

The Effects of Carbonic Anhydrase Inhibitors on Exercise Performance in Acute Hypoxia

by

Jou-Chung Chang

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Acute mountain sickness (AMS) can occur due to rapid altitude ascents and/or insufficient acclimatization. Acetazolamide (AZ) is commonly prescribed for AMS prophylaxis but appears to inhibit exercise performance. Methazolamide (MZ) has similar prophylactic benefits but does not have a similar decrement in isolated small muscle mass exercise in normoxia. We compared whole-body exercise performance in acute hypoxia ($F_{I}O_2 = 0.15$) between AZ and MZ and hypothesized that time trial duration will be the shortest in MZ compared with AZ and placebo (PLA). Fifteen young healthy participants completed 5 testing visits: day 1 maximal exercise test, day 2 a familiarization visit, and Day 3-5 were the experimental visits. Each experimental visit involved a 5-km hypoxic cycling time trial performed after a 2-day dosing protocol of either AZ (250mg t.i.d.), MZ (100mg b.i.d.) or PL (t.i.d.); the order was randomized and double-blinded. Before and after each experimental time trial, capillary blood samples were taken, and maximal voluntary contractions of the quadriceps were performed. AZ and MZ resulted in a partially compensated metabolic acidosis at rest (capillary H^+ 47 ± 3 , 43 ± 2 , 39 ± 2 nmol for AZ, MZ and PLA respectively, $p < 0.01$). Time to complete 5-km on PLA (562 ± 32 s, $p < 0.01$) was significantly faster than AZ and MZ (577 ± 38 vs. 581 ± 37 s respectively), with no differences between AZ and MZ ($p = 0.96$). The 5-km average ventilatory efficiency ($\dot{V}_E/\dot{V}CO_2$) listed from greatest to least was MZ, AZ and PLA (46 ± 6 , 43 ± 4 , 37 ± 3 respectively) and were all significantly different ($p < 0.05$). There were no differences in the average ventilation (124 ± 27 , 127 ± 24 , 127 ± 19 L/min respectively) and oxyhemoglobin saturation (87 ± 2 , 88 ± 2 , 88 ± 3 respectively) between PLA, AZ and MZ ($p > 0.05$). Peak quadricep torque before exercise was found to be significantly lower in AZ compared to PLA and MZ (543 ± 77 , 574 ± 76 , 552 ± 67 N respectively, $p < 0.05$). In conclusion, both AZ and MZ impaired whole-body exercise

performance in acute hypoxia and this finding might be important to consider for high altitude occupations.

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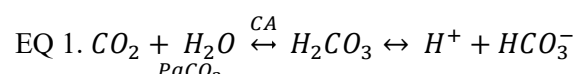
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List of Abbreviations

AZ	Acetazolamide
AMS	Acute mountain sickness
AaDO ₂	Alveolar-arterial oxygen difference
P _A CO ₂	Alveolar partial pressure of carbon dioxide
ANOVA	Analysis of variance
C _a O ₂	Arterial oxygen content
P _a O ₂	Arterial partial pressure of oxygen
P _a CO ₂	Arterial partial pressure of carbon dioxide
HCO ₃	Bicarbonate
BTPS	Body temperature pressure saturated
F _b	Breathing frequency
CO ₂	Carbon dioxide
\dot{V} CO ₂	Carbon dioxide output
CA	Carbonic anhydrase
Q	Cardiac output
EMG	Electromyography
P _{ET} CO ₂	End tidal partial pressure of carbon dioxide
P _{ET} O ₂	End tidal partial pressure of oxygen
FVC	Forced expired capacity
FEV ₁	Forced expired volume in 1 second
F _I O ₂	Fraction of inspired oxygen
hN	Handgrip torque
HR	Heart rate
Hct	Hematocrit
H ⁺	Hydrogen ions
HPV	Hypoxic pulmonary vasoconstriction
HVD	Hypoxic ventilatory decline
HVR	Hypoxic ventilatory response
HR _{max}	Maximal heart rate
MVC	Maximal voluntary contraction
MZ	Methazolamide
O ₂	Oxygen
\dot{V} O ₂	Oxygen uptake
S _p O ₂	Oxyhemoglobin saturation
PCO ₂	Partial pressure of carbon dioxide in the blood
PO ₂	Partial pressure of oxygen
\dot{V} O _{2peak}	Peak oxygen consumption
PO _{peak}	Peak power output
RPE-B	Perceived dyspnea
RPE-L	Perceived leg discomfort
PLA	Placebo
PV	Plasma volume
PAP	Pulmonary artery pressure
qN	Quadriceps torque
RPE	Rate of perceived exertion

RER	Respiratory exchange ratio
STPD	Standard pressure temperature dry
TT	Time trial
V_T	Tidal volume
\dot{V}_E	Ventilation
\dot{V}_A/\dot{Q}	Ventilation-perfusion mismatch

List of Equations



$$\text{EQ 2. } V_A = \frac{Pa\text{CO}_2}{V\text{CO}_2} \times k$$

1.0 LITERATURE REVIEW

As mountain tourism grows, the incidence of acute mountain sickness (AMS) will increase, due to rapid ascents and inadequate acclimatization to altitude (1, 2). The physiological challenge of an altitude ascent is physical exertion in a low oxygen (hypoxia) environment. The hypoxic stimuli itself challenges the cardiorespiratory system and limits one's ability to perform physical activity. The presence of AMS symptoms and insufficient acclimatization to altitude can further exacerbate the decline in exercise performance in such environments. Among humans, acetazolamide (AZ) is commonly prescribed for AMS prophylaxis and accelerating acclimatization to hypoxia. While these benefits can theoretically help with exercise performance in hypoxia by reducing AMS symptoms, AZ has been shown to provide inconclusive results, likely due to fatigue-inducing effects independent of the prophylactic pathway. Methazolamide (MZ) is a more potent analog of AZ. Unlike AZ, MZ is theorized to improve performance in hypoxia as it does not induce muscle fatigue. Thus far, a direct comparison of AZ and MZ on whole-body exercise performance in hypoxia have not been made, which will provide insight on factors impacting exercise performance and help determine whether MZ is a practical alternative for individuals rapidly ascending to altitude.

