabolic acidosis, driving ventilation and increasing arterial O_2 saturation. Though more recent studies suggest that it acts directly on the PA smooth muscle, reducing intracellular Ca^{2+} by an unknown mechanism unrelated to CA inhibition. Also through CA inhibition, MZ is able to achieve a similar improvement in arterial O_2 saturation during hypoxic exposure. It is not currently known if MZ will depress HPV in humans similar to AZ. It is also not known if MZ is able to act directly on PA smooth muscle cells. Future investigations comparing the effectiveness of AZ and MZ in attenuating HPV in humans as well as the effects of MZ on isolated PASMCs exposed to hypoxia could provide further insight into the mechanisms of CA inhibitors.

Chapter 2. Introduction

Approximately 10-25% of people ascending to altitudes above 2500 m experience symptoms associated with acute mountain sickness (AMS)(65, 95), which can be proactively mitigated by planning a slow ascent profile and with the prophylactic treatment of acetazolamide (AZ), a sulfonamide CA inhibitor (161). The mechanism whereby AZ relieves symptoms of altitude illness is not fully understood though it is believed that the systemic acidosis resulting from CA inhibition augments $\dot{V}_A$ and consequently improves oxygenation in hypoxia (161). In addition, AZ has been shown to reduce the severity of hypoxic pulmonary vasoconstriction (HPV) (176), a heterogeneous pulmonary vasoconstriction which leads to pulmonary hypertension and impaired ventilation-perfusion matching at high altitude (60). Many agree that AZ likely attenuates HPV by improving arterial O_2 saturation in hypoxia thereby reducing the stimulus for HPV (159) but there is evidence for a direct effect of AZ on HPV in humans (176), animals (123), and isolated PASMCs (148). For example in humans, HPV is attenuated with AZ compared to placebo while controlling arterial oxyhemoglobin saturation (176). In dogs, the administration of *N*-methyl-AZ, a sulfonamide analog to AZ with no CA inhibitory activity, caused a significant depression in the HPV response (123). Finally in isolated PASMCs, both AZ and NMA are able to reduce intracellular Ca^{2+}during hypoxic exposure through a mechanism that does not involve membrane depolarization or alterations in intracellular pH (148).

Although AZ is effective at preventing altitude illness, it is associated with reports of muscle fatigue (49, 76), impaired exercise performance (48), paraesthesias, mild nausea (100), headaches and drowsiness (23). MZ, a sulfonamide analog to AZ, has been shown to have similar potency in CA inhibition (100). In dogs, intravenously administered MZ attenuates HPV, but not to the same extent as AZ (123) suggesting that MZ may not share the same direct effect on HPV as AZ. Reports from those treated with both AZ and MZ indicates that MZ is associated with milder side effects which occur less frequently compared with AZ, and this is likely the result of MZ's superior distribution throughout the body, a longer half-life, and lower effective dose (100). Administration of MZ along with aminophylline actually improves endurance exercise at altitude (139). Furthermore MZ treatment has shown to activate nuclear factor (erythroid-derived 2)-related factor 2, a

transcription factor that regulates the majority of antioxidant gene expression, suggesting it may also attenuate HPV by upregulating scavengers of ROS (87). If MZ is able to improve oxygenation and attenuate HPV to a similar degree as AZ in humans, it could offer a more tolerable substitute to AZ for those sensitive to traditional altitude sickness drugs. The purpose of our study was to determine if MZ can improve oxygenation and attenuate HPV like AZ in healthy humans exposed to poikilocapnic hypoxia. We hypothesized that both AZ and MZ would improve arterial oxyhemoglobin saturation while attenuating HPV compared with placebo.

Chapter 3. Methods and Materials

This chapter consists of an overview of the experimental methods used to systematically answer our research questions. Details are provided on the recruited participants, prescreening measurements, the experimental protocols and procedures, measured variables, and data and statistical analyses.

3.1 Ethical Approval and Clinical Trial Registration

All experimental procedures and protocols were approved by the Clinical Research Ethics Board at the University of British Columbia (H16-00028) and conformed to the Canadian Government Tri- Council Policy Statement on research ethics (*see* Appendix A pg. 81). This study was registered with the U.S. National Institutes of Health (NCT02760121; *see* A.2, pg. 83) and was performed in compliance with the *Declaration of Helsinki.* Enrollment, randomization and transparency were performed in accordance with the CONSORT guidelines (144).

3.2 Participants

Experimental sessions were conducted in the Cardiopulmonary Laboratory for Experimental and Applied Physiology at the University of British Columbia's Okanagan Campus (Kelowna, BC, Canada; elevation = 344 m). Prior to the experimental day, participants (n=14) visited the lab to prescreen for history of disease, hypertension, normal pulmonary function and detectable TR (*see 3.3*, pg. 23). All participants provided written informed consent (*see* A.3, pg. 86). Participants were young (19-40 yrs.), healthy, normotensive (systolic <140 mmHg, diastolic <90 mmHg) men that underwent regular physical activity (2+ days per week). Participants were excluded if they were obese (body mass index >30 kg/m^2), smoked regularly, had a recent surgery, or were known to have a history of glaucoma, adrenocortical insufficiency, hepatic insufficiency, renal insufficiency, electrolyte imbalance, myocardial infarction, coronary artery disease, history of stroke, chronic obstructive pulmonary disease, asthma, taking anti-inflammatory medications or other

medications, have a clotting disorder, or have a known allergy to CA inhibitors or similar drugs.

3.3 Prescreening

On a separate day prior to experimentation, each participant completed pre-screening protocols for health history, hypertension, pulmonary function and suitable echocardiographic windows as well as the presence of a visible tricuspid regurgitant (TR) jet.

3.3.1 Health History Questionnaire

Health history questionnaire (*see* A.4, pg. 100) consisted of inclusion criteria questions that assessed age, sex and physical activity, as well as 11 exclusion criteria questions aimed to identify previous cardiovascular, pulmonary or other conditions that could pose a risk during experimentation. It also aimed to identify any allergies or contraindications with the drugs or blood sampling procedures.

3.3.2 Prescreening Protocol for Hypertension

Hypertension was assessed in accordance with the guidelines set forth by Hypertension Canada (84). Blood pressure was assessed with an automatic blood pressure cuff (Carescape V100 monitor, GE, Fairfield, CT) while participants remained in the seated position. Three consecutive measures were taken, separated by a minimum of one minute. If the average systolic blood pressure (SBP) value exceeded 140 mmHg and/or the average diastolic blood pressure (DBP) exceeded 90 mmHg, participants were considered to have hypertension and were excluded from the study.

3.3.3 Pulmonary Function Testing

Spirometry, lung volumes and diffusion capacity tests were conducted in agreement with the American Thoracic Society and European Respiratory Society's joint guidelines (92, 110, 187). Forced vital capacity (FVC) and forced expired volume in one second (FEV_1) were assessed using an FVC maneuver that involves a full inspiration followed by a forced expiration. A minimum of three repeatable maneuvers were performed and the largest FEV_1 and FVC value was selected (110). Vital capacity was assessed through a maneuver that involves a full inspiration followed by a full slow expiration; a minimum of three maneuvers

were performed and the largest vital capacity value was selected (110). A single breath carbon monoxide test was used to quantify diffusion capacity (D_LCO) on each individual (92). Body plethysmography was used to assess lung volumes, specifically total lung volume, functional residual capacity (FRC) and residual volume; panting maneuvers were performed until three tests were obtained with values ± 5% of the mean value (187). For each test, participants sat within the body plethysmography box (V6200, Vmax Sensormedics, Yorba Linda, CA, USA) with a rigid upright posture and their feet flat on the ground, whilst breathing through a spirometer and bacteriological filter with nose clamped. All pulmonary function measurements were compared against population-based predictions (18, 25, 111, 132).

3.3.4 Echocardiographic Screening

In the left lateral decubitus position participants were assessed for adequate imaging windows and whether it was possible to visualize a complete TR jet. Right atrial pressure was assessed using the collapsibility index of the inferior vena cava (IVC; *see* section 3.5, pg. 27) to ensure participants did not have atrial hypertension. The same research-trained sonographer performed all echocardiographic assessments.

3.4 Experimental Protocol

Participants were asked to abstain from alcohol, caffeine or strenuous exercise for 12 h prior to testing. Experimental protocol consisted of ten-minute baseline period followed by a sixty-minute exposure to poikilocapnic hypoxia. Respiratory and cardiovascular parameters were sampled continuously throughout the protocol. Arterial blood samples were obtained prior to instrumentation. Arterialized capillary blood samples and echocardiographic measurements were collected both at baseline and during the final five minutes of hypoxic exposure. Experimental methodology is described in detail in the following subsections.

3.4.1 Pharmacological Intervention

A double blind, placebo-controlled, crossover study design was used in which participants were randomized to treatment with MZ, AZ or placebo (PBO) for two days prior to obtaining outcome measures similar to previous work (176). Both AZ and MZ primarily target CA isoforms I, II, III, IV, XII, XIV; both drugs equally inhibit isoforms II, XII and XIV, whereas

MZ is three and five-fold more effective at inhibiting isoforms I and III, respectively. Isoform IV is inhibited 83-fold more effectively by AZ compared with MZ (162). The dosage was selected based on previous investigations into the effect of CA inhibitors on the physiological responses to hypoxia (81, 176, 179). For AZ, a dosage of 250 mg was administered orally three times daily. Due to its decreased protein binding (AZ: 97%; MZ: 55%), renal clearance (AZ: 200 ml/min; MZ: 20 ml/min), and longer half-life (AZ: 5 h; MZ: 14 h) (100), MZ was administered in lower doses (100 mg/dose) and less frequently (2 doses/day). To avoid any potential carry over effects and allow for sufficient number of half-lives to elapse (AZ: 48; MZ: 17), each intervention was separated by ten days. The interventions were prepared by a local pharmacist and placed in a gel capsule to ensure no observable difference due to dosage size; furthermore, identical capsules containing only microcrystalline cellulose were created for placebo trial. Gel capsules were then inserted into a blister package with labels outlining the dosing schedule. To match the dosing schedule between conditions, a placebo was inserted between the two doses of MZ so that capsule consumption appeared identical. The pharmacist completed the randomization and an identification code was generated for each of the three trials. The un-blinding code was kept in a sealed envelope and retained until all data analysis and statistical processing was complete.

3.4.2 Participant Instrumentation

Participants were instrumented with electrocardiogram electrodes in a lead-II configuration connected to a bio amp (FE132; ADInstruments, Colorado Springs, CO, USA), and a pulse oximeter on the left index finger (7500FO; Nonin Medical, Inc., Plymouth, Minnesota, USA) used to estimate peripheral oxyhemoglobin saturation (SpO_2). Beat-by-beat SBP and DBP were measured from a cuff placed on the mid-phalanx of the right middle finger using finger pulse photoplethysmography (Finometer PRO; Finapress Medical Systems, Amsterdam, the Netherlands), which has previously been validated against intrabrachial arterial pressure recordings (52). Return to flow calibration was performed prior to each trial to calibrate blood pressure to a reconstructed brachial waveform. An automated blood pressure cuff was placed on the right arm (Carescape V100 monitor, GE) to confirm reconstructed brachial measurements. Participants breathed through a mouthpiece (with nose clamp), bacteriological filter, and a two-way non-rebreathing valve (2700 series, Hans Rudolph,

Shawnee, KS, USA). Inspired port was connected, through wide bore tubing (I.D. 35 mm), to a 3 way, Y-shaped, stopcock type, manual valve (2100 series, Hans Rudolph), with one port open to room air and one connected to the hypoxic reservoir. The dead space between participant's mouth and non-rebreathing valve was measured to be ~ 250 ml. The expired port was connected to a 4.7 L mixing chamber (MLA246; ADInstruments), where mixed expired gases were drawn at 250 ml/min (Flow Control R-2, AEI Technologies, Pittsburgh, PA, USA) through two gas analyzer systems (S-3A & CD-3A, AEI Technologies) to measure the fraction of expired O_2 and CO_2 connected in series. Respiratory flow was measured near the mouth using a pneumotachograph (HR 800L; Hans Rudolph) and a differential pressure transducer (1110 series; Hans Rudolph), which was zeroed and calibrated using a 3-l syringe before experimentation. Respired gas partial pressures were sampled near the mouth, dried with nafion tubing in desiccant, and the percent concentration of O_2 and CO_2 was determined with an additional gas analyzer (ML206, ADInstruments). All Gas analyzers were calibrated prior to experimentation with gases of known concentration.

3.4.3 Poikilocapnic Hypoxia

The poikilocapnic hypoxia protocol was selected based on previous reports of HPV where 60 minutes of exposure elicited a significant increase in pulmonary pressure (33, 174, 176). A F_IO_2 of 0.12 was chosen, in an effort to mimic high altitude exposure of ~5000 m above sea level. Following instrumentation, participants lay supine on an echocardiographic table and breathed from a 150-l polyvinyl chloride collection reservoir (196-150, VacuMed, Ventura, CA, USA) containing hypoxic gas provided using an oxygen scrubber (HYP-123, Hypoxico Inc, New York, NY, USA). Respiratory and cardiovascular measurements were collected continuously during a baseline period of ten minutes breathing room air. In addition, an arterialized capillary sample was collected during the baseline period (*see* section 3.6.3.1, pg. 30). At the end of the baseline period, participants were repositioned in a left lateral decubitus position and echocardiographic images were acquired. Participants were returned to a supine position whereupon the three-way valve was switched from room air to supply the participant with hypoxic gas for 60-minutes. After ~50-minutes of exposure, arterialized capillary blood samples and echocardiographic images were obtained after which the hypoxic exposure was terminated and room air breathing was restored.

3.5 Primary Outcome Measurement – Pulmonary Artery Systolic Pressure

The primary response of interest is the magnitude of smooth muscle constriction within the pulmonary vasculature in response to hypoxia, understood as pulmonary vascular tone. Both pulmonary artery systolic pressure (PASP) and total pulmonary resistance (TPR; PASP/ $\dot{Q}$) have been used as a noninvasive indices for vascular tone (7, 33, 117). However, it has been suggested that PVR can change independently of vascular tone, for example when an increase in cardiac output ($\dot{Q}$) is accommodated by recruiting under perfused regions of the pulmonary vasculature a decrease in overall resistance may be observed without any change in vascular tone (86). For this reason it has been theorized that, PASP is more closely related to pulmonary vascular tone than PVR (6).

All echocardiographic measurements were collected on a commercially-available ultrasound system (Vivid E9; GE) using a broadband M5S 5 MHz transducer. Images were captured and saved for offline analysis using commercially available software (EchoPAC v.13; GE). All echocardiographic values represent an average from three cardiac cycles representing the clearest of five collected images for each experimental stage.

The collapsibility index of the IVC was assessed at baseline and used to estimate right atrial pressure as recommended by the American Society of Echocardiography (136). It is believed that right atrial pressure varies by less than 0.5 mmHg during hypoxic exposure compared with normoxia, therefore the baseline value was applied in the hypoxic condition under the assumption they were equal (151). The IVC diameter was measured from a subcostal acoustic window distal to the right atrial junction ($\approx$ 2 cm). The collapsibility index was calculated as the percentage of the difference between maximal and minimal size of the IVC before and during rapid inspiration, respectively. An IVC, with an initial diameter less than 1.7 cm, that collapses more than 50% is assumed to have a normal right atrial pressure of 1-5 mmHg; for the purposes of this study, normal right atrial pressure was assumed to be 3 mmHg (82). This method has been validated against right atrial pressure obtained directly by right heart catheterization (190). The peak velocity ($_V$) of the tricuspid regurgitant jet was identified from an apical four-chamber view using colour flow Doppler and was measured by continuous-wave Doppler ultrasound. The maximum pulmonary artery systolic pressure gradient (ΔPmax) could then be estimated from a previously

validated modified Bernoulli equation, ΔPmax (mmHg) = $4v^2$ (m/s) and PASP could be estimated by addition of the estimated right atrial pressure (125).

The same trained sonographer collected all ultrasound images for this study. The sonographer's reliability in measuring ΔPmax, diameter of left ventricular outflow tract and velocity time integral of the left ventricular outflow tract was determined through a reproducibility study involving within and between day comparisons during both normoxia and poikilocapnic hypoxia. The findings were statistically analyzed using the Cronbach's alpha reliability test intended to determine the correlation of two separate interrogations of the same construct. Based on a sample size of 18, the alpha values were all found to be >0.7 suggesting good consistency between measurements (Table 3.5-1) (118).

Table 3.5-1 Echocardiographer's between- and within-day reliability in normoxia and hypoxia

Condition	Comparison	$LVOT_D$	$LVOT_{VTI}$	ΔPmax
Normoxia	Within day	0.94	0.87	0.81
Hypoxia	Within day	0.96	0.74	0.91
Normoxia	Between day	0.92	0.86	0.76
Hypoxia	Between day	0.97	0.76	0.80

Abbreviations: $LVOT_D$, diameter of left ventricular outflow tract; $LVOT_{VTI}$, velocity-time integral of left ventricular outflow tract; ΔPmax, peak pressure gradient of across the tricuspid valve. Hypoxia administered for 30 minutes ($F_IO_2 \approx 0.12$). Within day trials separated by 1h.

3.6 Secondary Outcome Measurements

The following section elaborates on the collection and analysis of the study's secondary outcome measures including respiratory, cardiovascular, echocardiographic and blood sample variables. Each day prior to testing, atmospheric pressure, ambient temperature and relative humidity was recorded (RF Wireless Thermometer 683F03, RF-tech).

3.6.1 Continuous Measurements

All respiratory and cardiovascular parameters were acquired at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments) interfaced with a personal computer. Lab acquisition software was used to collect and analyze ventilatory and

cardiovascular variables (LabChart V7.1, ADInstruments). Cardiovascular data points were extracted from charting software at systole, triggered from the blood pressure systolic peak. Similarly, respiratory data points were selected at end expiration, triggered from the peak expiratory volume. Each individual data point for the continuous variables, during baseline and hypoxia, represents a 30 second average of the extracted data.

Instantaneous heart rate (HR) was calculated as 60/R-R interval taken from the electrocardiogram trace. Mean arterial pressure (MAP) was calculated as the sum of 1/3 SBP and 2/3 DBP. Inspired minute ventilation ($\dot{V}_I$) was calculated as a product of A) tidal volume (V_T), which was determined using an integral of the respiratory flow signal, and B) breathing frequency (f_B) and was converted from ambient temperature and pressure to body temperature and pressure saturated assuming body temperature to be 37°C. Expired minute ventilation ($\dot{V}_E$) was calculated using the Haldane transformation:

3-1
$$\dot{V}_E = \dot{V}_I \left(\frac{1 - F_I O_2 - F_I CO_2}{1 - F_E O_2 - F_E CO_2} \right)$$

Where F_IO_2 and F_ICO_2 are the inspired fraction of gases and F_EO_2 and F_ECO_2 are the mixed expired fraction of gases. The peak of the percent concentration of O_2 and the nadir of the percent concentration of CO_2 corresponded to the fractional inspired O_2 and CO_2 values from the respired gas analysis. The respired partial pressures of O_2 and CO_2 (PO_2; PCO_2) were time-corrected for gas analyzer sample delay such that the partial pressure of end-tidal O_2 and CO_2 ($P_{ET}O_2$; $P_{ET}CO_2$) values corresponded to the moment when the respiratory flow crossed zero in the positive to negative direction.