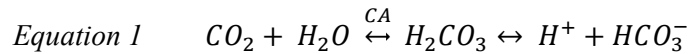
1.1 Resting Physiological Responses to Hypoxia

1.1.1 Respiratory Responses to Hypoxia

Hypoxia is defined as a reduction in the inspired partial pressure of oxygen (PO_2), which results in a decrease in arterial PO_2 . The drop in arterial PO_2 , termed arterial hypoxemia, is sensed by peripheral chemoreceptors found in the carotid and aortic bodies, which relays afferent feedback to the respiratory centers to increase ventilatory drive (3). The increased ventilation (\dot{V}_E) that occurs within seconds of hypoxic exposure is known as the hypoxic ventilatory response (HVR).

The increased \dot{V}_E improves arterial PO_2 and oxyhemoglobin saturation (S_pO_2), but lowers arterial carbon dioxide tension (PCO_2). Carbon dioxide plays an important role in blood pH regulation

through the reversible reaction, rapidly catalyzed by carbonic anhydrase (CA), that converts water and CO₂ to bicarbonate (HCO₃⁻) and hydrogen ions (H⁺) (Equation 1 below).



As a consequence of decreased arterial PCO₂, blood pH increases and blunts the initially higher hypoxic ventilatory drive, secondary to reduced stimulation of central chemoreceptors in the brainstem (4, 5). The decrease in \dot{V}_E , termed hypoxic ventilatory decline (HVD), occurs after 5-20 minutes of hypoxia and attenuates the initial rise in arterial PO₂ from HVR (6). The decline in \dot{V}_E is also present with isocapnic hypoxia, which indicates a mechanism that is independent of changes in arterial PCO₂, potentially due to desensitization of carotid chemoreceptor stimulation (7). With continued exposure, HVD can persist up to 8 weeks in humans, but \dot{V}_E will slowly increase over the course of 14 days due to acclimatization (6). With acclimatization, respiratory alkalosis and its suppression of \dot{V}_E is partially corrected by renal HCO₃⁻ excretion. Increased excretion of HCO₃⁻ results in greater H⁺ concentrations in the blood and tissues, which promotes stimulation of central chemoreceptors, thus improving arterial PO₂ and arterial oxygen content (C_aO₂). The time-dependent increase in \dot{V}_E response with acclimatization can also be attributed to increased O₂ sensitivity of the carotid chemoreceptors (8) and CO₂ sensitivity of central chemoreceptors (9).

The ventilatory response is also altitude dependent due to changes in PO₂. When inspired PO₂ drops below 100 mmHg or when arterial PO₂ falls below 50 mmHg, which is approximately 3000m in elevation, HVR will be stimulated (10). Above this altitude is where the greatest difference in alveolar gases between acclimatized and unacclimatized individuals was observed (11). According to the alveolar gas equation (Equation 2), increased \dot{V}_E would cause a subsequent fall in alveolar PCO₂ for a given level of $\dot{V}\text{CO}_2$.

$$\text{Equation 2} \quad V_A = \frac{P_a\text{CO}_2}{V\text{CO}_2} \times k$$

The drop in alveolar PCO_2 will then translate to a decrease in arterial PCO_2 . Upon exceeding 5400m, arterial PCO_2 will start to decrease linearly due to further increases in \dot{V}_E ; as a result, complete acclimatization is not possible beyond this point (11).

1.1.2 Cardiovascular Responses to Hypoxia

Upon immediate exposure to hypoxia, heart rate (HR) will increase (12); the extent of increase is proportional to the decrease in arterial PO_2 (13). Such rise in HR can be attributed to changes in the autonomic nervous system, specifically both an increase in sympathetic activity and parasympathetic withdrawal (14, 15). Stimulation of carotid chemoreceptors increases sympathetic outflow through the release of catecholamines (16, 17); which influences cardiac function and peripheral vasculature. In the heart, increased HR and inotropy (18) leads to an increase in cardiac output (\dot{Q}). Within the periphery, increased muscle sympathetic nerve activity with hypoxic exposure promotes systemic vasoconstriction (16). However, hypoxia causes dilation at the local tissue level, which counteracts the increase in global sympathetic activity to maintain perfusion and oxygen delivery of peripheral tissues (19).

The second mechanism to increase HR is through parasympathetic withdrawal. Parasympathetic blockade via atropine promotes increases in HR (14, 15), restoring maximal exercise HR in hypoxia (20). Unlike the systemic sympathetic response, parasympathetic outflow is limited to influencing the activity of pacemaker cells in the heart, thus affecting HR and not peripheral vasculature. In acclimatized individuals, the sustained rise in HR is attributed more to parasympathetic withdrawal than increased sympathetic outflow in comparison to activity done at sea level (21).

In attempt to maintain C_aO_2 and O_2 transport during decreased ambient PO_2 , \dot{Q} will increase with hypoxic exposure (22). When alveolar PO_2 is roughly around 70 mmHg, pulmonary blood flow and vascular resistance will begin to change (23). An increase in both pulmonary vascular resistance

and \dot{Q} will lead to an increase in pulmonary artery pressure (PAP), which is an indicator of hypoxic pulmonary vasoconstriction (HPV). Right ventricular afterload can increase with HPV, thus impairment of stroke volume (SV) can be expected. However, SV was found to be unaffected following improvements in HPV (24, 25). With SV remains unchanged during acute hypoxia, the increase in \dot{Q} is primarily due to changes in HR (26). As myocardial blood flow is dependent on the diastolic phase of cardiac cycle, the hypoxia-induced increase in HR reduces filling time and perfusion of the myocardium. However, at the level of the heart, hypoxia promotes vasodilation of the coronary arteries, which is beneficial in maintaining myocardial O_2 delivery during reduced ambient PO_2 (27, 28).

The acclimatization process to altitude consists of multiple physiological adjustments (11). Upon initial exposure, hypoxia-inducible factors will be released at the level of the cells, which stimulates synthesis of erythropoietin (EPO), and subsequently increase erythrocyte production. Since synthesis of cells takes time after increased EPO levels, hematocrit (Hct) only rises after a few days of continued hypoxia until it plateaus around 14 days. The second adjustment is diuresis, which allows excretion of HCO_3^- to adjust blood pH as mentioned previously. Diuresis can lead to a decrease in plasma volume (PV), resulting in SV decreases due to lower filling pressure. While PV expansion can restore SV, declines in SV cannot be reversed at elevations above 5000 m (29). Around 7 days, changes in PV and circulating catecholamine levels will stabilize, leading to the peak increase in \dot{Q} . Cardiac output will then fall afterwards due to hematological adjustments that maintain O_2 delivery, namely increased O_2 carrying capacity from EPO stimulation. As both renal excretion of HCO_3^- and RBC synthesis are not immediate responses to hypoxia, a normal healthy individual requires considerable time to sufficiently acclimatize to hypoxia, generally days to weeks depending on altitude.

1.2 Exercise in Acute Hypoxia

1.2.1 Increases in Ventilation and Associated Cardiovascular Effects

For a given level of exercise intensity or $\dot{V}O_2$, hypoxia will lead to significantly greater \dot{V}_E compared to normoxic conditions, as an attempt to meet oxygen demands of exercise when ambient PO_2 is reduced. However, the adjustment in \dot{V}_E does not completely compensate as arterial PO_2 and S_pO_2 are still lower than normoxia. In addition, the increase in \dot{V}_E may not be solely due to decreased PO_2 . With administration of 100% O_2 at 5800m, \dot{V}_E was still significantly greater than at sea level (36); which points to a possible O_2 -independent mechanism for the increase in \dot{V}_E during hypoxia. In hyperoxia, \dot{V}_E would be driven by acidosis at the level of central chemoreceptors.

Despite lower gas density at high altitudes, the work of breathing is greater for a given workload (37–39). Consequently, increased work of breathing induces the respiratory metaboreflex, where more blood flow are distributed to the respiratory muscles, and less to the peripheral exercising muscles (40). The effects of the respiratory metaboreflex are most prominent at intensities where \dot{Q} is limited; specific to hypoxia, this will be altitude dependent due to the relationship between ambient PO_2 and HVR as detailed previously. During whole-body hypoxic exercise, muscle blood flow can decrease by 20-30% due to the metaboreflex and the consequential increase in peripheral vascular resistance (41).

1.2.2 Gas Exchange Impairments

Gas exchange plays an important role in exercise performance as it directly affects O_2 uptake at the level of the lungs and peripheral tissues. Pulmonary gas exchange is indicated by the alveolar-arterial O_2 difference ($AaDO_2$), and has subsequent influence on arterial PO_2 and S_pO_2 ; both of which are found to decrease with hypoxic exercise (42). The factors contributing to $AaDO_2$ include ventilation-perfusion (\dot{V}_A/\dot{Q}) mismatch and diffusion capacity. The difference between alveolar-

arterial PO_2 widens with exercise intensity and increasing altitude, thus hypoxic exercise can lead to significant gas exchange impairments. As such, $AaDO_2$ corresponds with the decline of $\dot{V}O_{2max}$ in acute exposures to severe altitude (43).

The contribution of \dot{V}_A/Q inequality and diffusion capacity to gas exchange impairments varies by altitude and exercise intensity (42, 44). At rest, altitudes above 3000m show improved gas exchange compared to sea level and lower altitudes (42); such improvements can be attributed to better equilibration of \dot{V}_A/Q . When exposed to these altitudes, reduced arterial PCO_2 and respiratory alkalosis via hyperventilation results in a leftward shift of the oxyhemoglobin dissociation curve, increasing O_2 affinity for a given arterial PO_2 (44). With exercise at sea level, $AaDO_2$ progressively worsens with increasing workload (45), and the greatest $AaDO_2$ are observed in highly trained athletes that are capable of higher exercise intensities (34, 44). During normoxic exercise, impairments in gas exchange are mostly attributed to \dot{V}_A/\dot{Q} inequality and less to diffusion limitation until (near) maximal exercise intensities.

As one ascends to higher altitudes, $AaDO_2$ widens at lower exercise intensities. In terms of diffusion limitations, the specific combination of altitude and exercise intensity that brings significant changes to C_aO_2 largely depends on where arterial PO_2 is situated on the O_2 dissociation curve. When arterial PO_2 falls below 60 mmHg, it is situated on the steep portion of the curve, which is where diffusion limitation are more likely to arise due to impaired O_2 transport (44). Conversely, if arterial PO_2 falls on the flat portion of the curve, diffusion limitations can be present without changes in C_aO_2 (42).

Additionally, the rate of altitude ascent has a profound effect on \dot{V}_A/Q inequality (42). Immediately after a rapid ascent, resting \dot{V}_A/Q mismatch was significantly greater compared to sea level, while \dot{V}_A/Q mismatch after spending days at a given altitude was like that of sea level. Furthermore, chronic hypoxia was found to alter the pattern of \dot{V}_A/\dot{Q} inequality, where more

unventilated (i.e., shunts) and poorly ventilated regions develop in the lungs. As a result, \dot{V}_A/Q mismatch observed at a given altitude cannot be reversed with administration of 100% O₂ (42); pulmonary interstitial edema was postulated to be the cause of irreversible \dot{V}_A/\dot{Q} inequality with chronic hypoxia.

The reduction of inspired PO₂ and widening of AaDO₂ with exercise translates to a reduced pressure gradient, thus affecting driving pressure for O₂ diffusion at level of the tissue. While the leftward shift of oxyhemoglobin dissociation curve benefits pulmonary gas exchange, the increased O₂ affinity further impacts peripheral O₂ uptake. As O₂ molecules are more tightly bound to hemoglobin, O₂ unloading is impaired. Furthermore, decreases in O₂ affinity with administration of methylpropionic acid was associated with increases in $\dot{V}O_{2\max}$ (46). Overall, exercise intensity and altitude independently worsen gas exchange through changes in V_A/Q inequality and diffusion limitations. Thus, decrements in exercise performance with hypoxia can be attributed to impaired gas exchange in the lungs and peripheral tissues.