3.6.2 Hemodynamic Variables

The diameter of left ventricular outflow tract at the level of the aortic annulus was determined from the parasternal long axis view. Measurements were taken at the end of systole, representing the maximum diameter of the aorta. The velocity-time integral of left ventricular outflow tract was obtained from an apical five-chamber view by placing a pulsed wave Doppler sample volume (2.0 mm) inside the outflow tract at the level of the aortic valve. Stroke volume (SV) was calculated as the product of the velocity–time integral and

aortic cross-sectional area, and $\dot{Q}$ was obtained by multiplication with HR. These methods have been previously described and validated against thermodilution and direct Fick oximetry (22). TPR was estimated by indexing PASP to $\dot{Q}$. Left atrial pressure (LAP) was estimated using the systolic fraction of the pulmonary vein (ration of the systolic velocity-time integral to the sum of the systolic and early diastolic velocity-time integrals), which has been validated against both pulmonary capillary wedge pressure and LAP from left atrial catheterization (80).

3.6.3 Blood Samples

Baseline arterial blood was sampled from the left radial artery through an arterial puncture (n = 8) or arterial catheter (n = 2), described below. To minimize subject risk, patency of the radial and ulnar artery was confirmed with a negative modified Allen's test prior to arterial penetration (97). All participants provided a capillary sample at baseline and following 60 minutes of poikilocapnic hypoxia as described below. Blood samples were analyzed using a commercial blood gas analyzer (ABL90 FLEX, Radiometer, Copenhagen, Denmark) that aspirates blood samples into a chamber containing electrodes that are selective for the variables of interest. The analyzer was calibrated according to manufacturer specifications. Reported variables and analyses included: Arterial PO_2 (PaO_2), PCO_2 ($PaCO_2$), pH, H^+, Hct, Hb, concentration of bicarbonate ions ($[HCO_3^-]$), base excess (BE) and oxyhemoglobin saturation (SaO_2). For all measurements body temperature was assumed to be 37 °C.

3.6.3.1 Arterialized Capillary Samples

Capillary blood samples, obtained from a fingertip that has been heated, provide reasonable estimates of arterial pH and blood gases (106). Participants submerged their right hand into a water bath with a constant temperature (45°C) for five minutes. Skin around sample site was cleaned with an alcohol swab and an allowed to air-dry. A contact-activated lancet (BD Microtainer, BD, Mississauga, ON, Canada) was used to puncture the skin and a capillary blood sample was collected into a heparinized capillary tube (70 ml; safeCLINITUBES capillaries, Radiometer) and immediately analyzed using the blood gas analyzer.

3.6.3.2 Arterial Puncture

After application of a topical anesthetic (EMLA cream; 2.5% lidocaine, 2.5% prilocaine) an arterial puncture was performed in the supine position. The puncture site was heated with a dry warming pad prior to sterilization (2% chlorhexidine gluconate, 70% isopropyl alcohol SoluPrep Swab; 3M Canada, London, ON, Canada). A pre-heparinized, self-filling arterial blood syringe (PICO50, Radiometer) was inserted into the artery and 2 ml of blood was carefully collected. Air bubbles were immediately evacuated and the syringe was capped and analysis was performed within 30-seconds.

3.6.3.3 Arterial Catheter

Local anesthesia (2% lidocaine) was applied followed by the transcutaneous placement of a 20-gauge catheter (Radial artery catheter, Arrow International, Reading, PA, USA) into the left radial artery using a modified Seldinger technique guided by ultrasound (147). The catheter was connected to a commercially available arterial blood sampling kit (VP1, Edwards Lifescience, Irvine, CA, USA), allowing for repeated sampling and flushing with 0.9% saline. Prior to sampling, the dead space volume (<1 ml) was withdrawn and then an arterial sample (~1.5 ml) was collected into pre-heparinized syringes (safePICO syringes, Radiometer). Air bubbles were immediately evacuated from the syringe, the syringe was capped, and blood gas analysis was performed within 30-seconds of sampling.

3.7 Determination of Alveolar Gases and Alveolar Ventilation

Volume of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) per minute in standard temperature and pressure, dry was calculated using equations (3-2) and (3-3), respectively.

(3-2) $$(F_I O_2 \times \dot{V}_I) - (F_E O_2 \times \dot{V}_E)$$

(3-3) $$(F_E CO_2 \times \dot{V}_E) - (F_I O_2 \times \dot{V}_I)$$

Both $\dot{V}_I$ and $\dot{V}_E$ were expressed as body temperature and pressure saturated. The respiratory exchange ratio (RER) was calculated as a ratio of $\dot{V}CO_2$ to $\dot{V}O_2$.

The Bohr method was used to determine the ratio of dead space ventilation (V_D/V_T) which is equal to the ratio of the partial pressure gradient of arterial CO_2 ($PaCO_2$) to mixed expired CO_2 (P_ECO_2) to P_ACO_2 (3-4), under the assumption that none of the expired CO_2 comes from the physiological dead space and corrected for apparatus dead space volume. Arterialized capillary PCO_2 was thus used as an estimation of P_ACO_2.

(3-4) $$\frac{V_D}{V_T} = \frac{P_CCO_2 - P_ECO_2}{P_CCO_2}$$

$\dot{V}_A$ was determined by subtracting the product of V_D, which is comprised of both physiologic and apparatus dead space, and f_B from $\dot{V}_E$. The P_ACO_2 was estimated from $\dot{V}_A$ and $\dot{V}CO_2$ (3-5), where k is a constant (0.863).

(3-5) $$P_ACO_2 = \frac{\dot{V}CO_2}{\dot{V}_A} \times k$$

Using the alveolar gas equation, the P_AO_2 was estimated from the P_ACO_2, P_IO_2, F_IO_2 and RER (3-6).

(3-6) $$P_AO_2 = P_IO_2 - \frac{P_ACO_2}{RER} + \left[P_ACO_2 \times F_IO_2 \times \frac{1-RER}{RER}\right]$$

3.8 Sample Size Justification

A large effect size (f = 0.79 -1.09) was determined using previous data quantifying the attenuation of the pulmonary pressure response to acute hypoxia by AZ compared to placebo (176, 180). With an alpha value of 0.05 and given our study design, this data suggests that it

would take a minimum sample size of seven to resolve a difference between treatment and control with a power above 0.8.

3.9 Statistical Analysis

Statistical analysis was performed in R statistical language (R Foundation for Statistical Computing, Vienna, Austria). All trial data was tested for normality using the Shapiro-Wilk test. Each normally distributed outcome variable was compared within participants and between treatments (AZ, MZ, PBO) using a 2x3 repeated measures analysis of variance (ANOVA) with a significance level set at $P<0.05$. For non-parametric data, the Scheirer-Ray extension of the Kruskal-Wallis test was used with a significance level set at $P<0.05$. When a p-value less than 0.05 was achieved, *post hoc* comparisons were made using a Tukey HSD test corrected for pair-wise comparisons for parametric data and a Mann-Whitney U test for non-parametric data. Cardiovascular (HR, SaO_2 and MAP), respiratory ($\dot{V}_A$, $\dot{V}_E$, V_T, f_B, $P_{ET}O_2$, $P_{ET}CO_2$, P_AO_2, P_ACO_2, RER, $\dot{V}CO_2$, $\dot{V}O_2$ and V_D/V_T), blood sample (PaO_2, $PaCO_2$, pH, Hct, H^+, Hb, $[HCO_3^-]$, BE and SaO_2) and echocardiographic (PASP, $\dot{Q}$, TPR, TPR_{45} and SV) variables were included in the analyses. All values are presented as the mean values ± the standard error of the mean (SEM). Radial arterial blood sample data were compared between drug trials using a one way repeated measures ANOVA, followed by a Tukey's *post hoc* test when significant F-ratios were present ($P < 0.05$). Pulmonary vascular sensitivity was represented as changes in PASP against changes in P_CO_2, P_AO_2 and S_CO_2, from baseline to hypoxia.

Arterialized capillary samples were compared against arterial blood sample data and, in a subset of two participants, it was compared with arterial catheter data. Capillary data was plotted against arterial data, a line of identity was plotted and data was correlated using the Pearson r correlation coefficient. Furthermore, limits of agreement for capillary samples were estimated as being two standard deviations from the mean difference of all samples ($\mu_d \pm 2\sigma_d$; (104).

Previous reports of HPV have implicated a number of blood gas and hematological factors that contribute to changes in pulmonary vascular tone during hypoxic exposure (27). For this reason, a backward elimination of linear mixed effects regression approach was used to determine which parameters significantly contributed to the change in PASP and to what

degree. A linear mixed effects model approach was selected as the best method to account for individual variability associated with a repeated measures design. The fixed effects identified as potential contributors that were included in the model were $\dot{V}_{E,}$ P_ACO_2, H^+, $\dot{Q}$ and Hct. The model also included P_AO_2, but due to its curvilinear relationship with PASP, the log of the term was included (135). The model also contained a random effect for participants to account for the repeated measures study design. The tolerance to determine the inclusion criteria of an independent variable in the regression model was set at $P<0.05$. Standardized coefficients were calculated for the final model.

Chapter 4. Results

4.1 Participants

The flow of participants through enrollment to study completion is shown in Figure 4.1-1. Of the 14 participants recruited, one was excluded prior to randomization due to a lack of suitable imaging windows (the result of a prior musculoskeletal injury requiring surgery), and two participants were excluded from data analysis following successful completion of the study for (1) non-adherence to the experimental protocol, and (2) an inability to accurately image TR in one of three experimental conditions. Participants included in the mean data analysis (n=11) had an age of 25 ± 1 years (mean ± SEM), body mass index of 25.2 ± 0.6 kg/m^2, were all non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases, and were not taking any medications prior to testing. Participants were all normotensive men (mean systolic = 122 ± 3 mmHg, mean diastolic = 67 ± 4 mmHg) with normal pulmonary function (see Table 4.1-1).

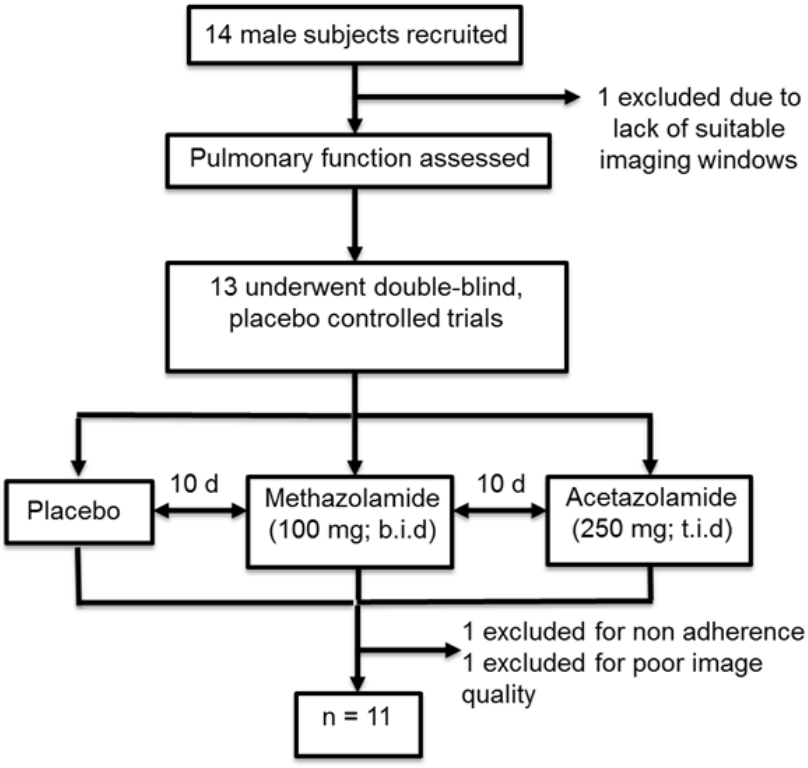

Figure 4.1-1 Participant flow chart

Table 4.1-1 Pulmonary function data

Variable	Mean ± SEM (% predicted)	Variable	Mean ± SEM (% predicted)
FVC (l)	6.0 ± 0.2 (114.8 ± 2.9)	**FRC (l)**	3.3 ± 0.1 (102.2 ± 3.4)
FEV_1 (l)	4.5 ± 0.2 (101.5 ± 4.6)	**D_LCO (ml/min/mmHg)**	33.9 ± 1.2 (93.9 ± 3.2)
FEV_1/FVC (%)	74.2 ± 3.2 (88.7 ± 4.1)	**V_A (l)**	6.2 ± 0.4 (92.5 ± 5.4)
TLC (l)	7.1 ± 0.2 (106.5 ± 3)	**D_LCO/V_A (ml/min/mmHg/l)**	5.1 ± 0.2 (94.5 ± 3)
VC (l)	6.1 ± 0.2 (116.5 ± 2.8)		

Abbreviations: FVC, forced vital capacity; FEV_1, forced expired volume in one second; TLC, total lung capacity; VC, vital capacity; RV, residual volume; FRC, functional residual capacity; D_LCO, diffusion capacity of the lung for carbon monoxide transfer; V_A, alveolar volume; D_LCO/V_A, D_LCO corrected for alveolar volume; SEM, standard error of the mean.

4.2 Influence of MZ and AZ on Acid-Base status, Blood Gases, and Cardiopulmonary Parameters at Baseline.

Baseline acid-base status, arterial blood gases, and cardiopulmonary data are summarized in Table 4.2-1 and individual data are presented in Appendix B B.3, pp 101-140. Treatment with MZ and AZ led to a 17.6 ± 4.3 % and 14.4 ± 6.4 % increase in $\dot{V}_E$ compared to PBO, respectively ($P < 0.05$). This effect on baseline $\dot{V}_E$ led to significant improvements in PaO_2 and reductions in $PaCO_2$ with MZ and AZ compared to PBO. There were no differences observed in resting $\dot{V}O_2$, $\dot{V}CO_2$, and RER between any of the treatments.

Treatment with AZ and MZ led to a hyperchloremic metabolic acidosis in all subjects. Arterial pH was decreased by both MZ and AZ treatment ($P < 0.001$) with pH being reduced the most by AZ treatment ($P < 0.001$). Similarly, arterial HCO_3^- concentration was reduced ($P < 0.001$) by both MZ (-24.7 ± 1.4 %) and AZ treatment (-33.6 ± 0.8 %) compared with PBO, but the effect of AZ treatment was superior ($P < 0.001$). BE was significantly decreased during MZ treatment compared to PBO ($P < 0.001$) and further depressed by AZ compared to MZ ($P < 0.001$). Blood concentration of chloride ions was higher in the MZ and AZ treatment compared

to PBO ($P < 0.001$). Hct was significantly increased by both the MZ (47.3 ± 1.0 %) and AZ (47.8 ± 0.8 %) treatments compared to PBO (45.2 ± 0.6 %; $P < 0.01$). Finally, baseline Hb and SaO_2 were similar across the three treatments.

There were no significant differences between treatments for any baseline cardiovascular measurements including MAP, $\dot{Q}$, SV, HR or PASP.

Table 4.2-1 Baseline acid-base status, arterial blood gases, and cardiopulmonary parameters for all treatment conditions.

	PBO	MZ	AZ
$\dot{V}_E$ (l/min)	13.2 ± 0.6	15.5 ± 0.9*	14.8 ± 0.6*
$\dot{V}O_2$ (l/min)	0.32 ± 0.02	0.32 ± 0.03	0.31 ± 0.01
$\dot{V}CO_2$ (l/min)	0.25 ± 0.01	0.25 ± 0.02	0.25 ± 0.01
RER	0.82 ± 0.06	0.82 ± 0.03	0.82 ± 0.02
PaO_2 (mmHg)	102.3 ± 4.8	103.8 ± 2.5*	104.7 ± 2.4*
$PaCO_2$ (mmHg)	38.1 ± 1.0	33.4 ± 0.8*	32.0 ± 0.7*
SaO_2 (%)	98.0 ± 0.2	98.0 ± 0.2	97.9 ± 0.1
pH	7.45 ± 0.01	7.37 ± 0.01*	7.33 ± 0*†
HCO_3^- (mmol/l)	25.2 ± 0.5	19.0 ± 0.5*	16.8 ± 0.4*†
BE (mEq/l)	0.5 ± 0.4	-5.7 ± 0.5*	-8.3 ± 0.4*†
Cl^- (mEq/l)	107.2 ± 0.5	112.7 ± 0.6*	114.5 ± 0.5*†
MAP (mmHg)	89.4 ± 2.7	89.5 ± 4.4	91.6 ± 2.5
$\dot{Q}$ (l/min)	4.8 ± 0.3	4.8 ± 0.4	4.5 ± 0.3
HR (/min)	56.3 ± 1.9	56.8 ± 3.4	55.1 ± 3.1
PASP (mmHg)	21.3 ± 0.9	21.0 ± 0.7	20.6 ± 0.8

All values are mean ± SEM. Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; $\dot{V}_E$, ventilation; $\dot{V}O_2$, volume of O_2 consumed; $\dot{V}CO_2$, volume of CO_2 produced; RER, respiratory exchange ratio; PaO_2, partial pressure of arterial O_2; $PaCO_2$, partial pressure of arterial CO_2; SaO_2, arterial oxyhemoglobin saturation; HCO_3^-, concentration of bicarbonate ions; BE, base excess; Cl^-, concentration of chloride ions; MAP, mean arterial pressure; $\dot{Q}$, cardiac output; HR, heart rate; PASP, pulmonary artery systolic pressure. *P<0.05 compared to PBO. †P<0.05 compared to MZ.

4.3 Influence of MZ and AZ on Pulmonary Gas Exchange during Poikilocapnic Hypoxia.

A representative breath-by-breath recording of $P_{ET}O_2$ and $P_{ET}CO_2$ from one subject for the duration of the hypoxic protocol is presented in Figure 4.3-1 for all three treatments. In addition, Table 4.3-1 summarizes the relevant pulmonary gas exchange variables, both at baseline and

after 60 minutes of poikilocapnic hypoxia. During hypoxia for all three trials, F_IO_2 was controlled at 0.120 ± 0.001. A main effect of treatment was detected for $\dot{V}_A$ ($P < 0.05$), indicating that it was greater in both MZ and AZ regardless of O_2 level. At baseline both the MZ and AZ treatment elevated P_AO_2 while depressing P_ACO_2 compared to PBO and this trend persisted in hypoxia. The differences in $\dot{V}_A$ and alveolar gases between treatments led to similar changes in arterialized capillary blood gases (i.e. PcO_2, $PcCO_2$ and ScO_2; see Figure 4.3-2). As expected, poikilocapnic hypoxia significantly reduced the P_CO_2 and P_CCO_2. Hypoxia led to a significant decrease in S_CO_2 from baseline with the absolute change dampened by both drugs. Both $P_{ET}O_2$ and $P_{ET}CO_2$, followed the same trend as P_AO_2 and P_ACO_2. At both O_2 levels, $P_{ET}O_2$ was elevated with MZ and AZ treatment compared to PBO. Conversely, an interaction effect was observed with $P_{ET}CO_2$ with a significant depression in the MZ and AZ treatment compared to PBO. No significant differences were observed for O_2 level or drug treatment for V_D/V_T, V_T or f_B.

A significant interaction ($P < 0.01$) effect was identified for blood pH indicating that in the MZ trial pH was lower during hypoxia (7.37 ± 0.01) compared to PBO ($P < 0.01$) and lower still in the AZ (7.33 ± 0.00) trial compared to MZ ($P < 0.01$).