1.2.3 Potential Factors Influencing Exercise Performance

For a given increase in workload, the rate of HR increase is significantly slower during hypoxia than normoxia (32). With exercise at sea level, HR normally increases primarily through parasympathetic withdrawal at low workloads (47), and a combination of parasympathetic withdrawal and sympathetic stimulation at higher workloads (48). As mentioned, HR increases with acute hypoxia due to increased sympathetic outflow. Although HR is significantly greater at a given workload of exercise, changes in \dot{Q} in hypoxic exercise closely follow that of sea levels (49), implying a decrease in SV. However, $\dot{V}O_{2\max}$ and exercise capacity in hypoxia are unaffected by SV changes via PV expansion (29).

Hemoglobin concentration is a major determinant of oxygen carrying capacity, which is a component of $\dot{V}O_2$. Increased hemoglobin concentration is expected to increase exercise capacity;

however, it was reported to not prevent the hypoxic decline in $\dot{V}O_{2\max}$. Despite improving arterial oxygenation, hemoglobin injections did not affect $\dot{V}O_{2\max}$ (12, 50, 51). Similar results can also be observed with hemodilution studies, where normalizing red blood cell volume to sea levels did not increase $\dot{V}O_{2\max}$ to sea level values (52).

Hypoxic pulmonary vasoconstriction can influence cardiac function by increasing PAP and thus right ventricular afterload. Normally, HPV helps with maintaining gas exchange efficiency by reducing blood flow to poorly ventilated areas in the lungs. With systemic hypoxia, HPV leads to extensive vasoconstriction in the pulmonary vasculature (53), thus increasing PAP for a given work load at altitude (54). Increased PAP is expected to impair exercise capacity due to its influence on right and left ventricle performance (55), but the results are not inconclusive (56, 57). As mentioned previously, SV is unaffected by changes in HPV, and increases in SV do not lead to improved exercise capacity in hypoxia (29). While decreasing pulmonary vascular resistance was found to increase maximum workload and \dot{Q} (58) and restore $\dot{V}O_{2\max}$ (59), exercise performance was unaffected by changes in PAP (60). Furthermore, administration of vasodilators did not restore maximal power output to that of sea level values (61).

Fatigue is known to be increased with hypoxic exposure (62, 63), thus potentially affecting exercise performance (37), however the exact mechanism is unclear. Peripheral fatigue is defined as reduced excitability of the muscle upon stimulation, and central fatigue as a decrease in voluntary activation (63). Despite no differences in end-exercise peripheral fatigue between normoxic and hypoxic time to exhaustion protocols, performance was significantly reduced with hypoxia (63). Central fatigue was hypothesized to be a significant contributor to the performance decline, as decreases in cerebral O_2 delivery correlated with reductions in cortical voluntary activation (64, 65). Despite improved cerebral blood flow and oxygenation with supplemental O_2 , peak work rate was still significantly lower compared to normoxia (66).

Substrate utilization can play a role in exercise performance as it can determine whether the exercise intensity is sustainable or not. Specifically, one of the primary fuels used in oxidative phosphorylation for ATP production is lipids, whereas carbohydrates are primarily utilized in glycolysis and produce fatigue-inducing metabolites. Hypoxia has been found to influence the contribution of substrates being used during exercise, in that more carbohydrates are utilized for a given relative exercise intensity (50% peak $\dot{V}O_2$) compared to normoxia (67). The shift to carbohydrate-dominant usage is evidenced by increased respiratory exchange ratio (RER) and plasma catecholamines and reduced free fatty acids. These findings suggest an explanation for the decline in performance, as certain glycolytic byproducts are associated with fatigue. However, the change in substrate utilization with hypoxia was confounded by nutritional status and exercise intensity. When participants avoided breakfast prior to testing, the hypoxic condition demonstrated significantly lower contribution of carbohydrate oxidation compared to normoxia for a given relative exercise intensity (68). On the contrary, these differences are not seen after breakfast consumption. (69)

1.3 Effects of Carbonic Anhydrase Inhibition

The most common method of AMS prophylaxis is the administration of carbonic anhydrase inhibitors. Carbonic anhydrase (CA) is an enzyme that catalyzes the reversible conversion of CO_2 and water to hydrogen and bicarbonate ions (equation 1), which is important for blood pH homeostasis. In the peripheral circulation, bicarbonate ions act as a buffer to maintain blood pH (70) and transport of CO_2 . In the pulmonary circulation, bicarbonate is dehydrated into water and CO_2 by CA, and CO_2 consequently is expired through ventilation (71). In the renal circulation, bicarbonate is also converted to CO_2 and water by CA to allow diffusion into renal tubular cells, and then reabsorbed back into the renal venous blood (72).

At sea level, blood pH homeostasis is maintained in a healthy individual by complete reabsorption and retainment of bicarbonate in the circulation (70). As one ascends to high altitudes, the respiratory alkalosis due to HVR will be compensated by renal excretion of bicarbonate. Intake of a CA inhibitor expedites this pH adjustment by inducing bicarbonate excretion and inhibiting its reabsorption. The subsequent metabolic acidosis offsets the respiratory alkalosis from HVR (70, 73), which restores blood pH to stimulate chemoreceptors, thus increasing alveolar and arterial oxygenation via hyperventilation (74, 75). The improvement in arterial PO_2 and S_pO_2 is hypothesized to be the primary mechanism of AMS prophylaxis by CA inhibition (75, 76) (Figure 1).

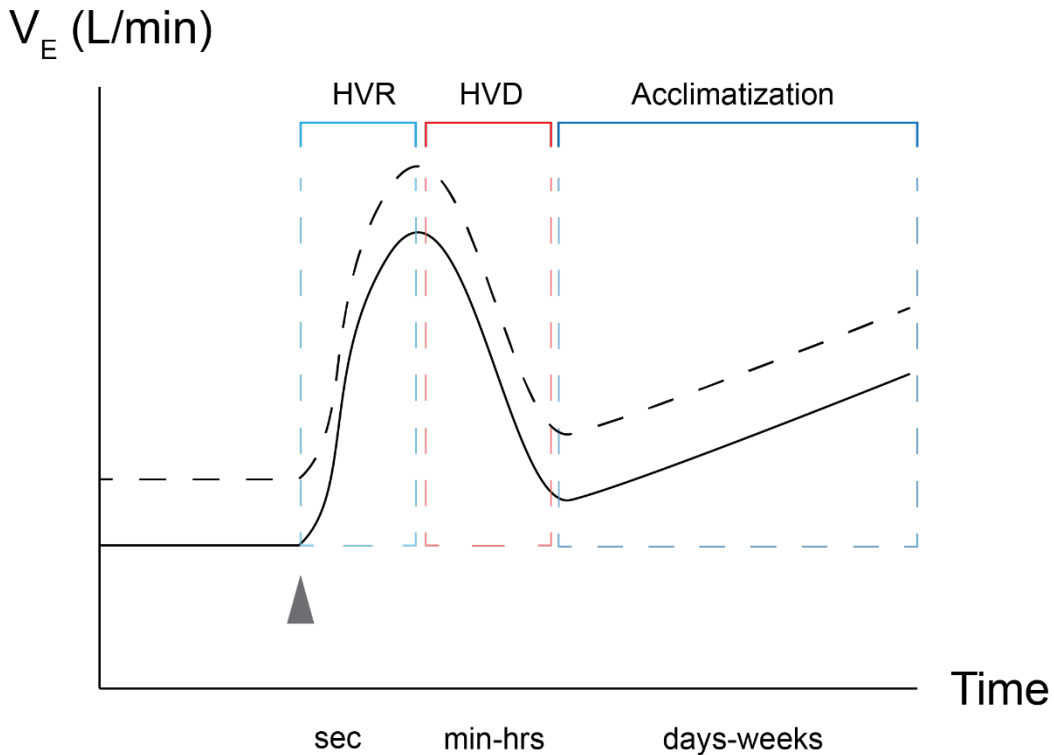


Figure 1: Time course of normal ventilatory response to hypoxia (solid line) and the associated effects of carbonic anhydrase inhibition (dashed line).

1.3.1 Differences in Dosing of Carbonic Anhydrase Inhibitors

The dose of CA inhibitors elicits varying physiological effects. At a low dose of AZ (>5 mg/kg), CA in the renal tissues will be inhibited (77); the mild metabolic acidosis is sufficient to counteract respiratory alkalosis without undesirable effects. At a higher dose (7-12 mg/kg), AZ can inhibit CA in erythrocytes and cross the blood-brain barrier, thus affecting the central nervous system. The higher level of CA inhibition can impede CO₂ transport and cause CO₂ retention, which can negatively impact exercise performance. Numerous studies have reported a significantly lower $\dot{V}CO_2$ with CA inhibition, but CO₂ retention in the tissues was not quantified nor measured (78–82).

1.4 Effects of Acetazolamide on Hypoxic Exercise Performance

While AZ negatively affects exercise performance in normoxia, the results are inconclusive with hypoxic exercise (83). Exercise performance is multifaceted (Figure 2) and can be influenced by physiological as well as psychological factors. In particular, perceived exertion during hypoxic exercise can be negatively influenced by the presence of AMS symptoms, such as headache, dizziness, and overall fatigue (1). Acetazolamide could potentially improve performance in hypoxia due to attenuation of AMS symptoms, rather than through physiological or biochemical alterations of performance *per se*. However, at a given exercise intensity in hypoxia, perceived leg discomfort increases with AZ compared to placebo (84). Thus, while AMS symptoms may be lessened, other exercise-limiting symptoms may be exacerbated with AZ.

Hypoxic exposure alone increases \dot{V}_E in the attempt to raise CaO_2 , but AZ causes a further 10-20% increase in \dot{V}_E compared to placebo, which can further increase $S_p\text{O}_2$ (84–89). Likewise, in instances of no \dot{V}_E increases with AZ intake, there were also no changes in $S_p\text{O}_2$ (82). While the relationship between \dot{V}_E and $S_p\text{O}_2$ are consistent across most studies on AZ, their impact on performance outcomes is more variable (Figure 3).