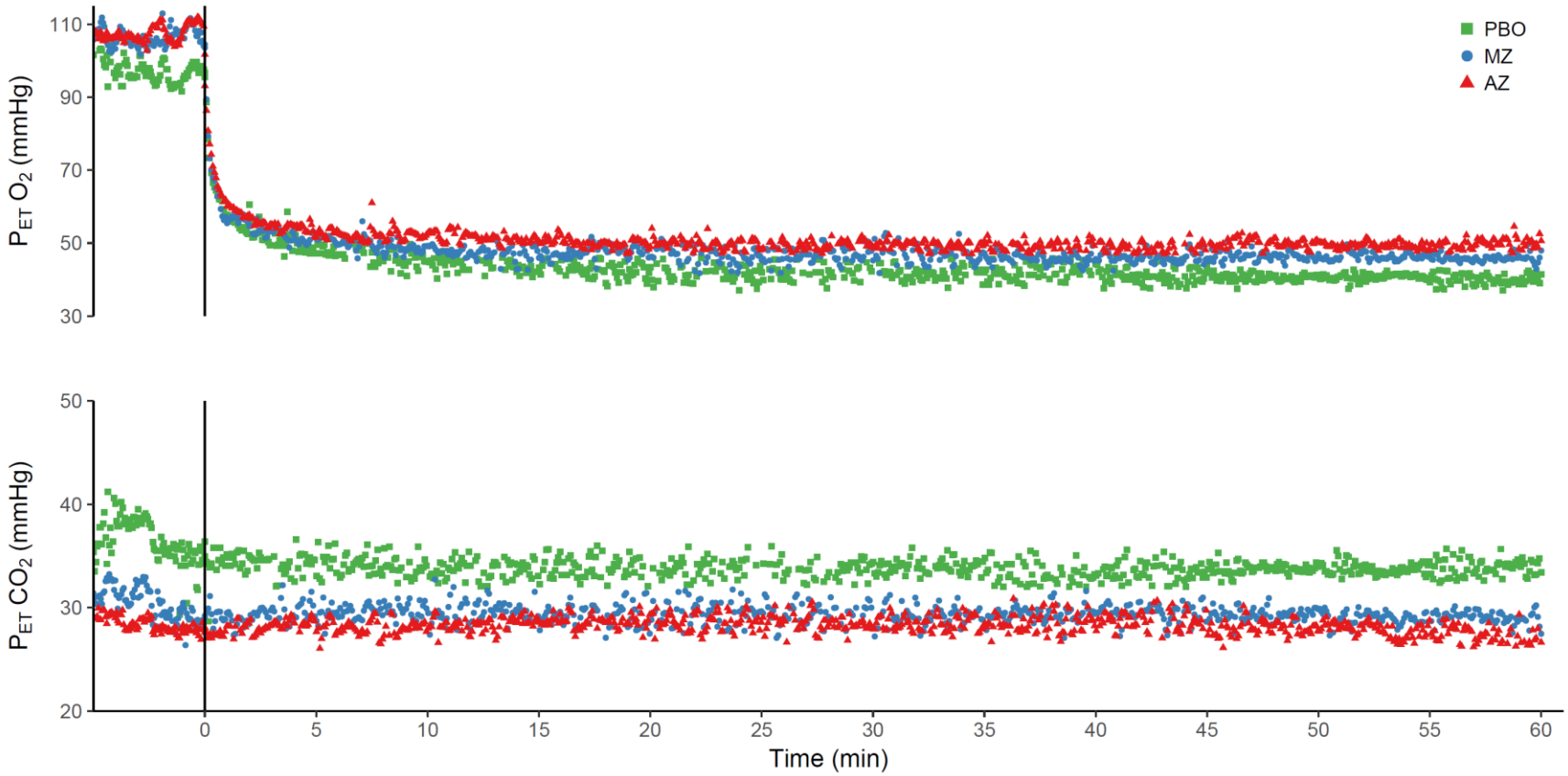

Figure 4.3-1 Representative trace of end-tidal gases during 60 minutes of poikilocapnic hypoxia. Breath-by-breath $P_{ET}O_2$ (upper) and $P_{ET}CO_2$ (lower) data from the three treatments for one participant during a five-minute baseline followed by 60 minutes of poikilocapnic hypoxia exposure, beginning at time = 0.

Table 4.3-1 Measures of pulmonary gas exchange at baseline and during hypoxia for all treatments

	Condition	PBO	MZ	AZ	O_2 Level	Treatment	Interaction
V_A (l/min)	**BL**	5.9 ± 0.2	7.1 ± 0.4*	7.2 ± 0.3*	P = 0.47	**P = 0.03**	P = 0.72
	HX	6.7 ± 0.4	7.5 ± 0.6*	7.6 ± 0.4*			
V_D/V_T (l)	**BL**	0.22 ± 0.02	0.22 ± 0.03	0.20 ± 0.03	P = 0.43	P = 0.23	P = 0.35
	HX	0.22 ± 0.02	0.20 ± 0.01	0.18 ± 0.01			
P_AO_2 (mmHg)	**BL**	95.9 ± 1.4	103.8 ± 0.9*	104.3 ± 1.1*	**P < 0.01**	**P < 0.01**	P = 0.72
	HX	40.2 ± 1.8	46.0 ± 1.6*	46.9 ± 1.5*			
P_ACO_2 (mmHg)	**BL**	37.3 ± 0.9	32.7 ± 0.8*	31.2 ± 0.6*	P = 0.08	**P < 0.01**	P = 0.62
	HX	32.9 ± 0.7	29.2 ± 0.8*	28.8 ± 0.7*			
V_T (l)	**BL**	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0	P = 0.12	P = 0.78	P = 0.93
	HX	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1			
f_B (/min)	**BL**	14.4 ± 1.3	15.7 ± 1.1	15.1 ± 0.8	P = 0.18	P = 0.66	P = 0.80
	HX	14.1 ± 1.1	14.4 ± 1.4	14.2 ± 1.2			
$P_{ET}O_2$ (mmHg)	**BL**	96.3 ± 1.1	104.7 ± 1.5*	105.2 ± 1.4*	**P < 0.01**	**P < 0.01**	P = 0.07
	HX	41.7 ± 1.0	47.0 ± 1.4*	46.9 ± 1.1*			
$P_{ET}CO_2$ (mmHg)	**BL**	38.9 ± 0.8	32.3 ± 0.9*	31.6 ± 0.9*	**P < 0.01**	**P < 0.01**	**P < 0.01**
	HX	29.5 ± 0.7	30.2 ± 0.7*	29.5 ± 0.7*			

All values are mean ± SEM. Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; V_A, alveolar ventilation; V_D/V_T, ratio of physiological dead space to tidal volume, P_AO_2, partial pressure of alveolar O_2; P_ACO_2, partial pressure of alveolar CO_2; V_T, tidal volume; f_B, breathing frequency; $P_{ET}O_2$, partial pressure of end tidal O_2; $P_{ET}CO_2$, partial pressure of end tidal CO_2. *P < 0.05 compared to PBO.

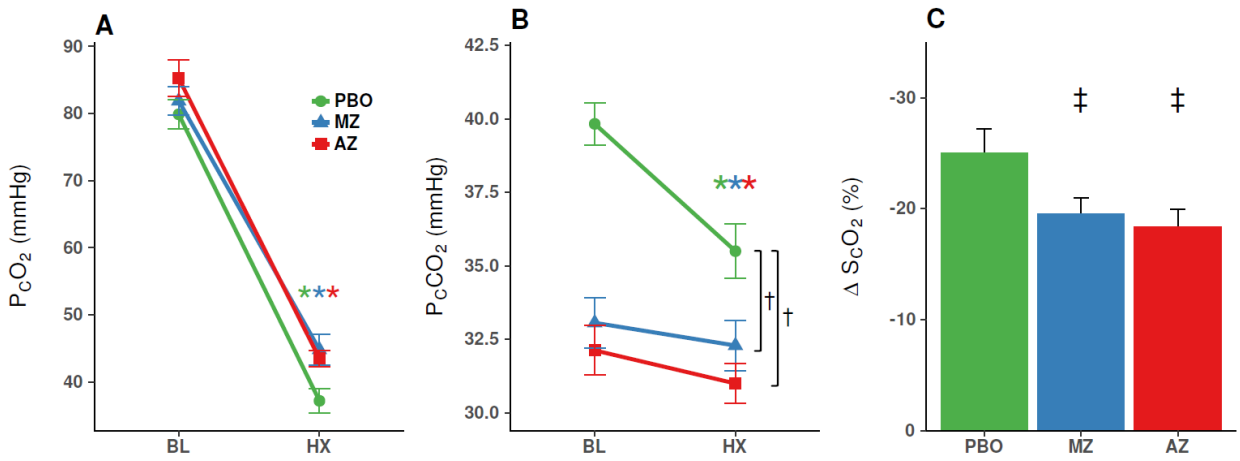

Figure 4.3-2 Changes in PO_2, PCO_2 and SO_2 from baseline to hypoxia measured from arterialized capillary samples.

Absolute P_CO_2 (A) and P_CCO_2 (B) at baseline and hypoxia; and absolute change in S_CO_2 from baseline to hypoxia. *Significant main effects of hypoxia ($P < 0.05$). †Significant main effects of treatment ($P < 0.05$). ‡ Significantly different compared to PBO.

4.3.1 Comparison of Capillary and Arterial Blood Samples

Capillary and arterial blood sample data were well correlated with each other at baseline for the following variables Hb, pH, PCO_2, BE, HCO_3^-, and Hct. At baseline, capillary samples were poor indicators of SaO_2 and PO_2. Figure 4.3-3 illustrate these relationships for select variables. Limits of agreement and mean bias for each variable are presented in Table 4.3-2. Overall, capillary measurements were a close approximation of arterial measures in baseline conditions with the exception of P_CO_2 consistently underestimating PaO_2.

Measurements from an arterialized capillary sample were compared with measurements obtained from a radial artery catheter at baseline and during hypoxia, in two participants treated with AZ. Similar to what was observed at baseline with arterial puncture and capillary comparison, most measurements (Hb, pH, PCO_2, BE, HCO_3^-, Hct, SO_2) were situated near the line of origin suggesting good agreement with the exception of PO_2, where the capillary sample underestimated the arterial sample by 30-34 mmHg at baseline. This underestimation was minimal during hypoxia with P_CO_2 measurements falling within 2-3 mmHg of PaO_2. For this reason, arterialized capillary PCO_2 was considered a reasonable estimate of arterial PCO_2 and

used to quantify alveolar gases during baseline and hypoxia, while arterialized capillary PO_2 was only considered an estimate of arterial PO_2 during hypoxia.

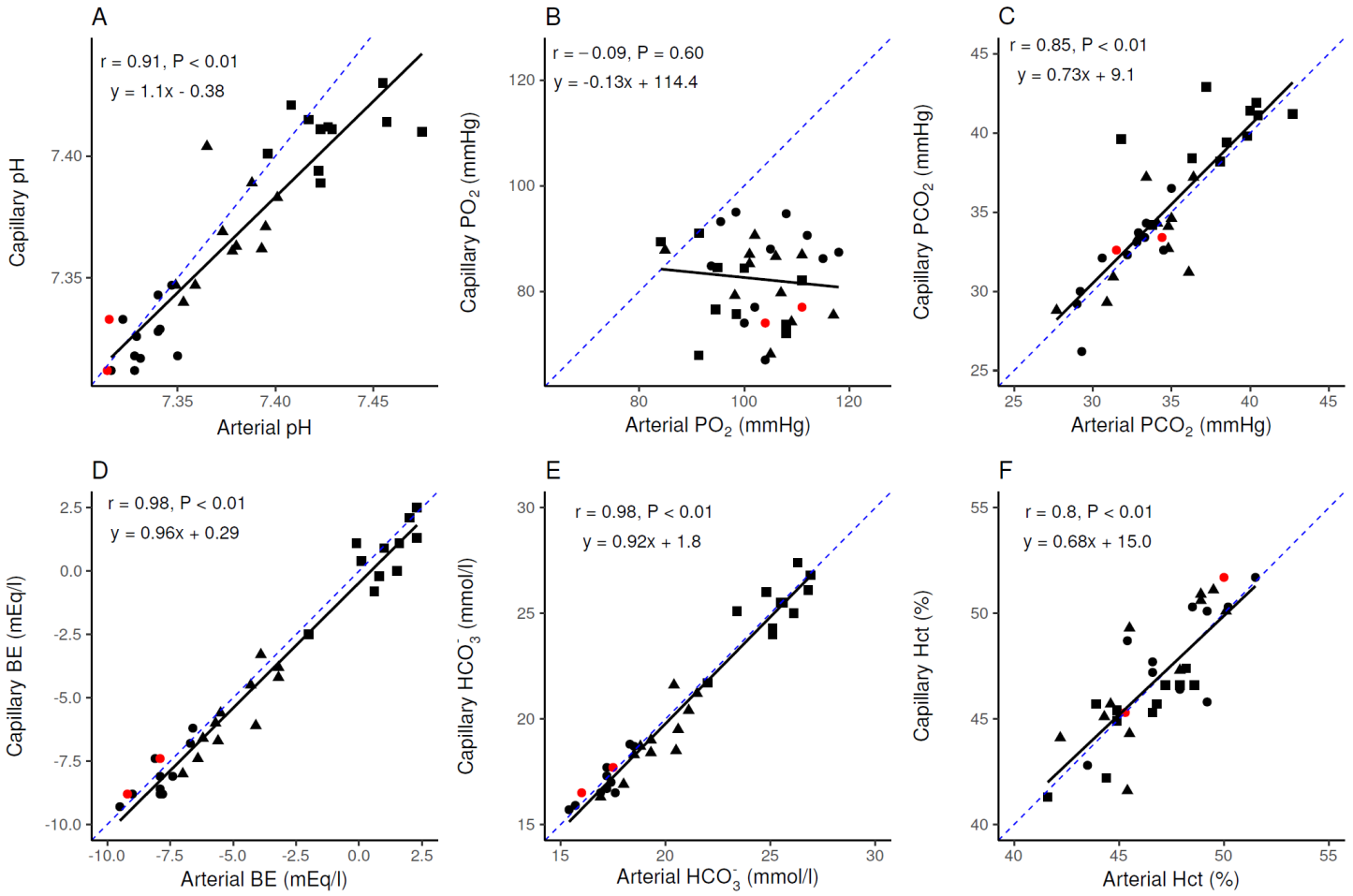

Figure 4.3-3 Correlation between arterial and capillary blood samples at baseline for each treatment condition.

(A) pH, (B) PO_2, (C) PCO_2, (D) BE, (E) HCO_3^-, (F) Hct data from a radial artery puncture (n = 11; black) and from participants with a radial artery catheter (n = 2; red). Capillary and arterial puncture data from all three trials (PBO: square; MZ: triangle; AZ: circle) correlated with the Pearson r. Linear regression (black line) including all trials was performed between capillary and puncture data. Line of identity presented in blue.

Table 4.3-2 Mean bias and limits of agreement for capillary and arterial blood samples at baseline.

Measure	Mean	Lower	Upper	Measure	Mean	Lower	Upper
Hb (g/dl)	-0.03	-1.9	2.0	SO_2 (%)	-2.6	-5.4	0.2
pH	-0.01	-0.06	0.04	BE (mEq/l)	-0.47	-4.6	3.7
PO_2 (mmHg)	-21.3	-45.2	2.6	HCO_3^- (mmol/l)	-0.27	-4.9	4.4
PCO_2 (mmHg)	0.50	-8.4	9.4	Hct (%)	0.08	-6.0	6.2

Abbreviations: PO_2, partial pressure O_2; PCO_2, partial pressure of CO_2; HCO_3^-, concentration of bicarbonate ions; BE, base excess; SO_2, oxyhemoglobin saturation; Hct, hematocrit; Hb, hemoglobin. Mean represents mean bias and upper and lower limits represent the 95% confidence interval of the mean bias.

4.4 Influence of MZ & AZ on Cardiovascular and Pulmonary Vascular Responses to Poikilocapnic Hypoxia

Cardiovascular measurements at baseline and during 60 minutes of hypoxia can be found in Table 4.4-1 and the absolute changes from baseline to hypoxia for PASP, $\dot{Q}$, and TPR_{45} are presented in Figure 4.4-1. A significant interaction for PASP suggests that the HPV response, as measured by the increase in PASP following hypoxic exposure, was significantly blunted in both AZ and MZ compared with PBO (Figure 4.4-1). Hypoxia led to a significant increase in $\dot{Q}$ regardless of treatment (BL: 4.7 ± 0.2 l/min; HX: 5.7 ± 0.2 l/min, $P < 0.01$). TPR increased in response to hypoxia ($P < 0.001$). There was an interaction trend for the TPR response to hypoxia and treatment, though it did not reach statistical significance ($P = 0.07$). However, a significant treatment and O_2 level effect for Hct suggests that measures of TPR should be corrected to a standardized Hct level. Correcting TPR to a standardized Hct of 45% (TPR_{45}) produced similar results and preserved the interaction trend. When comparing the change in TPR_{45} from baseline to hypoxia between treatments, AZ significantly blunted the TPR_{45} response to hypoxia compared to placebo, while MZ only trended towards blunting TPR_{45} though it did not achieve significance (Figure 4.4-1). During hypoxia HR, SV and MAP were significantly elevated, with no effect of treatment. A significant main effect of O_2 level was found for systolic fraction, where it was elevated with hypoxia compared to baseline ($P = 0.01$).

Pulmonary vascular sensitivity to hypoxia was presented as mean change in PASP against mean change in P_AO_2, P_CO_2 and the ideal end capillary oxyhemoglobin saturation calculated from P_AO_2 using the Severinghaus transform and can be found in Figure 4.4-2.

Sensitivity was significantly lower in the AZ and MZ treatments compared to PBO, when presented as PASP/P_AO_2 or P_CO_2 ($P < 0.01$). No significant difference in sensitivity was observed when presented as PASP against the ideal end capillary oxyhemoglobin saturation.

Table 4.4-1 Cardiovascular and pulmonary vascular responses to hypoxia.

	Condition	PBO	MZ	AZ	O_2 Level	Treatment	Interaction
PASP	**BL**	20.9 ± 0.7	20.5 ± 0.6	20.1 ± 0.6	**P < 0.001**	**P < 0.001**	**P < 0.001**
(mmHg)	**HX**	35 ± 1.5	29.5 ± 1*	28.1 ± 1*			
$\dot{Q}$	**BL**	4.8 ± 0.3	4.8 ± 0.4	4.5 ± 0.3	**P < 0.001**	**P = 0.05**	P = 0.50
(l/min)	**HX**	6.1 ± 0.5	5.6 ± 0.5	5.6 ± 0.4			
TPR	**BL**	4.6 ± 0.3	4.7 ± 0.4	4.8 ± 0.4	**P < 0.001**	P = 0.41	P = 0.07
(mmHg/l/min)	**HX**	6.2 ± 0.6	5.6 ± 0.5	5.6 ± 0.4			
TPR_{45}	**BL**	4.5 ± 0.3	4.6 ± 0.4	4.7 ± 0.4	**P < 0.001**	P = 0.11	P = 0.07
(mmHg/l/min)	**HX**	6.1 ± 0.5	5.6 ± 0.4	5.3 ± 0.4			
SV	**BL**	85.6 ± 5.2	84.9 ± 5.6	82.4 ± 3.9	**P = 0.02**	P = 0.75	P = 0.65
(ml)	**HX**	91.1 ± 5.0	88.6 ± 6.2	90.6 ± 3.8			
HR	**BL**	56.3 ± 1.9	56.8 ± 3.4	55.1 ± 3.1	**P < 0.001**	P = 0.41	P =0.20
(bpm)	**HX**	66.8 ± 3.5	63.9 ± 3.3	61.7 ± 3.6			
MAP	**BL**	89.4 ± 2.7	89.5 ± 4.4	91.6 ± 2.5	**P = 0.001**	P = 0.59	P = 0.22
(mmHg)	**HX**	92.8 ± 3.0	99.3 ± 4.1	94.5 ± 3			
Hct	**BL**	45.2 ± 0.6	47.3 ± 1.0*	47.8 ± 0.8*	**P = 0.004**	**P = 0.001**	P = 0.62
(%)	**HX**	46.9 ± 0.7	48.5 ± 0.9*	49.0 ± 0.8*			
Systolic	**BL**	44.1 ± 0.9	43.7 ± 1.3	43.7 ± 1.4	**P = 0.014**	P = 0.60	P = 0.33
Fraction (%)	**HX**	40.2 ± 1.1	39.4 ± 2.3	41.9 ± 1.9			

All values are mean ± SEM. Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; PASP, pulmonary artery systolic pressure; $\dot{Q}$, cardiac output; TPR, total pulmonary resistance; TPR_{45}, total pulmonary resistance corrected to a hematocrit of 45%; SV, stroke volume; HR, heart rate; MAP, mean arterial pressure; Hct, hematocrit. *P < 0.05 compared to PBO.