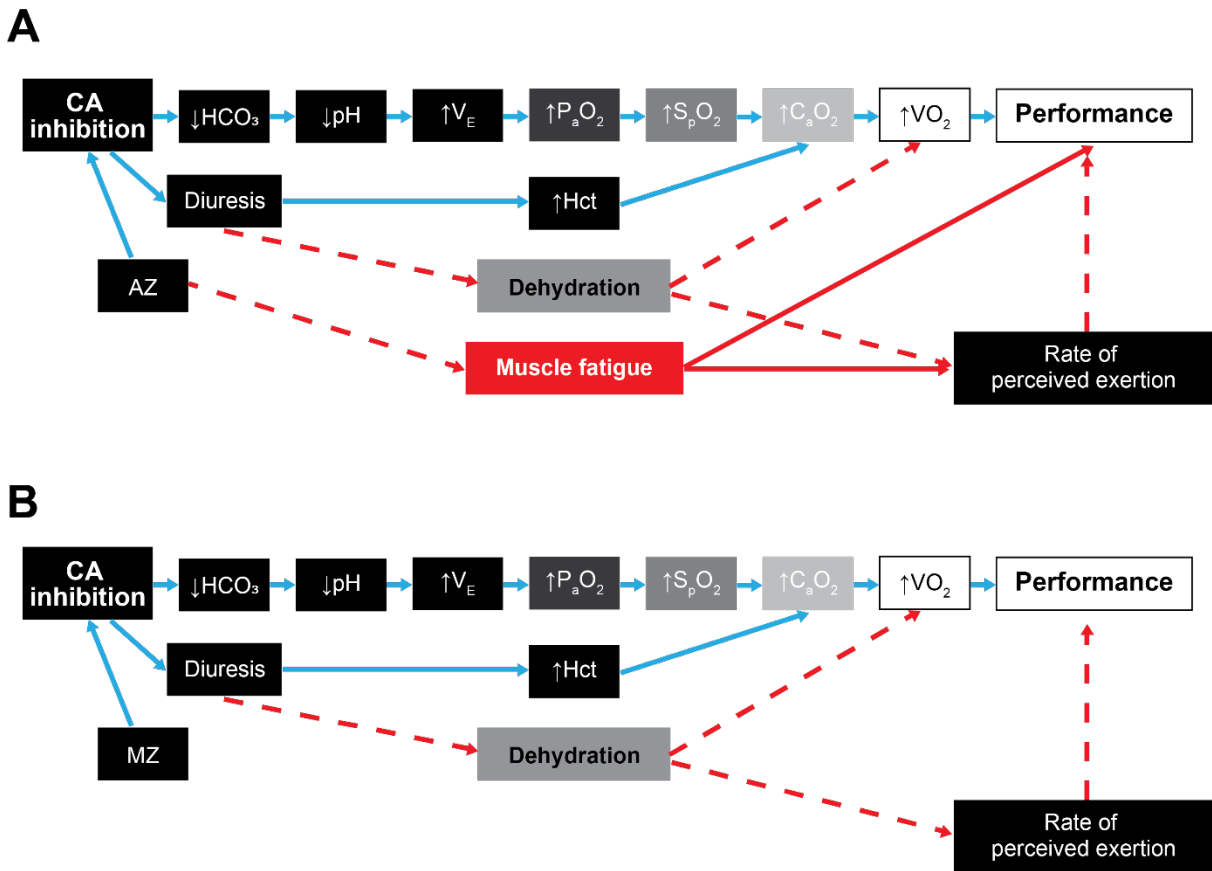


Figure 2: Pathways which carbonic anhydrase inhibitors can impact exercise performance. Panel A: acetazolamide (AZ). Panel B: methazolamide (MZ). The solid arrows represent direct relationships, while dashed arrows represent indirect relationships. The opaqueness of the textbox indicates the strength of the finding (i.e., black = observed in majority of studies; white = observed in few studies).