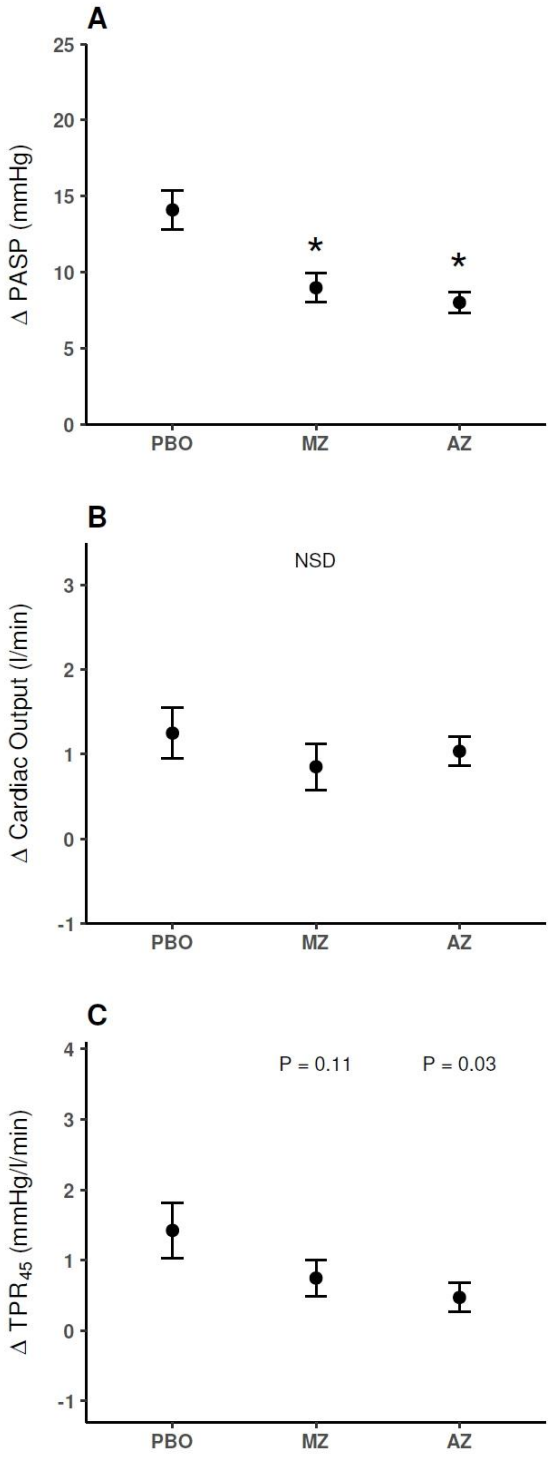

Figure 4.4-1 Absolute mean and individual changes in (A) pulmonary artery systolic pressure (PASP), (B) cardiac output ($\dot{Q}$) and (C) total pulmonary vascular resistance corrected to a hematocrit of 45% (TPR_{45}). Values are mean ± SEM. Color identifies individual subjects. *P < 0.05. All P-values are compared to PBO.

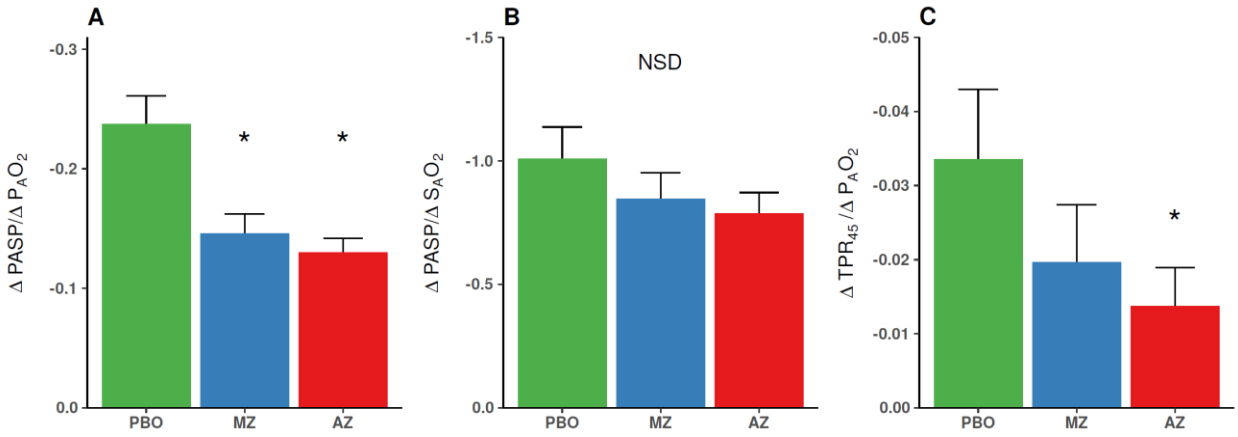

Figure 4.4-2 HPV Reactivity between treatments. Change in pulmonary pressure from baseline to hypoxia, indexed against (A) alveolar P_AO_2 and (B) ideal alveolar end capillary SO_2 calculated from P_AO_2 using the Severinghaus transform function as well as (C) change in TPR_{45} against .

4.5 Determination of the Contributing Factors to HPV using Backwards Elimination of Linear Mixed Effects Model

Stepwise mixed effects modeling was performed to determine how metabolic acidosis, ventilatory drive and changes in hemodynamics contribute to the PASP response to poikilocapnic hypoxia. Subjects were included in the initial full model as a random effect to account for this study's repeated measures design. $\dot{V}_{E,}$ P_ACO_2, H^+, $\dot{Q}$, Hct and the logarithm of P_AO_2 were identified as physiological contributors to the pulmonary pressure response to hypoxia and were selected to be included in the full model. Backwards-stepwise elimination of non-significant effects was performed on the full model to select a model with the most explanatory power, which identified H^+ and log P_AO_2 as significant contributors to the PASP response. Fitting the model determined that H^+ and log P_AO_2 were significant effects with coefficients of -0.26 mmHg/mmol and -28.9 mmHg/log(mmHg). The model passed assumption tests for linearity, homoscedasticity and normality. The effects, coefficients, associated P-values, standardized coefficient and final model is presented in Table 4.5-1.

Table 4.5-1 Coefficients, P-values and standardized beta weights of significant predictors of HPV

Effects	Coefficients	P	β
$\mathbf{H^+}$	-0.26	< 0.01	-0.18
$\mathbf{\log P_AO_2}$	-28.9	< 0.01	-0.76

$$\mathbf{PASP} = 91.8 - 0.26\,\mathbf{H^+} - 28.9 \log \mathbf{P_AO_2};\ R^2 = 0.81$$

Abbreviations: P_AO_2, partial pressure of alveolar O_2; P, coefficient p-value; β, standardized coefficient. Final linear mixed effects model selected through backward elimination is found below coefficients.

Chapter 5. Discussion

5.1 Summary of Main Findings

The purpose of this study was to determine if MZ can improve oxygenation and attenuate HPV to a similar extent as AZ in healthy humans exposed to poikilocapnic hypoxia. This is the first study to demonstrate that MZ increases $\dot{V}_A$ and improves oxygenation to the same extent as AZ during exposure to poikilocapnic hypoxia in healthy humans. Furthermore, this is the first report illustrating a comparable attenuation of HPV in healthy humans administered MZ and AZ. Modeling the pulmonary vascular response to hypoxia suggests that the effects of AZ and MZ are largely attributable to improved P_AO_2; however, both AZ and MZ led to reduced pulmonary vascular reactivity through mechanisms independent of changes in P_AO_2.

5.1 Effects of AZ and MZ on Acid-Base Status and Pulmonary Gas Exchange in Hypoxia

Oral administration of both AZ and MZ induced a metabolic acidosis through renal bicarbonate excretion that increased ventilatory drive and P_AO_2 in normoxia. This ultimately resulted in a higher SaO_2 during hypoxia. This shift in acid-base status improves pulmonary gas exchange through multiple effectors and is thought to alleviate the symptoms associated with AMS.

5.1.1 Normoxic Condition

Renal CA inhibition limits bicarbonate reabsorption in the proximal tubules and H^+ secretion in the distal tubules, resulting in an observable decrease in plasma pH within hours of treatment (158). It is thought that through this metabolically-induced acidosis, CA inhibitors mimic renal acclimatization prior to altitude ascent by increasing bicarbonate excretion and H^+ retention (161). This occurred with both treatments though the acidosis was more severe with AZ compared to MZ. The acidotic state that is induced through CA inhibition activates the central and peripheral chemoreceptors to augment basal ventilation (158), the influence on $\dot{V}_A$ was similar in magnitude for both AZ and MZ in the current study. This acidosis is the

primary and major mechanism through which CA inhibition increases ventilation. Inhibition of CA isoforms on the endothelial cells near chemoreceptors results in local CO_2 retention and ventilatory stimulation in normoxia (168). Despite the observed differences in plasma bicarbonate and H^+ between AZ and MZ, the augmented ventilation was similar between treatments which could be accounted for by the difference in the pharmacokinetic and pharmacodynamic properties of each drug. Both drugs have similar potency on all CA isoforms found in the kidneys besides CA IV where AZ is 83 times more potent, possibly accounting for the augmented acidosis observed. Conversely, the elevated lipophilicity of MZ allows it to cross the blood-brain barrier more readily than AZ, a suggested mechanism for the increased Nrf-2 activation observed in the brain with MZ (87). The increased penetrance of MZ could amount to a greater central chemoreceptor activation compared to AZ, allowing for the MZ treatment to achieve similar ventilatory responses with reduced acidosis.

5.1.2 Hypoxic Condition

A greater ventilatory drive, improved PaO_2 and a resetting of the operating point on the metabolic hyperbola are the primary reasons for the success of AZ as a treatment for altitude illness (165). Our results confirmed this effect of AZ and showed that MZ led to similar increases in $\dot{V}_A$, P_AO_2, and PaO_2 in both normoxia and hypoxia. This effect has been observed in a number of altitude studies using different dosages of AZ and other potent CA inhibitors which is further discussed in section 5.2.3, pg. 57. Given the synergistic effects of an O_2-CO_2 interaction at the peripheral chemoreceptor, the acidotic effect to AZ might be expected to augment HVR though this is not the case in humans due to its concomitant depression of PCO_2 that inhibits the central chemoreceptors (11, 165). Moreover, in cats when administered intravenously, AZ has been shown to reduce HVR through direct carotid inhibition, though this effect does not extend to the more lipophilic sulfonamide MZ, which would be expected to penetrate the carotid body more efficiently, suggesting AZ may act on the carotid bodies through a pathway independent of CA inhibition (177). This alternative mechanism could also account for the comparable ventilation and SO_2 between AZ and MZ observed in this study, despite the differences in acid-base status, though previous work suggests that direct inhibition of chemoreceptors does not play a significant role in ventilation when drugs are administered orally (165). Ultimately, it is the shift in the setpoint

of the metabolic hyperbole caused by CA inhibition that is advantageous at altitude; by shifting to a steeper position on the metabolic hyperbole, AZ is able to mitigate the inhibitory effects of hypocapnia caused by hypoxic hyperventilation (170).

5.2 Effects of AZ and MZ on Hypoxic Pulmonary Vasoconstriction

The hypoxic stimulus in this study led to expected increases in PA pressure in all three trials and treatment with MZ showed a significant reduction of the PA pressor response, similar to AZ. The equivalent P_AO_2 between the two drug treatments suggest that the HPV stimulus is approximately equal with both AZ and MZ. The study was designed to assess the HPV response in simulated environmental conditions (i.e. poikilocapnic hypoxia), for this reason there is a treatment induced effect on the hypoxic stimulus in that it is less potent in the drug trials compared to placebo. This important therapeutic feature proves to be a confounder in determining the specific effects of sulfonamides with CA inhibitory activity on the HPV response. Through the use of an end-tidal forcing system Teppema et al demonstrated that, when $P_{ET}O_2$ was held constant during a bout of poikilocapnic hypoxia, AZ is still able to significantly attenuate HPV compared to placebo likely through a mechanism unrelated to improvements in SO_2 alone (176). Mixed effects modeling indicates that though P_AO_2 is the primary determinant of HPV in this study, there is unaccounted error in the model, allowing room for other unknown mechanisms. This hypothesis is supported by a study in spontaneous breathing dogs whereby an engineered sulfonamide with no CA activity, known as NMA, was able to attenuate HPV to the same degree as MZ and AZ when administered intravenously (123). Further support comes from studies of PASMCs treated with either NMA or AZ that showed a similar inhibition of intracellular Ca^{2+} during hypoxic exposure with significant differences in intracellular pH (148). These data infer the existence of an alternative sulfonamide receptor within the pulmonary artery smooth muscle. Benzolamide, a sulfonamide analog to AZ with increased CA potency but less lipophilicity, is not able to attenuate hypoxic increases in intracellular Ca^{2+} though they do achieve a similar decrease in pH as seen with AZ. This could indicate that either AZ has a unique feature, not shared by other CA inhibiting sulfonamides, that allows it to bind to a receptor involved in the control of hypoxic Ca^{2+} release or the other drugs tested did not penetrate the PASMC sufficiently to

have the same effect. It is unknown whether MZ has a similar effect on Ca^{2+} in the PASMCs.

The effects of pH on the HPV response are somewhat contradictory. As previously mentioned, acidosis is a powerful ventilatory driver that ultimately reduces the HPV stimulus in hypoxic conditions. However, elevated levels of plasma H^+ concentration have been shown to have potentiating effects on HPV(89). The net physiological effect of acidosis is not well understood. Tremblay et al have demonstrated that eliminating the metabolic acidosis caused by AZ treatment, through an intravenous bicarbonate infusion, results in a partial restoration of the HPV response in healthy humans (180). Their data indicate that AZ attenuates HPV, at least in part, through the acidotic effect, though it is unlikely through an elevated ventilatory drive alone considering ventilation and SpO_2 was equal between trials (180). Data from the present study also suggests that the acidotic effect is not likely only due to an increased ventilation given that ventilation and HPV were equal between the AZ and MZ trials despite the differences in pH. The lack of an observable consensus regarding the integrative role of H^+ on the pulmonary vascular response could be due to the many complex interactions between AZ and HPV, including sulfonamide activity that is independent of CA inhibition such as what has been observed in both the PCR and the PA (123, 148, 177).

The diuretic action of the treatments was the likely cause of the elevated Hct in this study (133). Hemodilution in animals with polycythemia, has been shown to drastically reduce the slope of the pressure-flow relationship in the pulmonary vasculature, indicating that elevated Hct is correlated to elevated PA pressures (35). The exact mechanism behind this relationship is not well understood but it could be due to a scavenging of vasodilators by the erythrocytes such as cyclooxygenase byproducts (108) or NO (30). The differences in Hct between treatments and placebo could confound the response though, were this the case, it would suggest that the observed attenuation of HPV by AZ and MZ is somewhat underestimated. As Hct can impact pulmonary resistance simply due to the elevated viscosity, the TPR value that is presented in Figure 4.4-1 is corrected to a Hct of 45% and still found a similar trend to what was observed with PASP. Further discussion of whether changes in TPR or changes in PASP more accurately represent the HPV response can be found in section 5.2.3 pg. 57.

There is an inverse relationship between hypoxic PA pressure and pulmonary gas exchange efficiency (89). As a result, the reduction in HPV observed with both AZ and MZ (Figure 4.4-1) could potentially improve pulmonary gas exchange efficiency. The HPV mechanism plays an important role in driving ideal $\dot{V}_A/\dot{Q}$ matching and by dampening this response in normoxia, CA inhibitors have been shown to reduce gas exchange efficiency in dogs (168). This worsening of gas exchange may be less relevant when the lung is exposed to a global hypoxic stimulus; as demonstrated by the results of this study, CA inhibition has a positive net effect on pulmonary gas exchange resulting in higher oxyhemoglobin saturations that occurred in equal magnitude with both AZ and MZ treatments (Figure 4.3-2). With multiple effector pathways identified alongside the complex integrative role of CA inhibition within the pulmonary vasculature and the many processes of gas exchange, it is apparent that future research is needed to further characterize the mechanisms of HPV and how they interact with sulfonamides.

5.2.1 Changes in Pulmonary Vascular Sensitivity to Hypoxia

Graded changes in altitude alone have no effect on the pulmonary vasculature's sensitivity to hypoxia, though this study suggests there could potentially be a decrease in sensitivity with both AZ and MZ treatments (Figure 4.4-2). The known stimulus for HPV is an additive effect of P_AO_2, mixed venous PO_2 and bronchial PO_2, which is the equivalent of PaO_2 (102, 103). Changes in hypoxia affect all three of these parameters similarly but to different magnitudes. Given the integrative role of CA in gas exchange, it could be suggested that CA inhibition in the pulmonary circulation results in a local accumulation in CO_2 and results in a Bohr shift that impairs the flux of O_2 across the alveoli to the capillaries (71, 179). Evidence of AZ induced impairments in intracellular Ca^{2+} suggest that sulfonamides may reduce hypoxic sensitivity from within the PASMCs by impairing ion channels or through other unknown pathways (148). The change in cellular redox state due to decreased pH could impact a signal sent through gap junctions, a form of signal transduction that has previously been implicated in the HPV response (186). If this alteration of the signal is enough to attenuate or disrupt HPV, it could play a role in the depressed sensitivity to hypoxia with AZ and MZ.

The differences in pulmonary sensitivity to hypoxia disappears when PA pressures are indexed against the ideal end capillary saturation calculated from P_AO_2 using the

Severinghaus transform. This loss of sensitivity is likely due to a rightward shift in the oxyhemoglobin dissociation curve caused by the systemic acidosis associated with renal CA inhibition (179). A rightward shift moves the steep region of the curve closer to the hypoxic stimulus applied in this study and therefore given changes in PO_2 with CA inhibition manifest as larger changes in SO_2.

5.2.2 Mixed Effects Model of the Physiological Contributors to PA Pressure during Treatment with CA Inhibitors in Normoxia and Hypoxia

We generated a mixed effects model to identify the important physiological contributors to the pulmonary response to hypoxia and its attenuation by AZ and MZ. Contributors to the full mixed effects model ($\dot{V}_E$, P_ACO_2, H^+, $\dot{Q}$, Hct and P_AO_2) were selected based on their identification as primary or secondary physiological contributors to the HPV response (27). Backwards stepwise elimination of the full mixed effects model identified both H^+ and P_AO_2 as the most important contributors to the observed differences in PA pressures. Standardizing the regressing coefficients to the same scale reveals that changes in P_AO_2 has a four-fold larger impact than changes in H^+ on deviations in PA pressures (Table 4.5-1). The negative coefficient associated with H^+ infers an inverse relationship with PASP and though this contradicts reports that the relationship is positive (135), it is important to note that this term is reflective of the changes induced by AZ and MZ treatment. In order to demonstrate reproducibility and validate our experimental model against the work of others, we applied our algorithm to a data set obtained using nearly identical experimental conditions to the current study (176). The data that was used in the prediction and the results can be found in Table 5.2-1.

Table 5.2-1 Predicted PASP values using mixed effects model and pulblished data

PASP = 91.8 – 0.26 $\mathbf{H^+}$ – 28.9 log $\mathbf{P_AO_2}$				
	AZ		**PBO**	
	BL	**HX**	**BL**	**HX**
$P_{ET}O_2$ (mmHg)	94.1	50.0	85.6	50.0
H^+ (nmol)	45.8	45.8	37.2	37.2
PASP Measured (mmHg)	20.9	27.2	23.3	34.8
PASP Predicted (mmHg)	23.6 ± 0.6	30.7 ± 0.9	26.1 ± 0.8	33.0 ± 0.8
95% CI PASP Predicted	22.4 – 24.8	28.9 – 32.5	24.5 – 27.7	31.4 – 34.6

Abbreviations: $P_{ET}O_2$, partial pressure of end-tidal O_2; H^+, hydrogen ions; PASP, pulmonary artery systolic pressure; CI, confidence interval. Measured PASP is a sum of mean ΔPmax values from previous study (176) and an estimation of normal right atrial pressure in healthy individuals (3 mmHg). Red font indicates predicted values. Predicted values are mean ± SEM.

The algorithm was not able to reasonably predict PASP values where all of the measured values fell outside the 95% confidence interval of the prediction. There are a number of sources of error that could contribute to the discrepancies between predicted and measured, for example there was no P_AO_2 data available so in normoxia $P_{ET}O_2$ was used as a surrogate. The data set used in the prediction algorithm was collected at an altitude that was approximately 700 m above where the experimental data was collected. For this reason, participants living at the slightly higher altitude may be acclimatized to have a similar resting PA pressures at lowered $P_{ET}O_2$ values, leading to a slight overestimation in the predicted value. Finally, only ΔPmax was available from the data set used, so an estimate of right atrial pressure, typical of healthy individuals (3 mmHg), was added to the measured value to obtain PASP. This could account for some discrepancy in the predicted value if the subject pool contained individuals with elevated right atrial pressure.

5.2.3 PASP and TPR as an Index of Pulmonary Vascular Tone

Hypoxic induced increases in pulmonary vascular tone is thought to be the mechanical component of the HPV response that leads to both increased PA pressure and resistance. Pressure-flow relationships, obtained though graded increases in perfusion rate, offer the most accurate representation of vascular tone (86) though this requires continuous measures

of pressure and flow while making graded changes to flow through mechanical or pharmacological means. This study found significant attenuation in HPV with both AZ and MZ but found that only AZ reduced TPR significantly while only a downward trend was observed with MZ. This somewhat conflicting data suggests that the indices are not in perfect agreement and it brings about the question as to which is more appropriate. The intrinsic function of vascular tone is to impede flow, and therefore it stands to reason that TPR would act as an appropriate indicator of the HPV response. TPR is predicated on Ohm's law and the assumption that the relationships between flow, pressure and resistance are linear (107). This is only true when dealing with rigid vessels, the highly compliant and under perfused pulmonary vasculature exhibits a curvilinear pressure-flow relationship, that increases in slope with hypoxic exposure (83, 107). Small changes in $\dot{Q}$ can impact TPR, when in reality they may have been accommodated for by vessel compliance without any change in the underlying tone (86, 107). Moreover, TPR does not account for any potential changes in LAP, an important determinant of pulmonary vascular resistance (86). Recent studies have inferred that PASP is linearly related to pulmonary vascular tone and is likely the better estimate of the HPV response (6).

5.3 Limitations

Dosages for this study (MZ: 100 mg, B.I.D; AZ: 250 mg, T.I.D) were selected based upon previous work regarding the effectiveness of AZ at blunting HPV (158, 166, 176) as well as commonly used dosages for glaucoma treatment (28, 100) . The differences in plasma H^+ and bicarbonate concentration could suggest that the AZ dosage was higher than necessary. It could also be suggested that a lower dosage of AZ would achieve a comparable acid/base status and therefore the effects of MZ observed in this study may underestimate the actual response compared to AZ. When the treatment responses are compared in terms of pulmonary gas exchange, the data suggests that since the MZ dosage achieved equal P_AO_2, P_ACO_2 and SO_2 values that there is no significant difference in regards to arterial oxygenation improvement when compared to AZ. This study aimed to compare the integrative ventilatory and cardiovascular effects of AZ and MZ during hypoxic exposure that simulates environmental hypobaria. For this reason, the specific effects of either treatment on HPV are confounded by the concomitant increases in $\dot{V}_A$, P_AO_2 and pH.

Due to constraints of the study design, blood samples from arterial puncture were obtained at a time point approximately 90 mins before baseline respiratory and metabolic variables were collected. For this reason, it was not possible to reasonably quantify the alveolar-to-arterial difference of oxygen and further probe mechanisms of gas exchange efficiencies between trials.

Selection of the most appropriate index of HPV has a small effect on the interpretation of these results; MZ loses some of its potency in attenuating HPV when compared to AZ using TPR. Considering that this effect is due to slight non-significant variations in $\dot{Q}$ between trials leading to the observed difference in TPR, it could be suggested that PASP is perhaps a better representation in this study. However, since the increases in $\dot{Q}$ and systolic fraction (a surrogate of LAP) due to hypoxia were not different between trials, the trend in TPR attenuation by MZ could become more pronounced with a slightly larger sample size or larger dose of MZ.

5.4 Conclusion

The findings of this study are the first to show a similar augmentation of ventilatory drive with AZ and MZ that results in an elevation of P_AO_2 and ultimately improves oxygenation during hypoxia. Furthermore, this is the first time that MZ has been shown to have a similar efficacy in attenuating HPV in healthy humans. Collectively, the effects of MZ on ventilation, oxygenation and pulmonary vascular response to hypoxia amount to an improvement in gas exchange that likely increases an individual's tolerance to environmental hypoxia.

Chapter 6. Extended Discussion

6.1 Changes in capillary ion and erythrocyte concentration

Part of the renal inhibition that occurs though the application of sulfonamides includes an inhibition of chloride reabsorption in the distal tubule (133). The resultant increase in renal chloride excretion led to the passive excretion of water which accounts for the diuretic properties of sulfonamides. Data from this study confirms the chloride excretory effects in both AZ and MZ. The increased hematocrit observed at baseline with both treatments is also likely an indirect effect of the natriuresis (158).

6.2 Expanded Limitations

A measure of iron status could have helped tackle some of the broader mechanism questions. Individuals suffering from polycythemia due to CMS have shown a reduction in erythrocytes through renal inhibition with AZ (128). Furthermore, treatment with AZ resulted in elevated iron levels which has been identified as a modulator of HPV. It could be suggested that one of the alternative mechanisms by which AZ attenuates HPV is through the HIF pathway, though as the authors of the study have suggested, the elevated iron levels could be simply due to the abrupt halt of erythropoiesis by AZ which would not occur in individuals who were not suffering from erythropoiesis.

6.3 Future Directions

There is still much work needed to characterize and understand the effects of CA inhibiting sulfonamides on pulmonary gas exchange and PA responses to hypoxia; the field could benefit from further investigations into the separate effect of acidosis from those of CA inhibition and the effects of MZ on ventilatory responses and fatigue.

The complex and integrative effects of CA inhibition are surely the reason for lack of evidence for the specific sulfonamide mechanisms that alter ventilatory and PA responses to hypoxia, particularly due to the significant acidosis associated with treatment. Inducing an acidosis independent of CA inhibition followed by hypoxic exposure could elucidate the magnitude of the contribution of plasma pH. Furthermore, large doses of sulfonamides

administered intravenously could add more insight by fully and systemically inhibiting CA rapidly, without any major fluctuations in pH. Finally, understanding the unique effects possessed by individual sulfonamides could be obtained by developing a dose-response curve for sulfonamides to bicarbonate excretion and plasma pH. The differences between sulfonamides can be further investigated by using doses that match acid/base status between treatments. Finally, pending approval for use in humans, administration of NMA could help identify the unique effects of a sulfonamide that lacks CA inhibitory action; this data could not only help determine the contributions of acidosis to the development of HPV but also the effects of other CA enzymes that may play a role.

Previous work has identified a different effect between intravenously administered MZ and AZ on the peripheral chemoreceptors of sedated cats (177). Predicated on the increased lipophilicity of MZ, the authors suggested that since AZ blunts the HVR and MZ does not, it must be due to a mechanism independent of CA inhibition as there should have been sufficient MZ diffusion into the carotid body to cause complete inhibition. It would be interesting to determine if this effect is observed in humans as well, either via intravenous or oral administration of both drugs. Furthermore, the study in cats observed significant differences in pH so a careful matching of doses in humans or abolishment of pH disturbances through bicarbonate infusion could offer insight into the acidotic effects and whether or not the attenuation of HVR by AZ is underrepresented due to the systemic acidosis.

Finally, more work regarding the negative side effects of CA inhibition could help determine not only which treatment is most effective but also which is more tolerable among most individuals. With numerous reports of muscular fatigue and diminished exercise performance with different sulfonamides (24, 46, 48, 49, 76, 191), it could be useful to test the specific effects of these drugs on muscle function and exercise performance in humans in a controlled laboratory setting.

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Appendices

Appendix A Forms

A.1 University of British Columbia Ethics Certificate of Full Board Approval

The University of British Columbia
Office of Research Ethics
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

ETHICS CERTIFICATE OF FULL BOARD APPROVAL

PRINCIPAL INVESTIGATOR:	INSTITUTION / DEPARTMENT:	UBC CREB NUMBER:
Glen E. Foster	UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences	H16-00028

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Okanagan

Other locations where the research will be conducted:
N/A

CO-INVESTIGATOR(S):
Giulio Dominelli
Philip Ainslie
Jonathan Little
Chris McNeil
Paolo B. Dominelli

SPONSORING AGENCIES:
- British Columbia Lung Association - "The effect of carbonic anhydrase inhibitors on the pulmonary system response to hypoxia and muscle fatigue "
- Natural Sciences and Engineering Research Council of Canada (NSERC) - "Intermittent Hypoxia and Cardiopulmonary Adaptation "
- UBCO Faculty of Health and Social Development - "Start-Up Funds"

PROJECT TITLE:
The effect of carbonic anhydrase inhibitors on the pulmonary system response to hypoxia

THE CURRENT UBC CREB APPROVAL FOR THIS STUDY EXPIRES: March 8, 2017

The full UBC Clinical Research Ethics Board has reviewed the above described research project, including associated documentation noted below, and finds the research project acceptable on ethical grounds for research involving human subjects and hereby grants approval.

This approval applies to research ethics issues only. The approval does not obligate an institution or any of its departments to proceed with activation of the study. The Principal Investigator for the study is responsible for identifying and ensuring that resource impacts from this study on any institution are properly negotiated, and that other institutional policies are followed. The REB assumes that investigators and the coordinating office of all trials continuously review new information for findings that indicate a change should be made to the protocol, consent documents or conduct of the trial and that such changes will be brought to the attention of the REB in a timely manner.

REB FULL BOARD MEETING REVIEW DATE: March 8, 2016	

DOCUMENTS INCLUDED IN THIS APPROVAL:	DATE DOCUMENTS APPROVED: April 18, 2016

Document Name	Version	Date
Protocol:		
Proposal	2	April 12, 2016
Consent Forms:		
Hypoxia Consent	2	April 12, 2016
Investigator Brochures:		
Methazolamide Monograph	1	July 1, 2010
Xylocaine monograph	1	March 3, 2009
Acetazolamide Monograph	1	June 24, 2010
Advertisements:		
Hypoxia Ad	3	April 18, 2016
Questionnaire, Questionnaire Cover Letter, Tests:		
Hypoxia Questionnaire	2	April 12, 2016
ParQ	1	January 1, 2002
Hypoxia Pre Screen	2	April 12, 2016
Other Documents:		
External Review	1	January 23, 2016
Response to Provisos	1	April 12, 2016

CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.

2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.

3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

Approval of the Clinical Research Ethics Board by:

Dr. Stephen Hoption Cann, Co-Chair

A.2 U.S. National Institutes of Health Registration (NCT02760121)

ClinicalTrials.gov

IMPORTANT: Listing of a study on this site does not reflect endorsement by the National Institutes of Health. Talk with a trusted healthcare professional before volunteering for a study. Read more...

Trial record **3 of 199** for: Carbonic Anhydrase Inhibitors

Previous Study | Return to List | Next Study

AZ, MZ, and the Pulmonary System Response to Hypoxia

This study has been completed.

Sponsor:
University of British Columbia

Information provided by (Responsible Party):
University of British Columbia

ClinicalTrials.gov Identifier:
NCT02760121

First received: April 20, 2016
Last updated: October 18, 2016
Last verified: October 2016
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

The purpose of this proposal is to compare the physiological effects of acetazolamide (AZ) and methazolamide (MZ) on the control of breathing and hypoxic pulmonary vasoconstriction. The first objective is to assess the effects of AZ and MZ on the control of breathing in normoxia and hypoxia. To achieve this the ventilatory interaction between oxygen and carbon dioxide will be measured and effects compared between placebo, AZ, and MZ conditions. In addition, the isocapnic and poikilocapnic hypoxic ventilatory response and hypercapnic ventilatory response will be measured with each drug. The second objective is to assess the effects of AZ and MZ on the control of the pulmonary vasculature during hypoxia. Pulmonary pressure and cardiac output will be measured during 60 minutes of poikilocapnic hypoxia.

Condition	Intervention	Phase
Altitude Sickness Hypertension, Pulmonary	Drug: Acetazolamide Drug: Methazolamide Drug: Placebo	Phase 4

Study Type: Interventional
Study Design: Allocation: Randomized
Intervention Model: Crossover Assignment
Masking: Double Blind (Participant, Investigator)
Primary Purpose: Basic Science

Official Title: The Effect of **Carbonic Anhydrase Inhibitors** on the Pulmonary System Response to Hypoxia

Resource links provided by NLM:

Drug Information available for: Acetazolamide Methazolamide Acetazolamide sodium

Genetic and Rare Diseases Information Center resources: Acute Mountain Sickness

U.S. FDA Resources

Further study details as provided by University of British Columbia:

Primary Outcome Measures:

- Change in ventilation [Time Frame: Baseline and 60 minutes of poikilocapnic hypoxia]

To quantify the isocapnic hypoxic ventilatory response, the hypercapnic ventilatory response, and the hypercapnic hypoxic ventilatory response, ventilation will be measured throughout controlled changes in end-tidal gas levels. Each protocol will consist of 90s steps in end-tidal oxygen partial pressure from baseline through 65, 57, and 47 mmHg. For hypercapnic hypoxia, the end-tidal partial pressure for carbon dioxide will be increased from baseline to +6 mmHg for 7 minutes before reducing the end-tidal partial pressure of oxygen as above. The poikilocapnic hypoxic ventilatory response will be determined by measuring the change in ventilation from baseline throughout 60 minutes of poikilocapnic hypoxia (fraction of inspired oxygen = 0.12)

- Change in pulmonary artery pressure [Time Frame: Baseline and 60 minutes of poikilocapnic hypoxia]

  Pulmonary artery systolic pressure (PASP) will be derived using the modified Bernoulli equation and the regurgitant velocity across the tricuspid valve. Estimates of right atrial pressure will be evaluated based upon the collapsibility index of the inferior vena cave during a sniff test. The pulmonary artery pressure response will be measured during 60 minutes of exposure to poikilocapnic hypoxia (fraction of inspired oxygen = 0.12)

Secondary Outcome Measures:

- Change in cerebral blood velocity [Time Frame: Baseline and 60 minutes]

  To quantify the isocapnic hypoxic cerebral blood velocity response, the hypercapnic cerebral blood velocity response, and the hypercapnic hypoxic cerebral blood velocity response, cerebral blood velocity in the middle and posterior cerebral arteries will be measured throughout controlled changes in end-tidal gas levels. Each protocol will consist of 90s steps in end-tidal oxygen partial pressure from baseline through 65, 57, and 47 mmHg. For hypercapnic hypoxia, the end-tidal carbon dioxide partial pressure will be increased from baseline to +6 mmHg for 7 minutes before reducing the end-tidal oxygen partial pressure as above. The poikilocapnic hypoxic ventilatory response will be determined by measuring the change in ventilation from baseline throughout 60 minutes of poikilocapnic hypoxia (fraction of inspired oxygen = 0.12)

Other Outcome Measures:

- change in arterial oxygen partial pressure [Time Frame: Baseline and 60 minutes]
- Change in arterial carbon dioxide partial pressure [Time Frame: Baseline and 60 minutes]
- Change in arterial pH [Time Frame: Baseline and 60 minutes]
- Change in heart rate [Time Frame: Baseline and 60 minutes]
- change in blood pressure [Time Frame: Baseline and 60 minutes]
- change in end-tidal oxygen and carbon dioxide partial pressure [Time Frame: Baseline and 60 minutes]
- Change in arterial oxygen saturation [Time Frame: Baseline and 60 minutes]
- Change in cardiac output [Time Frame: Baseline and 60 minutes of poikilocapnic hypoxia]

  Cardiac output will be determined using the aortic time integral velocity and the diameter of the aortic valve annulus. Data will be collected at baseline and throughout exposure to poikilocapnic hypoxia (fraction of inspired oxygen = 0.12)

- Change in pulmonary venous blood velocity [Time Frame: Baseline and 60 minutes of poikilocapnic hypoxia]

  Doppler ultrasound will be used to measure the velocity of blood draining from the pulmonary vein at baseline and throughout exposure to poikilocapnic hypoxia (fraction of inspired oxygen = 0.12)

- Hemoglobin [Time Frame: Baseline]
- albumin [Time Frame: Baseline]
- iron [Time Frame: Baseline]

Enrollment: 14
Study Start Date: May 2016
Study Completion Date: August 2016
Primary Completion Date: August 2016 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Experimental: Acetazolamide Participants will be dosed 250mg Acetazolamide (p.o.) three times per day for two days prior to and a single dose on the day of study.	Drug: Acetazolamide
Experimental: Methazolamide Participants will be dosed 100mg Methazolamide (p.o.) twice daily separated by a placebo for two days prior to and a	Drug: Methazolamide

Additional relevant MeSH terms:

Carbonic Anhydrase Inhibitors	Acetazolamide
Enzyme **Inhibitors**	Methazolamide
Hypertension, Pulmonary	Anticonvulsants
Altitude Sickness	Molecular Mechanisms of Pharmacological Action
Lung Diseases	Diuretics
Respiratory Tract Diseases	Natriuretic Agents
Respiration Disorders	Physiological Effects of Drugs

ClinicalTrials.gov processed this record on June 08, 2017

A.3 Participant Consent Form

T H E U N I V E R S I T Y O F B R I T I S H C O L U M B I A

Subject Information and Consent Form

The effect of carbonic anhydrase inhibitors on the pulmonary system response to hypoxia

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1. INVITATION

You are being invited to take part in this research study because you are a healthy male between the ages of 19-40 with no history of cardiopulmonary ailments, wrist injury/surgery, or contraindications to drugs that alter the way you process carbon dioxide, referred to as carbonic anhydrase inhibitors

2. YOUR PARTICIPATION IS VOLUNTARY

Your participation in this study is completely voluntary. You have the right to refuse participation in this study. Should you choose to participate, you may choose to withdraw from the study at any time without penalty.