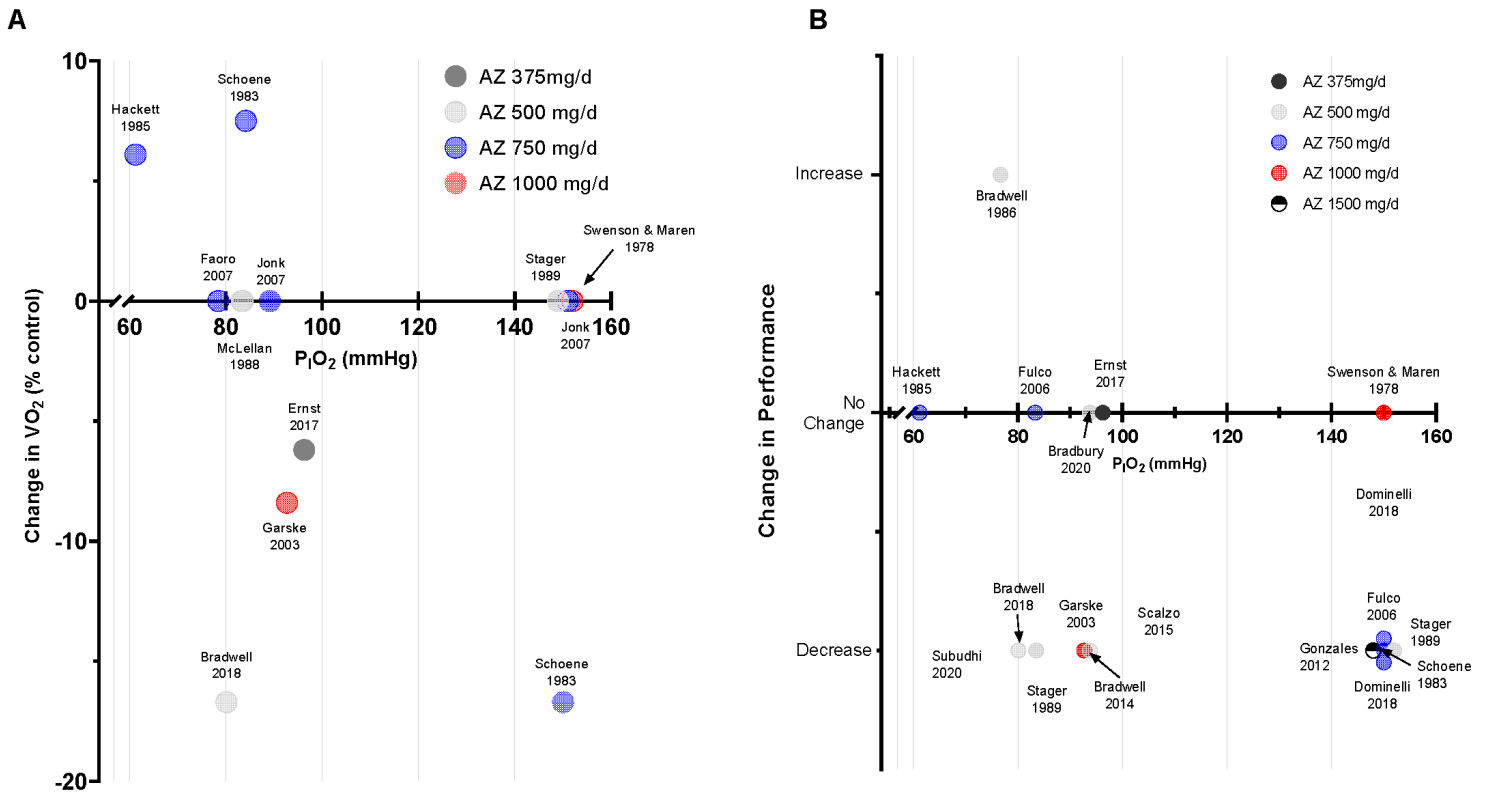


Figure 3: Summary of studies assessing the effect of acetazolamide on maximal oxygen uptake ($\dot{V}O_{2max}$) and performance outcomes (i.e. Time to exhaustion, exercise duration, maximal power output). Panel A: The y-axis represents the change in $\dot{V}O_{2max}$. Panel B: The y-axis represents the change in performance in a binary fashion (i.e. increased or decreased). AZ, acetazolamide; P_iO_2 , partial pressure of inspired oxygen; $\dot{V}O_2$, oxygen uptake. (83)

1.4.1 Influences on Ventilation and Oxygen Uptake in Hypoxic Exercise

It is hypothesized that CA inhibitors are beneficial to exercise performance in hypoxia due to increased \dot{V}_E , improved pulmonary gas exchange and arterial oxygenation (88). While increased \dot{V}_E can increase $\dot{V}O_2$ by raising S_pO_2 and C_aO_2 , this is not a direct relationship (Figure 3a). Despite higher peak \dot{V}_E and S_pO_2 during maximal exercise, the AZ group can show no significant differences in $\dot{V}O_{2max}$ (85, 87, 88, 90), an increase in $\dot{V}O_{2max}$ (7.5%) (89), or a decrease (8.7%) (84). The improvement in $\dot{V}O_2$ could be attributed to greater increases in C_aO_2 for a given \dot{V}_E increase, due to the shape and steeper slope of the oxyhemoglobin dissociation curve. For instance, at an inspired PO_2

of 84 mmHg, resting S_pO_2 is around 66% (89), while it is 79% at an inspired PO_2 of 94 mmHg (85). At a S_pO_2 of 66%, arterial PO_2 falls on the steeper portion of the oxyhemoglobin dissociation curve. Due to greater oxyhemoglobin affinity, relatively minor increases in arterial PO_2 will have greater increases in C_aO_2 , thus improving $\dot{V}O_{2max}$ (89). In contrast, at a S_pO_2 of 79%, oxyhemoglobin affinity is closer to the flat portion of the curve, thus a similar change in arterial PO_2 brings about smaller improvements in C_aO_2 , which may result in no difference in $\dot{V}O_{2max}$ (85). As mentioned, higher altitudes will lead to a lower arterial PCO_2 and higher pH from hyperventilation, causing a relative left-shift on the oxyhemoglobin dissociation curve. Thus, increases in \dot{V}_E with AZ may lead to a further leftward shift and increase binding affinity of O_2 to hemoglobin, which is to the benefit of O_2 loading in the lungs, but at the cost of O_2 unloading in the tissues.

At submaximal intensities, many studies observe an increase in \dot{V}_E and S_pO_2 at any given work rate with AZ intake. It was expected that $\dot{V}O_2$ will increase consequently due to greater O_2 loading, but submaximal $\dot{V}O_2$ did not differ from placebo (81, 84, 86, 88). In part, this may be attributed to partial red blood cell CA inhibition, which yields lesser O_2 unloading during capillary transit (91). Similarly, at the level of the active muscles, there are no differences in leg $\dot{V}O_2$ despite a 10% increase in \dot{V}_E for a given submaximal workload (88). These results further suggest that increases in \dot{V}_E arising from CA inhibition do not translate to increases in $\dot{V}O_2$. Another potential explanation is that the magnitude of S_pO_2 increases is insufficient to improve $\dot{V}O_2$. Specifically, to affect $\dot{V}O_2$, there needs to be at least a ~3% change in S_pO_2 (92).

However, it is important to note that $\dot{V}O_{2max}$ is not the definitive predictor of exercise performance (Figure 3b). Despite no difference in time to exhaustion compared to placebo, $\dot{V}O_{2max}$ can increase by 7.5% (89) or decrease by 6% (93) with AZ intake. Conversely, with no significant changes in $\dot{V}O_{2max}$, time to exhaustion can decrease by 26% (82) or remain unchanged (85). A possible explanation for changes in $\dot{V}O_{2max}$ but similar performance outcome may be a result of

respiratory metaboreflex (92), where higher \dot{V}_E requires greater blood flow and total O_2 delivery to respiratory muscles, and consequently decreased O_2 delivery to locomotor muscles. At a given submaximal exercise intensity where there is no change in $\dot{V}O_2$, increased \dot{V}_E coincides with reduced time to exhaustion (82, 86). The findings support the hypothesis that increased \dot{V}_E , and thus respiratory metaboreflex, can decrease whole-body exercise performance. However, leg $\dot{V}O_2$ and blood flow were observed to be similar during exercise with AZ compared to placebo (88), which suggests that the increased \dot{V}_E with AZ is insufficient to affect blood flow to locomotor muscles. The disparity between $\dot{V}O_2$ and performance can be partially attributed to the dissociation between oxygenation and performance. Although all participants taking AZ showed an increase in resting S_pO_2 , ~56% of participants were unable to complete the exercise protocol (79). Thus, while maximal oxygen uptake is an important indicator of aerobic exercise capacity, these findings suggest that O_2 delivery and consumption cannot fully account for the changes in exercise performance at altitude caused by AZ (Figure 3).