Before you decide, it is important for you to understand what the 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.

3. WHO IS CONDUCTING THE STUDY?

This study is being conducted by Dr. Glen Foster, Paolo Dominelli and other investigators at the Cardiopulmonary Laboratory for Experimental and Applied Physiology at the University of British Columbia Okanagan campus. The study is funded by the Natural Science and Engineering Council of Canada

4. BACKGROUND

The human body requires enough oxygen to survive. Hypoxia is a condition where oxygen levels are lower than normal. This is a common symptom of many diseases involving the respiratory (breathing) system. Hypoxia can lead to high blood pressure in the vessels that move blood to the lungs. This happens because hypoxia causes squeezing of blood vessels inside the lungs and decreased movement of oxygen and carbon dioxide between the air and the blood. Research on hypoxia caused by being at high altitude shows that a drug called 'acetazolamide (AZ)' can lower blood pressure and vessel squeezing in the lungs. Unfortunately, this drug also has side effects such as increased fatigue (tiredness) of muscles. In research done on animals, a drug called 'methazolamide (MZ)' lowered blood pressure in the lungs without fatiguing the muscles. Methazolamide is used clinically to treat patients who have high pressure in their eyes and is used to help people get used to travelling to high

altitude and prevent altitude illness. It is unknown if methazolamide will also work better than acetazolamide in humans.

5. WHAT IS THE PURPOSE OF THE STUDY?

The purpose of this study is to determine the effect of two similar medications on (i) the control of breathing (how much and how fast we breathe) in response to hypoxia and (ii) If both medications are able to reduce the pressure in blood vessels in and around the lungs when you are exposed to hypoxia.

6. WHO CAN PARTICIPATE IN THIS STUDY?

You may be able to participate in this study if:

You are male between the ages of 19-40yrs

You do not smoke

You have normal lung function

You have no symptoms of cardiopulmonary disease (this includes exercise-induced asthma)

Regularly participate in aerobic physical activity

7. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You cannot participate in this study if:

You have had recent wrist surgery/injury

You have any contraindications to carbonic anhydrase inhibitors

You have allergies to latex or lidocaine or sulfa drugs

Have any cardiovascular or respiratory ailments

Have clotting disorders

Take anti-inflammatory, diuretics or blood thinners/anti-platelet medication

8. WHAT DOES THE STUDY INVOLVE?

Overview of the study:

You are being invited to participate in four data collection test days and your participation in the study is entirely voluntary. The sessions will take place at the Cardiopulmonary Laboratory for Experimental and Applied Physiology at the Arts Building (Room 185) on the University of British Columbia campus. The study will require 4 days of testing totaling 12 hours. The first day will take 2hrs and the subsequent 3 days will each take ~3-3.5 hours.

The first day will be a screening and familiarization day. The 2nd, 3rd and 4th day will all be identical except for the medication or placebo you take beforehand. Each of these days will consist of three different breathing tasks that are separated by 10-30 minutes of rest.

If You Decide to Join This Study:

Specific Procedures

Day 1

The first day of the experiment is for screening and familiarization. First, your height and weight will be measured and you will fill in a questionnaire. You will then undergo a simple, non-invasive breathing test to ensure that you do not have any obstructive lung disease (i.e., asthma). This requires you to breathe in deeply and breathe out quickly through a mouthpiece. During some of these tests you will be seated in an airtight container. The container allows for the precise measurement of pressure. The container has windows on all sides and you are able to see and communicate with the investigators at all times. You will only be in the container for ~1min at a time and are able to leave in between tests.

The diffusion capacity of your respiratory system will then be determined. Diffusion capacity is a measure of how well your lungs can transfer molecules (mainly oxygen) from the air in your lungs into the small blood vessels in your lungs. You will be required to inhale a very small amount of carbon monoxide, hold your breath, and then exhale. The amount of carbon monoxide is less than what most people are exposed to by driving on the road. There are no uncomfortable sensations associated with this test.

A thin flexible tube will then be placed in a large vein in your forearm. The small tube in the vein in your forearm will be used to inject agitated saline. The agitated saline is used as contrast to better view structures of your heart together with a probe on an ultrasound machine. To obtain the images of your heart an ultrasound machine will non-invasively emits and detect sound waves (which cannot be heard) to develop the image it is scanning. All together this technique is called echocardiography and is used worldwide to non-invasively image the heart. The device that emits sound will need to be placed on your chest over your heart. To obtain the best image a bit of gel is applied to your skin. The gel should not irritate your skin in any way, but may feel cold.

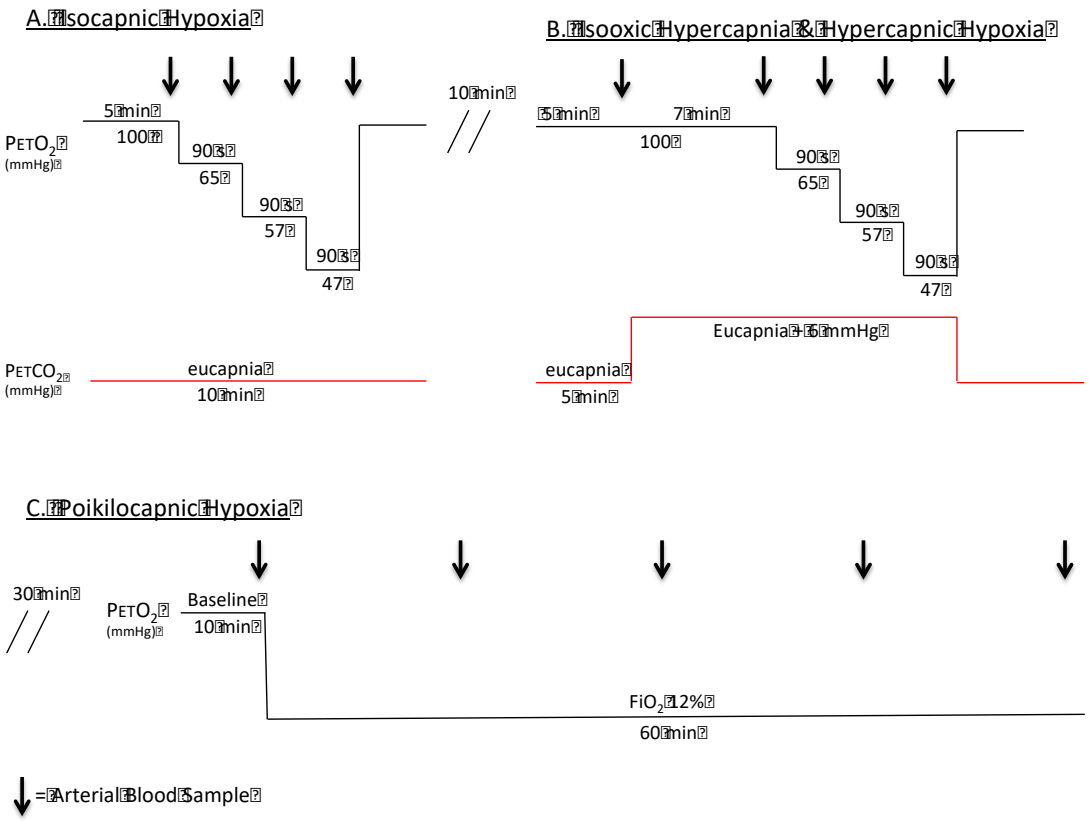
You will then be given the doses of Acetazolamide by a qualified physician (co-investigator Dr. Dominelli) and specific instructions as to when to take them.

Acetazolamide is a carbonic anhydrase inhibitor. This is a class of drugs that suppress the activity of carbonic anhydrase an enzyme responsible for speeding the reaction of carbon dioxide with water in blood that normally occurs. Their clinical use has been established as antiglaucoma agents, diuretics, antiepileptics, management of mountain sickness, gastric and duodenal ulcers, neurological disorders, or osteoporosis. The dose of acetazolamide will be 250 mg every 8 hours This dose is the recommended clinical dose.

Acetazolamide administration

At the end of the first day you will be given seven 250 mg pills of acetazolamide. You will be asked to take 1 pill every 8 hours for 2 days prior to your second day of testing. You will take the last pill 1 hour before the start of the 2nd testing day.

Below is a flow chart with displays how the following testing days will be completed



Day 2

This testing day will be split into three different breathing tests. Before any of them, we will have to place to catheters (small tubes) in blood vessels in your arm.

The first is identical to the one done on the first day (into a large vein in your forearm). It is used to inject saline contrast in an identical fashion as Day 1.

After this, the physician who is inserting the other tube will perform a test to verify you have good circulation in your hand. The test is called the "Allan Test" not painful. If the physician deems your circulation adequate and is satisfied the study can be performed safely, you will be able to have the catheter used in the study. If you are cleared to participate, a thin flexible tube will be placed in the artery near your wrist in your non-dominant hand. Prior to insertion a local anesthetic (numbing liquid) will be injected near the site the tube will go in your wrist to minimize any discomfort. The small tube near your wrist will be used to withdraw blood during exercise and monitor your blood pressure.

If you do not wish to have the tube in the artery near your wrist, but still wish to participate, we can perform an alternate method. In this alternate method, we only take one blood sample (via one single puncture) from the artery in your wrist. After that, we only take small blood samples from the tip of a finger. This is achieved by using a device called a lancet. A lancet contains a small needle that pierces your skin and allows a small drop of blood out. We collect the blood and analyze it for similar things as the other blood samples. The device and method of collecting blood is similar to how people who do that on a daily basis (those who have diabetes) and is minimally painful. You may choose this option before the any of the test and do a different option for each testing day. For example, you may decide to have an arterial catheter before the 2nd day, but not the 3rd or 4th.

Before you start the three trials we will place several other instruments which non-invasively measure parameters. They are as follows:

-A small cuff will be placed on one of your fingers. The cuff will inflate during testing and squeeze your finger. This allows us to measure the pressure in your blood vessels. You will only notice the squeezing around your finger. The cuff deflates between trials

-A transcranial Doppler will be placed on your head. The Doppler uses the same sound waves as the machine that imaged your heart. This allows us to visualize blood flow in vessels inside your head. There is no pain or discomfort associated with this. The probe is only on you during each trial

-An duplex ultrasound will be placed over a blood vessel in your neck. This too uses the same sound waves as the above devices and measures blood flow in your neck. There is no pain or discomfort associated with this. The probe is only on you during each trial.

-A pulse oximeter on your finger. This is a device that emits and receives light and allows for an estimation of the amount of oxygen in your blood. There is no pain or discomfort associated with this

Test A- Isocapnic hypoxia

For this test you will lie supine on a bed and breathe though a mouthpiece. During this test you will be asked to lie quietly and breathe normally as you feel. During the test, a device will lower the amount of oxygen you breathe in. This is referred to as hypoxia. There will be four steps of the hypoxia as shown in the above figure. During the whole time, the device

you breathe through ensure the other gases in your blood stay the same. Each step will be 90 seconds long and we will take a blood sample at the end of each. You may notice that you feel the need to breathe more during this test, this is normal. If you are uncomfortable at any point you can remove the mouthpiece and any negative sensations will immediately resolve as you will be breathing room air. After this test you will rest for 10 mins and will not have any of the devices on you.

Test B- Isooxic hypercapnia & Hypercapnic hypoxia

For this test you will lie in the same bed and breathe through the same mouthpiece with all the same devices. The test is similar in that we will change the amount of oxygen you breathe in, except we will also change the amount of carbon dioxide in your blood. Similar to the other test, you may feel like you need to breathe more, this is normal. During each step in which we change the air composition you breathe in, we will take a blood sample. If you are uncomfortable at any point you can remove the mouthpiece and any negative sensations will immediately resolve as you will be breathing room air. After this test you will rest for 30 mins and will not have any of the devices on you

Test C- Poikilocapnic Hypoxia

For this test you will lie in the same bed and breathe through the same mouthpiece with all the same devices. For this test you will breathe a constant level of hypoxia for 60 mins. This hypoxia is generated from a commercially available device that decreases the amount of oxygen you breathe in. Similar to the other test, you may feel like you need to breathe more, this is normal. During this trial, we will take a blood sample every 15 mins. At the same time we will have the device on your chest and will takes images of your heart. You will not notice any discomfort during this. At the same time, we will inject the agitated saline contrast in your large forearm vein. This is the exact same as we did on the first day of testing. If you are uncomfortable at any point you can remove the mouthpiece and any negative sensations will immediately resolve as you will be breathing room air.

After completing the last test, you will be given one of the other medications (or placebo pill) and instructions on when you should begin taking the pills. You will have to wait a minimum of 1 week between testing days to ensure there is enough time between tests.

Day 3 & 4

The third and fourth testing days will be identical to the second, except you will have either a different pill before the trial. After the third day of testing, identical instruction and a different pill will be provided to take in advance of the fourth day of testing. You will again have to wait a week before starting the next trial.

Methazolamide is a carbonic anhydrase inhibitor. This is a class of drugs that suppress the activity of carbonic anhydrase an enzyme responsible for speeding the reaction of carbon dioxide with water in blood that normally occurs. Their clinical use has been established as antiglaucoma agents, diuretics, antiepileptics, management of mountain sickness, gastric and duodenal ulcers, neurological disorders, or osteoporosis. The dose of methazolamide will be 100 mg every 12 hours. This dose is the recommended clinical dose.

Some of the blood samples will be immediately analyzed for arterial blood gases and electrolytes, after which they will be immediately discarded. One sample will be stored in a freezer where it will be later analyzed for albumin, iron and reactive oxygen species. The sample will only be identified by your unique code. After analysis, any remaining blood will be destroyed. No genetic testing or banking of you blood samples will be done.

9. WHAT ARE MY RESPONSIBILITIES?

You will be expected to participate in 4 testing sessions and to avoid exercise, food, and caffeine for at least 2 hours prior to each testing day. You will be expected to take the medication (or placebo) as prescribed.

10. WHAT ARE THE POSSIBLE HARMS AND DISCOMFORTS?

During the control of breathing tests, subjects make experience headache or may feel lightheaded. These symptoms will pass after they have returned to breathe room air (after a couple of minutes).

The two drugs used in this study are from a class called “carbonic anhydrase inhibitors”. They are approved for use in humans and have been used for >70 years and millions of people have taken them. Acetazolamide, in particular, is prescribed commonly to healthy humans travelling to high altitude to minimize the risk of acute mountain sickness. The discomforts and possible side effects of acetazolamide and methazolamide are similar. While on these medications you will find your urine output is increased, you may feel thirsty,

and you may feel tingling in your hands and feet. You may find yourself breathless more often as a result of an increase in your resting drive to breathe. While taking these medications, carbonated beverages will taste "flat" and the taste of some foods may be altered. All medicines may cause side effects, but many people have no, or minor, side effects. It is important to note that many side-effects are only reported in studies that used patients who have other health problems which make the side-effects worse. As such, it is difficult to determine how likely you will be to experience any of the side effects. You should consult with your doctor if any of these most COMMON side effects persist or become bothersome: Blurred vision; constipation; diarrhea; drowsiness; loss of appetite; nausea; vomiting. The most common side effect (30-40% of patients) is general malaise, fatigue, weight loss, nausea, anorexia, depression and loss of libido. You may also notice that you are breathing more at rest and/or during activities. You should seek medical attention IMMEDIATLY if any of these SEVERE side effects occur: Severe allergic reactions (rash; hives; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); blood in urine; changes in hearing; convulsions; dark, bloody stools; dark urine; fast breathing; fever; lack of energy; lower back pain; red, swollen, or blistered skin; ringing in the ears; sore throat; tingling of the arms or legs; unusual bleeding or bruising; vision changes; yellowing of the skin or eyes. In this investigation your exposure to acetazolamide and methazolamide is minimal.

The side effects from both acetazolamide and methazolamide may be worsened by alcohol or other medication. Please limit alcohol consumption when on any of the drugs and do not drive or other usage tasks until you know how you react.

Potential risks associated with the catheter in your arm include bleeding (less than 1%), bruising (14%), and infection (less than 1%). Other potential risks associated with an arterial line include artery aneurysms (0.09%), arterial laceration (under 1%), blood clotting (less than 1%), brief tightening of a blood vessel (5%), death of skin tissue over the catheter site (0.09%), and line disconnection (lower 1%). The reported risk are from clinical studies where co-morbities may be present. We will numb the area in your hand to minimize discomfort for the arterial catheter. When collecting blood, the utmost care will be taken to ensure your comfort. Catheter insertion and maintenence will be performed by one of the trained physicians who will have clinical experience placing and maintaining the catheters.

You will be advised to refrain from strenuous exercise or heavy lifting for the remainder of the day after testing is complete. The following day(s) you may want to refrain from racquet sports that use the arm where the arterial catheter was located, but otherwise will be able to return to normal activity levels.

Intravenous Catheter and Saline Bubble Injection: Contrast echocardiography with agitated saline injection is a standard clinical technique used to diagnose intracardiac and intrapulmonary shunting and carries essentially no risk. The subject may experience mild discomfort due to the catheter in their arm. Potential risks associated with the intravenous catheter include bleeding (less than 1%), bruising (14%), and infection (less than 1%). Saline injection into a vein carries a risk of temporary dizziness, confusion, difficulty breathing and a risk of brain injury or stroke.

Agitated saline, either alone or mixed with 5% dextrose in water has been used as an echo contrast agent for over thirty years (Gramiak and Shah, Invest Radiol 3:356-66, 1968). In 1984, the first (and only) survey of the safety of these early echo contrast agents was conducted by the Committee on Contrast Echocardiography for the American Society of Echocardiology (Bommer et al J Am Coll Cardiol 3:6-13, 1984). They evaluated a retrospective survey of 363 physicians who routinely used echo contrast agents including agitated indocyanine green and various saline solutions. Of 51,180 patients undergoing contrast echocardiography, only 32 cases of side effects were reported. The majority of the reported side effects involved reactions to indocyanine green and saline solutions containing preservatives or bacteriostatic agents. Subsequently the only reports of side-effects that appear in the literature are two letters reporting transient dizziness associated with agitated sterile saline injection in patients with cardiac shunting (Dittrich Ann Int Med 123: 731-732, 1995; Srivastava and Undesser Ann Int. Med 122: 396, 1995). All of the side effects in all of the reports were transient. We will use a minimal volume (3-4 mL) of sterile saline, without preservatives.

11. WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING?

There are no direct benefits for this study.

12. 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. If your participation in this study includes enrolling in any optional studies, or long term follow-up, you will be asked whether you wish to withdraw from these as well

If you consent, we will use parts of you data in the results (example: Days 1-3 are completed, but not Day 4). This will be your choice, and if you wish to, all data will be deleted.

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

Your confidentiality will be respected. However, research records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada, and UBC Clinical Research Ethics Board 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] 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 investigators, 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.

Some of the blood samples will be immediately analyzed for arterial blood gases and electrolytes, after which they will be immediately discarded. One sample will be stored in a freezer where it will be later analyzed for albumin, iron and reactive oxygen species. The sample will only be identified by your unique code. After analysis, any remaining blood will be destroyed. No genetic testing or banking of you blood samples will be done.

14. WHAT HAPPENS IF SOMETHING GOES WRONG?

Signing this consent form in no way limits your legal rights against the investigators, or anyone else, and you do not release the study doctors or participating institutions from their legal and professional responsibilities.

In the unlikely event of a medical emergency during the study, immediate care will be provided by researches who have current first aid certificates. There is an automated emergency defibrillator and first aid supplies (including airway management material) in the study area and the distance to the nearest hospital emergency room is 15 kilometers.

15. WHAT WILL THE STUDY COST ME?

Remuneration: You will not be paid for participation in this study.

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 *Paolo Dominelli at p.dominelli@alumni.ubc.ca or (604)992-2071*.

17. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT?

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 Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598).

My signature on this consent form means:

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 results 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

In signing this form you are consenting to participate in this research project and acknowledge receipt of a copy of this form. Signing this consent form in no way limits your legal rights against the investigators, or anyone else.

I will receive a signed copy of this consent form for my own records.

I consent to participate in this study.

Subject's Signature	Printed name	Date
Investigator's Signature	Printed name	Date

My signature above signifies that the study has been reviewed with the study subject by me and/or by my delegated staff. My signature may have been added at a later date, as I may not have been present at the time the subject's signature was obtained.

A.4 Subject Demographics and Health History Questionaire

The effect of carbonic anhydrase inhibitors on the pulmonary system response to hypoxia

Subject Identification Code: _____

Weight (Kg): _____ Height (cm):_____ BMI: _____

Gender: _____ Age (years): _____

Time of last meal: _____ Pulmonary function:_____

Please answer **Yes/No** for each question. If yes, please explain, as you are responsible for answering the questions:

Have you refrained from caffeine, alcohol and vigorous exercise 12 hours prior to the experimental day?
YES NO

Do you have a history of fainting or have ever experienced a syncopal episode (e.g., fainting)?
YES NO

Do you have a previous history of or a current respiratory disease or abnormality (e.g., asthma, chronic bronchitis, cystic fibrosis)?
YES NO

Do you have a previous history of or a current cardiovascular disease or abnormality (e.g., cardiac arrhythmia, hypertension, myocardial infarction, blood clotting problems) or have a cardiac pacemaker?
YES NO

Do you have a previous history of or a current neurological disease or abnormality (e.g., epilepsy, chronic migraines, stroke)?
YES NO

Have you recently (within 6 months) injured either wrist or lower leg?

YES NO

Have you had surgery on or near (i) Either wrist (ii) Lower leg (iii) Nose, throat or nasopharynx

YES NO

Are you currently on any kind of medication, over the counter or prescribed?
YES NO

Do you smoke?
YES NO

Do you have any drug or latex allergies?
YES NO

Have you had all of your questions or concerns addressed?

YES NO

Appendix B Individual Raw Data

B.1 Individual Raw Data for Participant Characteristics and Outcome Variables

Table B.1-1 Participant Characteristics

ID	Age (year)	Height (m)	Weight (kg)	BMI (kg/m^2)	Systolic (mmHg)	Diastolic (mmHg)	MAP (mmHg)
001	23	1.74	77	25.4	107	57	74
002	31	1.75	83	27.0	128	64	85
004	29	1.65	69	25.2	113	79	91
005	20	1.78	73	23.0	119	58	78
006	22	1.88	90	25.5	128	77	94
007	28	1.74	72	23.8	116	65	82
008	26	1.74	74	24.5	127	69	89
010	22	1.65	62	22.7	115	57	76
013	26	1.77	89	a28.4	128	72	91
014	24	1.77	75	23.8	127	77	93
015	25	1.8	89	27.5	135	63	87
Mean	**25**	**1.75**	**77**	**25.2**	**122**	**67**	**85**
SEM	**1**	**0.02**	**2.8**	**0.6**	**3**	**2**	**2**

Abbreviations: BMI, body mass index; MAP, mean arterial pressure; SEM, standard error of the mean

Table B.1-2 Pulmonary artery systolic pressure (PASP; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	19.5	32.7	18.1	27.0	19.0	28.5
002	24.3	42.6	22.9	29.8	22.3	29.0
004	17.7	29.9	18.8	26.9	17.5	26.6
005	22.1	34.0	21.4	25.1	20.8	29.2
006	23.8	36.5	22.9	30.0	22.4	32.3
007	21.9	33.3	21.7	30.1	21.6	30.7
008	22.6	35.3	19.5	32.3	21.3	31.3
010	18.0	27.5	19.2	27.4	18.6	28.8
013	21.4	44.7	22.5	37.8	21.1	28.2
014	17.6	36.5	17.2	28.7	17.0	22.9
015	20.9	31.9	21.3	29.2	19.8	22.2
Mean	20.9	35.0	20.5	29.5	20.1	28.1
SEM	0.7	1.4	0.6	0.9	0.5	0.9

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-3 Left ventricular outflow tract - velocity time integral ($LVOT_{VTI}$; cm)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	23.8	30.2	26.2	27.3	26.1	27.9
002	20.4	20.5	17.1	22.0	21.5	21.4
004	25.1	23.3	23.0	24.5	22.4	26.2
005	28.1	31.0	24.3	21.6	25.1	29.7
006	29.7	30.2	29.5	34.3	27.5	26.4
007	25.2	28.0	27.0	25.6	25.0	28.8
008	29.8	23.2	29.5	30.6	27.6	29.3
010	23.1	27.4	21.6	25.9	23.4	24.2
013	27.3	26.3	27.5	29.1	27.5	30.1
014	24.4	26.3	24.1	19.7	20.3	20.9
015	21.4	30.4	26.1	27.7	22.6	32.7
Mean	**25.3**	**27.0**	**25.1**	**26.2**	**24.5**	**27.1**
SEM	**0.9**	**1.0**	**1.0**	**1.2**	**0.7**	**1.0**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-4 Stroke Volume (SV; ml)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	81.8	103.8	90.1	94.1	89.7	96.0
002	61.4	61.8	51.6	66.4	64.9	64.6
004	83.6	77.7	76.5	81.5	74.7	87.2
005	83.3	92.0	72.0	64.2	74.4	88.1
006	118.8	120.8	117.9	137.2	109.7	105.5
007	78.2	86.9	83.7	79.4	77.4	89.3
008	108.5	84.4	107.3	111.4	100.6	106.7
010	76.8	91.1	71.7	86.0	77.8	80.3
013	78.2	75.3	78.6	83.2	78.7	86.1
014	101.8	109.7	100.5	82.1	84.8	87.4
015	69.4	98.3	84.5	89.7	73.3	105.9
Mean	**85.6**	**91.1**	**84.9**	**88.6**	**82.4**	**90.6**
SEM	**4.7**	**4.6**	**5.1**	**5.7**	**3.6**	**3.5**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-5 Cardiac Output ($\dot{Q}$; l/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	4.1	4.7	3.8	4.1	3.9	4.6
002	3.9	4.6	3.1	4.2	3.3	3.7
004	4.3	5.0	4.2	5.2	3.9	5.1
005	4.3	5.1	5.1	4.5	3.9	4.6
006	7.1	9.0	7.0	9.6	7.0	7.5
007	4.5	6.2	4.9	6.1	4.6	5.5
008	4.9	4.2	4.0	5.6	3.5	4.4
010	4.7	7.0	4.9	6.2	5.4	6.4
013	4.2	6.2	3.5	4.4	4.0	6.1
014	6.8	7.8	6.8	6.3	5.6	6.5
015	4.1	6.9	5.2	5.7	4.6	6.7
Mean	**4.8**	**6.1**	**4.8**	**5.6**	**4.5**	**5.6**
SEM	**0.3**	**0.4**	**0.3**	**0.4**	**0.3**	**0.3**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-6 Total pulmonary resistance (TPR; mmHg/l/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	4.7	6.9	4.8	6.6	4.9	6.2
002	6.2	9.3	7.4	7.1	6.7	7.8
004	4.1	6.0	4.5	5.2	4.4	5.2
005	5.2	6.7	4.2	5.6	5.4	6.4
006	3.4	4.0	3.3	3.1	3.2	4.3
007	4.9	5.4	4.4	4.9	4.8	5.5
008	4.6	8.3	4.9	5.8	6.1	7.1
010	3.8	3.9	3.9	4.4	3.5	4.5
013	5.1	7.3	6.4	8.6	5.3	4.6
014	2.6	4.7	2.5	4.6	3.0	3.5
015	5.1	4.6	4.1	5.1	4.3	3.3
Mean	4.5	6.1	4.6	5.6	4.7	5.3
SEM	0.3	0.5	0.4	0.4	0.3	0.4

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-7 Heart Rate (HR; beats/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	51	45	42	43	43	48
002	63	74	60	63	51	58
004	52	64	55	63	53	59
005	51	55	71	70	52	52
006	59	75	60	70	64	71
007	57	71	58	77	59	62
008	46	50	37	50	35	41
010	61	77	69	72	69	80
013	54	82	45	53	51	71
014	66	71	68	76	67	74
015	59	70	61	63	63	63
Mean	**56**	**67**	**57**	**64**	**55**	**62**
SEM	**2**	**3**	**3**	**3**	**3**	**3**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-8 Peripheral oxyhemoglobin saturation (SpO_2; %)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	106.1	79.1	98.6	87.5	98.4	86.3
002	98.4	76.8	98.4	84.0	98.4	85.8
004	97.4	67.3	96.6	84.0	96.4	79.3
005	97.4	74.3	98.4	80.9	98.2	85.5
006	96.8	75.5	98.4	80.6	98.4	81.4
007	97.4	70.3	98.1	74.8	98.4	82.4
008	99.4	72.0	98.4	78.7	99.4	83.2
010	95.6	85.5	98.2	86.1	97.4	81.2
013	97.5	67.9	98.4	77.1	97.4	74.9
014	98.4	75.0	97.4	83.5	97.4	78.3
015	97.6	70.4	98.4	76.0	98.8	81.4
Mean	98.4	74.0	98.1	81.2	98.1	81.8
SEM	0.8	1.5	0.2	1.2	0.2	0.9

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-9 Systolic blood pressure (SBP; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	132	140	133	148	129	124
002	147	155	132	144	131	149
004	134	120	116	133	121	128
005	107	138	136	125	124	114
006	135	141	144	150	150	157
007	127	133	141	146	129	130
008	147	158	124	145	138	155
010	117	104	112	117	112	126
013	126	115	100	135	138	125
014	141	141	144	163	135	140
015	118	143	153	166	137	137
Mean	**130**	**135**	**130**	**143**	**131**	**135**
SEM	**3**	**5**	**4**	**4**	**3**	**4**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-10 Diastolic blood pressure (DBP; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	67	77	77	82	72	76
002	62	69	75	85	70	86
004	81	67	70	87	62	70
005	54	72	79	67	66	65
006	70	71	67	68	89	86
007	73	82	93	96	78	73
008	83	85	68	81	75	87
010	64	56	42	51	64	62
013	72	67	42	57	79	72
014	70	74	82	92	68	70
015	63	67	67	86	67	70
Mean	**69**	**72**	**69**	**77**	**72**	**74**
SEM	**2**	**2**	**4**	**4**	**2**	**2**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-11 Mean arterial pressure (MAP; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	88.7	97.6	95.5	104.2	90.8	92.1
002	90.5	97.8	94.0	104.4	90.6	107.4
004	98.5	84.5	85.1	102.4	81.9	89.4
005	71.7	94.2	97.8	86.2	85.1	81.0
006	91.3	94.5	92.6	95.3	109.3	109.9
007	91.3	98.9	108.8	112.3	94.9	91.8
008	104.5	109.6	86.6	102.6	95.9	109.5
010	81.5	72.2	65.0	73.2	79.7	83.3
013	90.0	82.7	61.2	83.4	98.3	89.4
014	93.9	96.5	102.5	115.9	90.6	93.4
015	81.5	92.0	95.8	112.8	90.3	92.4
Mean	**89.4**	**92.8**	**89.5**	**99.3**	**91.6**	**94.5**
SEM	**2.4**	**2.7**	**4.0**	**3.7**	**2.3**	**2.8**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-12 Tidal volume (V_T; l)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	0.9	1.1	1.1	1.0	0.9	1.0
002	0.9	0.9	1.0	0.9	1.1	1.4
004	0.7	0.8	0.7	0.6	0.8	0.7
005	0.8	0.9	0.8	0.9	0.9	0.9
006	0.8	1.0	0.9	1.0	0.9	1.1
007	1.3	1.6	1.0	1.5	0.9	1.1
008	1.0	0.9	0.8	0.9	0.8	0.8
010	0.6	0.7	0.7	0.7	0.9	0.7
013	0.7	1.0	0.7	0.8	0.8	0.8
014	0.8	0.9	1.2	1.0	1.0	1.0
015	1.3	1.0	1.5	1.5	1.2	1.2
Mean	**0.9**	**1.0**	**0.9**	**1.0**	**0.9**	**1.0**
SEM	**0.1**	**0.1**	**0.1**	**0.1**	**0.0**	**0.1**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-13 Breathing frequency (f_B; breath/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	13.9	11.1	14.7	15.5	17.5	13.7
002	13.3	16.6	12.8	15.8	12.9	8.5
004	18.3	17.8	20.4	7.7	16.4	19.7
005	13.3	12.1	18.1	14.4	18.9	16.0
006	16.7	15.0	17.9	14.4	18.1	13.9
007	6.4	5.9	10.8	7.4	13.6	8.9
008	11.9	14.0	16.6	18.7	14.6	14.7
010	20.3	16.1	17.4	19.3	15.5	20.7
013	17.0	13.7	18.9	18.5	14.9	14.7
014	18.7	19.1	17.5	18.4	14.7	15.9
015	8.8	13.9	8.2	8.2	9.7	9.7
Mean	**14.4**	**14.1**	**15.7**	**14.4**	**15.1**	**14.2**
SEM	**1.2**	**1.0**	**1.0**	**1.3**	**0.7**	**1.1**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-14 Inspired ventilation ($\dot{V}_I$; l/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	12.8	12.7	16.4	17.4	17.1	14.6
002	12.9	15.7	13.9	16.4	15.1	12.9
004	14.6	14.9	15.2	6.7	13.6	16.0
005	12.4	12.3	16.5	14.7	17.8	16.2
006	15.1	16.0	17.1	15.5	18.0	15.7
007	9.0	9.5	12.1	11.1	13.6	10.9
008	12.5	13.7	15.4	17.6	13.3	13.1
010	14.2	12.3	13.6	15.1	14.4	16.9
013	13.8	14.1	15.4	16.5	12.8	13.5
014	16.7	18.8	22.6	21.2	16.3	18.5
015	12.3	14.3	12.6	13.4	12.1	13.1
Mean	**13.3**	**14.0**	**15.5**	**15.1**	**14.9**	**14.7**
SEM	**0.5**	**0.7**	**0.8**	**1.0**	**0.6**	**0.6**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-15 Expired ventilation ($\dot{V}_E$; l/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	12.7	12.6	16.4	17.3	17.1	14.6
002	12.7	15.6	13.8	16.3	15.0	12.8
004	14.5	14.8	15.2	6.7	13.6	15.9
005	12.3	12.2	16.4	14.6	17.8	16.2
006	15.0	15.9	17.0	15.4	17.9	15.6
007	8.9	9.4	12.0	11.0	13.5	10.9
008	12.5	13.6	15.4	17.5	13.2	13.0
010	14.2	12.3	13.5	15.0	14.3	16.8
013	13.7	14.0	15.3	16.4	12.7	13.4
014	16.6	18.7	22.5	21.1	16.2	18.4
015	12.1	14.2	12.6	13.3	12.0	12.9
Mean	**13.2**	**13.9**	**15.5**	**15.0**	**14.8**	**14.6**
SEM	**0.6**	**0.7**	**0.8**	**1.0**	**0.6**	**0.6**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-16 Fraction of inspired O_2 (F_IO_2; %)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	20.9	11.6	20.8	12.2	20.9	12.1
002	21.1	12.0	20.8	11.7	20.9	12.2
004	20.5	11.6	20.8	11.7	20.9	11.9
005	21.0	12.2	20.8	11.8	20.7	12.1
006	20.8	12.2	21.1	12.1	21.0	12.1
007	21.0	12.0	20.9	12.0	20.7	12.0
008	20.5	11.8	20.9	12.0	20.8	12.0
010	20.4	11.9	20.8	12.0	20.8	11.8
013	20.6	11.8	20.7	12.1	20.9	11.3
014	20.8	11.9	20.9	12.1	20.6	12.2
015	21.1	12.0	20.7	12.0	20.9	12.4
Mean	**20.8**	**11.9**	**20.8**	**12.0**	**20.8**	**12.0**
SEM	**0.1**	**0.1**	**0.0**	**0.0**	**0.0**	**0.1**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-17 Fraction of expired O_2 (F_EO_2; %)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	17.98	8.90	18.64	9.87	18.61	9.60
002	17.43	9.07	18.35	9.14	18.35	9.16
004	18.66	9.24	18.80	10.01	18.64	9.73
005	17.73	9.25	18.17	8.99	18.50	9.78
006	17.90	8.95	18.29	9.35	18.36	9.14
007	16.14	8.05	17.52	8.30	17.40	8.80
008	17.50	8.55	18.61	9.52	18.22	9.69
010	18.34	10.55	18.52	9.97	18.05	9.71
013	17.85	8.54	18.54	9.55	18.24	8.57
014	18.32	9.56	18.27	9.63	17.99	9.66
015	16.50	8.54	17.09	8.20	17.17	8.76
Mean	**17.67**	**9.02**	**18.25**	**9.32**	**18.14**	**9.33**
SEM	**0.21**	**0.18**	**0.14**	**0.17**	**0.13**	**0.13**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-18 Fraction of mixed expired CO_2 (F_ECO_2; %)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	2.31	2.03	1.83	1.95	2.10	2.15
002	2.77	2.45	2.32	2.06	2.00	2.43
004	1.80	1.88	1.90	1.87	1.93	1.91
005	2.52	2.25	2.28	2.18	2.14	2.14
006	2.53	2.51	2.40	2.43	2.10	2.37
007	3.65	3.09	2.74	3.03	2.90	2.82
008	2.86	2.48	2.14	2.09	2.24	2.07
010	2.08	1.95	2.04	1.76	2.25	1.93
013	2.24	2.58	1.90	2.01	2.15	2.19
014	2.13	2.05	2.22	2.06	2.33	2.24
015	3.18	2.87	3.31	3.11	2.97	2.50
Mean	**2.55**	**2.38**	**2.28**	**2.23**	**2.28**	**2.25**
SEM	**0.15**	**0.11**	**0.12**	**0.12**	**0.09**	**0.07**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-19 Partial pressure of end-tidal O_2 ($P_{ET}O_2$; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	97.1	43.5	106.8	52.1	114.2	52.5
002	97.9	40.6	103.8	45.3	111.0	49.2
004	98.1	41.1	104.3	49.5	104.1	44.3
005	99.6	41.4	101.9	55.9	103.5	48.2
006	93.7	40.4	106.9	45.5	103.8	47.0
007	92.9	38.3	102.3	40.7	103.1	45.1
008	96.8	40.7	108.5	45.3	105.6	47.2
010	94.3	48.1	101.9	47.6	102.3	45.8
013	89.0	36.6	97.3	43.1	97.7	39.1
014	103.1	46.1	116.9	50.3	108.7	50.6
015	96.0	42.0	101.1	41.2	103.1	47.0
Mean	**96.3**	**41.7**	**104.7**	**47.0**	**105.2**	**46.9**
SEM	**1.0**	**0.9**	**1.4**	**1.3**	**1.2**	**1.0**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-20 Partial pressure of end-tidal CO_2 ($P_{ET}O_2$; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	35.9	30.2	29.8	27.7	28.0	26.7
002	38.7	33.3	32.2	28.6	28.3	27.7
004	40.4	34.1	33.7	28.7	32.2	30.2
005	39.4	35.0	34.8	30.5	34.0	31.3
006	39.6	33.4	32.6	29.1	31.6	28.3
007	41.7	35.3	35.3	34.8	33.3	31.8
008	36.9	33.0	30.3	30.2	32.0	31.0
010	40.9	35.9	33.7	31.1	32.0	28.6
013	42.3	36.8	34.8	32.3	36.8	33.3
014	33.1	28.8	24.6	26.2	26.8	26.2
015	39.1	33.9	33.9	32.7	32.6	29.2
Mean	**38.9**	**33.6**	**32.3**	**30.2**	**31.6**	**29.5**
SEM	**0.7**	**0.7**	**0.9**	**0.7**	**0.8**	**0.6**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-21 Volume of O_2 consumed per minute ($\dot{V}O_2$; l/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	0.31	0.28	0.29	0.32	0.32	0.29
002	0.38	0.37	0.28	0.34	0.32	0.32
004	0.22	0.29	0.24	0.09	0.26	0.28
005	0.34	0.29	0.36	0.34	0.32	0.30
006	0.36	0.41	0.40	0.34	0.38	0.37
007	0.36	0.30	0.34	0.33	0.36	0.28
008	0.30	0.36	0.28	0.35	0.29	0.24
010	0.23	0.14	0.25	0.25	0.33	0.29
013	0.31	0.37	0.27	0.34	0.27	0.29
014	0.34	0.36	0.49	0.43	0.35	0.38
015	0.48	0.41	0.37	0.41	0.37	0.39
Mean	**0.33**	**0.32**	**0.32**	**0.32**	**0.32**	**0.31**
SEM	**0.02**	**0.02**	**0.02**	**0.02**	**0.01**	**0.01**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-22 Volume of carbon dioxide produce per minute ($\dot{V}CO_2$; l/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	0.23	0.20	0.23	0.26	0.28	0.24
002	0.28	0.30	0.25	0.26	0.23	0.24
004	0.20	0.22	0.22	0.09	0.20	0.23
005	0.24	0.21	0.29	0.24	0.29	0.27
006	0.29	0.31	0.32	0.29	0.29	0.28
007	0.25	0.22	0.26	0.26	0.30	0.24
008	0.28	0.26	0.25	0.28	0.23	0.21
010	0.23	0.19	0.21	0.20	0.25	0.25
013	0.24	0.28	0.23	0.26	0.21	0.23
014	0.28	0.30	0.39	0.34	0.30	0.32
015	0.30	0.32	0.32	0.32	0.28	0.25
Mean	**0.25**	**0.25**	**0.27**	**0.25**	**0.26**	**0.25**
SEM	**0.01**	**0.01**	**0.01**	**0.02**	**0.01**	**0.01**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-23 Respiratory exchange ratio (RER)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	0.72	0.72	0.80	0.81	0.90	0.83
002	0.84	0.80	0.88	0.77	0.75	0.76
004	0.90	0.74	0.93	1.10	0.80	0.83
005	0.72	0.73	0.81	0.73	0.93	0.90
006	0.82	0.75	0.80	0.87	0.75	0.76
007	0.69	0.75	0.76	0.78	0.84	0.86
008	0.94	0.73	0.90	0.82	0.80	0.86
010	1.00	1.40	0.86	0.82	0.76	0.88
013	0.76	0.76	0.85	0.75	0.77	0.78
014	0.81	0.84	0.79	0.79	0.85	0.87
015	0.63	0.79	0.87	0.78	0.75	0.65
Mean	**0.80**	**0.82**	**0.84**	**0.82**	**0.81**	**0.82**
SEM	**0.03**	**0.05**	**0.01**	**0.03**	**0.02**	**0.02**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-24 Partial pressure of alveolar O_2 (P_AO_2; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	105.0	46.1	117.6	58.1	123.3	59.4
002	115.3	50.5	116.0	51.2	114.3	57.0
004	116.2	43.0	120.8	63.4	116.5	54.5
005	105.4	46.7	112.6	48.5	118.8	56.3
006	107.2	48.3	117.8	57.6	110.8	54.3
007	109.7	44.4	120.5	48.2	119.1	53.4
008	109.2	45.1	115.1	53.5	116.7	55.0
010	112.0	60.9	116.0	53.9	117.3	56.3
013	103.4	42.6	114.2	50.1	116.2	43.3
014	105.0	55.9	112.0	58.8	115.7	61.9
015	105.7	49.5	114.3	49.7	112.8	51.5
Mean	**108.6**	**48.5**	**116.1**	**53.9**	**116.5**	**54.8**
SEM	**1.2**	**1.5**	**0.8**	**1.4**	**0.9**	**1.3**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-25 Partial pressure of alveolar CO_2 (P_ACO_2; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	35.1	27.7	28.2	25.3	26.5	24.4
002	33.8	30.4	30.9	26.3	30.2	25.2
004	29.3	31.3	28.6	23.5	30.0	27.5
005	36.5	32.0	31.9	27.8	30.1	28.7
006	37.0	30.5	30.1	26.7	31.8	25.8
007	30.7	32.2	25.3	31.5	26.8	29.2
008	36.7	30.1	33.1	27.5	29.3	28.3
010	35.3	33.4	31.1	28.3	26.6	26.1
013	36.9	33.6	31.7	29.4	27.9	30.4
014	39.0	26.3	33.3	23.9	30.6	24.0
015	33.1	31.0	32.1	29.9	31.4	26.5
Mean	**34.9**	**30.8**	**30.6**	**27.3**	**29.2**	**26.9**
SEM	**0.8**	**0.6**	**0.7**	**0.7**	**0.5**	**0.6**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-26 Alveolar ventilation ($\dot{V}_A$; l/min)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	5.6	6.2	7.1	8.9	9.0	8.6
002	7.0	8.4	6.9	8.5	6.6	8.3
004	6.0	5.9	6.7	3.2	5.8	7.4
005	5.7	5.8	7.8	7.6	8.4	8.1
006	6.8	8.7	9.1	9.4	7.8	9.5
007	7.0	6.0	8.7	7.0	9.8	7.0
008	6.5	7.4	6.6	8.8	6.7	6.3
010	5.5	4.8	5.9	6.2	8.0	8.3
013	5.5	7.2	6.1	7.5	6.5	6.4
014	6.1	9.7	10.0	12.2	8.3	11.6
015	7.9	8.9	8.7	9.2	7.6	8.2
Mean	**6.3**	**7.2**	**7.6**	**8.0**	**7.7**	**8.1**
SEM	**0.2**	**0.4**	**0.4**	**0.6**	**0.3**	**0.4**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-27 Partial pressure of capillary O_2 (P_CO_2; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	75.7	37.8	86.9	53.3	86.2	48.0
002	67.9	37.1	75.5	49.9	90.6	47.6
004	91.0	34.1	86.6	62.2	94.7	42.9
005	82.1	39.2	68.1	38.2	87.4	48.2
006	73.7	36.2	85.2	41.5	77.0	44.5
007	72.1	33.6	79.7	40.3	74.0	40.6
008	81.4	33.7	90.6	41.6	67.0	41.3
010	76.6	53.6	79.2	45.7	84.8	41.5
013	84.5	30.6	87.0	37.3	88.0	34.9
014	84.4	38.2	74.2	44.3	93.2	41.4
015	89.4	35.1	87.8	38.7	95.0	47.5
Mean	**79.9**	**37.2**	**81.9**	**44.8**	**85.3**	**43.5**
SEM	**2.0**	**1.6**	**1.9**	**2.1**	**2.5**	**1.1**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-28 Partial pressure of capillary CO_2 (P_CCO_2; mmHg)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	38.2	32.7	29.3	29.1	29.2	28.1
002	39.4	33.7	34.3	30.8	30.0	28.4
004	41.9	37.8	34.1	28.5	32.1	33.7
005	41.2	36.1	37.2	33.5	34.3	31.5
006	38.4	34.0	32.7	31.5	32.6	29.5
007	42.9	38.6	37.2	36.7	33.4	30.8
008	39.6	35.0	30.9	33.9	33.7	32.7
010	41.4	39.5	34.6	33.5	33.1	31.2
013	41.1	38.9	33.4	35.4	36.5	35.4
014	34.2	29.3	28.8	28.3	26.2	29.6
015	39.8	34.9	31.2	34.0	32.3	30.1
Mean	**39.8**	**35.5**	**33.1**	**32.3**	**32.1**	**31.0**
SEM	**0.7**	**0.8**	**0.8**	**0.8**	**0.8**	**0.6**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-29 Hematocrit (Hct; %)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	45.7	47.1	49.3	48.6	48.7	47.9
002	45.3	47.4	44.3	47.9	45.8	48.6
004	42.2	44.0	41.6	45.4	42.8	44.5
005	46.6	50.7	51.1	52.7	50.1	51.6
006	45.4	45.8	50.6	49.3	51.7	53.4
007	44.9	45.9	45.7	50.4	45.3	48.0
008	41.3	42.6	44.1	42.8	47.2	46.1
010	46.6	48.2	47.3	48.1	46.4	49.5
013	47.4	47.2	45.1	46.1	47.7	48.0
014	45.7	49.2	50.1	51.7	50.3	50.4
015	46.6	48.1	50.9	50.4	50.3	51.2
Mean	**45.2**	**46.9**	**47.3**	**48.5**	**47.8**	**49.0**
SEM	**0.5**	**0.6**	**0.9**	**0.8**	**0.7**	**0.7**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-30 Hemoglobin (Hb; g/dl)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	14.3	15.4	14.8	15.9	14.8	15.6
002	14.3	15.4	14.8	15.9	14.8	15.6
004	14.3	15.4	14.8	15.9	14.8	15.6
005	14.3	15.4	14.8	15.9	14.8	15.6
006	14.3	15.4	14.8	15.9	14.8	15.6
007	14.3	15.4	14.8	15.9	14.8	15.6
008	14.3	15.4	14.8	15.9	14.8	15.6
010	14.3	15.4	14.8	15.9	14.8	15.6
013	14.3	15.4	14.8	15.9	14.8	15.6
014	14.3	15.4	14.8	15.9	14.8	15.6
015	14.3	15.4	14.8	15.9	14.8	15.6
Mean	**14.3**	**15.4**	**14.8**	**15.9**	**14.8**	**15.6**
SEM	**14.3**	**15.4**	**14.8**	**15.9**	**14.8**	**15.6**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-31 Bicarbonate ion (HCO_3^-; mmol/l)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	24.3	23.5	16.9	16.9	15.9	14.9
002	24.0	24.3	20.4	19.6	15.7	15.3
004	26.0	25.6	18.4	16.8	16.5	16.9
005	26.8	27.2	21.2	20.5	18.8	18.0
006	25.5	24.3	18.5	19.7	16.5	16.0
007	27.4	27.2	21.6	20.9	17.7	16.2
008	25.1	24.1	18.7	19.2	17.3	17.1
010	25.0	24.6	19.0	19.1	16.7	16.6
013	26.1	25.8	18.3	18.7	18.7	18.8
014	21.7	19.6	16.3	15.8	13.7	15.3
015	25.5	24.4	19.5	20.1	17.0	15.3
Mean	**25.2**	**24.6**	**19.0**	**18.8**	**16.8**	**16.4**
SEM	**0.4**	**0.6**	**0.4**	**0.5**	**0.4**	**0.3**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-32 pH from capillary sample (pH)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	7.41	7.47	7.37	7.37	7.34	7.33
002	7.39	7.47	7.38	7.41	7.33	7.34
004	7.40	7.44	7.34	7.38	7.32	7.31
005	7.42	7.49	7.36	7.40	7.35	7.37
006	7.43	7.46	7.36	7.41	7.31	7.34
007	7.41	7.46	7.37	7.36	7.33	7.33
008	7.41	7.45	7.39	7.36	7.32	7.33
010	7.39	7.40	7.35	7.37	7.31	7.34
013	7.41	7.43	7.35	7.33	7.32	7.33
014	7.41	7.43	7.36	7.36	7.33	7.32
015	7.42	7.45	7.40	7.38	7.33	7.32
Mean	**7.41**	**7.45**	**7.37**	**7.37**	**7.33**	**7.33**
SEM	**0.00**	**0.01**	**0.01**	**0.01**	**0.00**	**0.00**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

Table B.1-33 Base excess (BE; mEq/l)

	PBO		MZ		AZ	
ID	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
001	-0.2	0.1	-7.4	-7.3	-8.8	-9.9
002	-0.8	0.8	-4.2	-4.3	-9.3	-9.3
004	1.1	1.4	-6.7	-7.4	-8.8	-8.6
005	2.1	3.7	-3.8	-3.8	-6.2	-6.5
006	1.1	0.7	-6.1	-4.3	-8.8	-8.6
007	2.5	3.2	-3.3	-4.0	-7.4	-8.7
008	0.4	0.2	-5.6	-5.6	-8.1	-8.1
010	0.0	-0.2	-6.0	-5.6	-8.6	-8.2
013	1.3	1.4	-6.6	-6.6	-6.8	-6.4
014	-2.5	-3.9	-8.0	-8.5	-10.9	-9.7
015	0.9	0.7	-4.5	-4.4	-8.1	-9.7
Mean	0.5	0.7	-5.7	-5.6	-8.3	-8.5
SEM	0.4	0.5	0.4	0.4	0.3	0.3

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; BL, baseline; HX, hypoxia; SEM, standard error of the mean.

B.2 Individual data for arterial puncture at baseline

Table B.2-1 Partial pressure of arterial O_2 (PaO_2; mmHg)

ID	PBO	MZ	AZ
001	98.5	111.0	115.0
002	91.3	117.0	112.0
004	91.4	106.0	108.0
005	111.0	105.0	118.0
006	108.0	101.0	102.0
007	108.0	107.0	100.0
008	144.0	102.0	104.0
010	94.5	98.2	93.7
013	94.9	101.0	105.0
014	100.0	109.0	95.5
015	84.1	84.9	98.4
Mean	**102.3**	**103.8**	**104.7**
SEM	**4.4**	**2.3**	**2.2**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean.

Table B.2-2 Partial pressure of arterial CO_2 ($PaCO_2$; mmHg)

ID	PBO	MZ	AZ
001	38.1	30.9	29.0
002	38.5	34.1	29.2
004	40.4	34.8	30.6
005	42.7	36.4	33.4
006	36.3	34.8	34.5
007	37.2	33.4	33.3
008	31.8	31.3	32.9
010	40.0	35.0	32.8
013	40.5	32.9	35.0
014	33.8	27.7	29.3
015	39.8	36.1	32.2
Mean	**38.10**	**33.40**	**32.02**
SEM	**0.88**	**0.71**	**0.59**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean.

Table B.2-3 Hematocrit (Hct; %)

ID	PBO	MZ	AZ
001	43.9	45.5	45.4
002	46.6	45.5	49.2
004	44.4	45.4	43.5
005	47.9	49.5	49.2
006	44.9	48.9	51.5
007	44.9	44.6	45.0
008	41.6	42.2	46.6
010	48.6	47.9	47.9
013	48.2	44.3	46.6
014	46.8	50.1	50.2
015	47.2	48.9	48.5
Mean	**45.91**	**46.62**	**47.60**
SEM	**0.59**	**0.70**	**0.67**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean.

Table B.2-4 Hemoglobin (Hb; g/dl)

ID	PBO	MZ	AZ
001	14.3	14.8	14.8
002	15.2	14.9	16.1
004	14.5	14.8	14.2
005	15.6	16.1	16.1
006	14.6	16.0	16.8
007	14.7	14.6	14.7
008	13.6	13.8	15.2
010	15.9	15.6	15.6
013	15.7	14.5	15.2
014	15.3	16.3	16.4
015	15.4	15.9	15.8
Mean	**15.0**	**15.2**	**15.5**
SEM	**0.2**	**0.2**	**0.2**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean.

Table B.2-5 Bicarbonate (HCO_3^-; mmol/l)

ID	PBO	MZ	AZ
001	25.1	18.0	15.7
002	25.1	21.1	15.4
004	24.8	19.3	16.9
005	26.9	21.5	18.3
006	25.5	20.5	17.6
007	26.3	20.4	17.2
008	23.4	18.8	17.2
010	26.1	19.3	17.2
013	26.8	18.5	18.5
014	22.0	16.9	15.8
015	25.6	20.6	17.4
Mean	**25.24**	**19.54**	**17.02**
SEM	**0.40**	**0.39**	**0.28**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean.

Table B.2-6 Arterial oxyhemoglobin saturation (SaO_2; %)

ID	PBO	MZ	AZ
001	97.8	98.3	98.4
002	97.7	98.8	98.5
004	97.0	98.0	98.1
005	98.3	98.0	98.3
006	98.7	98.4	97.9
007	98.4	98.2	97.5
008	99.5	97.9	97.8
010	97.6	97.8	97.3
013	97.5	97.7	97.7
014	98.2	98.5	97.6
015	96.9	96.5	97.8
Mean	**97.96**	**98.01**	**97.90**
SEM	**0.21**	**0.16**	**0.11**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean.

Table B.2-7 Arterial pH

ID	PBO	MZ	AZ
001	7.43	7.37	7.34
002	7.42	7.40	7.33
004	7.40	7.35	7.35
005	7.41	7.38	7.35
006	7.46	7.38	7.32
007	7.46	7.40	7.32
008	7.48	7.39	7.33
010	7.42	7.35	7.33
013	7.43	7.36	7.33
014	7.42	7.39	7.34
015	7.42	7.37	7.34
Mean	**7.43**	**7.38**	**7.33**
SEM	**0.01**	**0.00**	**0.00**

Abbreviations: PBO, placebo; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean.

B.3 Raw arterial blood data from subset of subjects (n = 2)

Table B.3-1 Arterial blood data at baseline and hypoxia (Hb, pH, $PaCO_2$, PaO_2)

		Hb (g/dl)		pH		P_aCO_2 (mmHg)		PaO_2 (mmHg)	
ID	**Drug**	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
006	AZ	16.3	16.6	7.314	7.348	31.5	28.5	111.0	47.1
007	AZ	14.8	15.1	7.315	7.308	34.4	34.3	104.0	42.2

Abbreviations: Hb, hemoglobin; $PaCO_2$, partial pressure of arterial CO_2; P_aO_2, partial pressure of arterial O_2; BL, baseline; HX, hypoxia; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean

Table B.3-2 Arterial blood data at baseline and hypoxia (SaO_2, BE, HCO_3^-, Hct)

		SaO_2 (%)		BE (mEq/l)		HCO_3^- (mmol/l)		Hct (%)	
ID	**Drug**	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**	**BL**	**HX**
005	MZ	98.4	95.3	-4.6	-5.3	20.0	19.0	49.2	50.6
006	AZ	98.4	83.9	-9.2	-8.8	16.0	15.7	50.0	50.9
007	AZ	97.8	76.5	-7.9	-8.3	17.5	17.2	45.3	46.3

Abbreviations: Hb, hemoglobin; $PaCO_2$, partial pressure of arterial CO_2; PaO2, partial pressure of arterial O_2; BL, baseline; HX, hypoxia; MZ, methazolamide; AZ, acetazolamide; SEM, standard error of the mean