In addition, while increased \dot{V}_E due to AZ is beneficial to arterial oxygenation, it can be a possible hindrance to exercise performance by increasing perceived dyspnea (87). However, this mechanism during exercise is not fully supported. During hypercapnic ventilatory response testing, AZ reduced dyspnea perception for a given \dot{V}_E compared to placebo (84). While AZ increased \dot{V}_E by 16% at a given workload, perceived dyspnea did not increase (84). The observed dissociation between perceived dyspnea and \dot{V}_E may be explained by AZ's ability to affect neural afferent transmission, as evidenced by greater reflex latencies but similar efferent conduction velocities with AZ intake (94). As perceived exertion is also attributed to afferent feedback from active muscles, impairing transmission from afferent receptors will reduce sensory information and alter perceived exertion. For instance, there were no differences in maximal \dot{V}_E and end-exercise dyspnea, there was a 10% decrease in exercise duration with AZ (84). Similarly, where there were no significant

differences in \dot{V}_E , time to exhaustion was reduced by 26% in AZ compared to placebo (82). Contrary to the original hypothesis, a drop in exercise performance can still be present without significant changes in \dot{V}_E and/or perceived dyspnea.

1.4.2 Mechanism of Muscle Fatigue

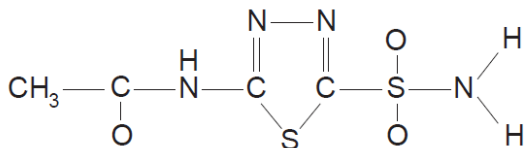
As decreased arterial oxygenation contributes to muscle fatigue in hypoxic exercise (95), CA inhibition may attenuate muscle fatigue development by improving arterial oxygenation through increased \dot{V}_E . However, AZ was found to induce muscle fatigue through other mechanisms. Muscle fatigue may be associated with changes in acid-base status induced by AZ. Carbonic anhydrase inhibition with AZ significantly lowers blood pH and plasma bicarbonate (HCO_3^-) concentration both at rest and during exercise (82, 86, 88, 96). Lower blood lactate concentrations with AZ intake can be explained by more intracellular retention of H^+ and lactate in the muscle (97). Inducing metabolic alkalosis with HCO_3^- supplementation was found to increase total work performed during supramaximal exercise (98). The greatest improvements in exercise performance with bicarbonate were observed in subjects with lower levels of proteins (i.e., monocarboxylate transporter -1, CA II, CA III and Na^+/H^+ exchanger) mediating muscle cell pH regulation and transport of lactate/protons, suggesting that acid-base balance in skeletal muscle plays a role in muscle fatigue (98). However, the extent of fatigue development is not proportional to reductions in blood pH (99). With increasing exercise intensity, greater amounts of metabolites, such as lactate and H^+ , are produced. While the accumulation of metabolites coincides with the onset of muscle fatigue, the rate of perceived exertion was not altered with changes in blood lactate (100). Likewise, there are no changes in $\dot{V}\text{O}_{2\text{max}}$ with AZ despite a 50% reduction in peak blood lactate (81). Altogether, acid-base status within the muscle appears to be an important contributor to muscle fatigue while the role of blood acid-base status remains unclear.

The next plausible mechanism for inducing muscle fatigue with AZ is the drug's influence on the big potassium (BK) channels (101). BK channels are located in T-tubules of skeletal myocytes and are integral to maintaining the electrochemical gradient and electrolyte homeostasis. The restoration of resting membrane potential in skeletal muscle is essential to initiation of a muscle contraction. If BK channels remain open after a depolarization event, such as a contraction, the probability of the subsequent depolarization reduces. As a result, increased BK channel activity decreases muscle force during continuous contractions (102). AZ is known to be an effective BK channel opener (101, 103), thus supporting the hypothesis that AZ causes muscle fatigue through alterations of the electrochemical gradient.

1.5 Types of Carbonic Anhydrase Inhibitors used in Humans

In humans, AZ is the most common CA inhibitor, and it is widely prescribed for prophylaxis of AMS symptoms and was extensively researched. Methazolamide (MZ) has a similar chemical structure to AZ, but has an additional methyl group on the thiadiazole ring, which increases its lipophilicity (72) (Figure 4).

acetazolamide



methazolamide

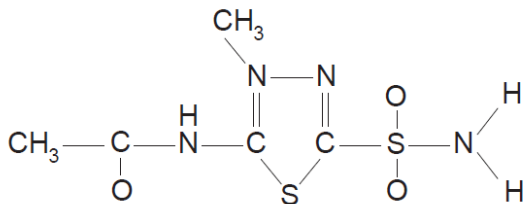


Figure 4: Chemical structure of acetazolamide and methazolamide.

Due to greater lipophilic properties, MZ has a longer half-life and greater potency than AZ (104), and also is more localized within the brain (105). With differences in potency, a relatively smaller dose of MZ is required to achieve the same level of CA inhibition as a given dose of AZ (e.g., 200-250 mg/day of MZ vs 500-750 mg/day of AZ).

1.6 Effects of Methazolamide on Hypoxic Exercise Performance

Compared to AZ, there is limited research on MZ and its effects on hypoxic exercise performance. A comparison between MZ, theophylline, and a combination of MZ and theophylline was made for their effects on 12.5km cycling time trials in normoxia and hypoxia (inspired PO_2 150 mmHg and 105 mmHg respectively) (106). Theophylline is a phosphodiesterase inhibitor and adenosine receptor blocker, and is suggested to improve exercise performance when combined with MZ (107). Between individual treatments of MZ and theophylline, there was no significant differences in the change in time trial performance from normoxia to hypoxia when compared to control. In the combined treatment of MZ and theophylline, the decrement in time trial performance was attenuated relative to control. The proposed mechanism is an increase in arterial oxygenation by MZ and systemic circulation via adenosine receptors by theophylline (74, 108). However, this study has certain limitations. No measurements of \dot{V}_E or blood hemoglobin concentrations were made, thus the mechanism for oxygenation improvement cannot be properly discerned. In addition, a randomized cross-over controlled trial design was not employed, which makes it difficult to attribute the observed changes to the effect of the drug intervention.

In a subsequent study, Subudhi et al. (109) implemented a repeated measures crossover design and assessed time trial performance in hypobaric hypoxia (inspired $PO_2 \sim 80$ mmHg) with and without combined MZ and theophylline. Contrary to the initial study, time trial performance decreased compared to control, despite significant increases in S_pO_2 . Increased fatigability and

reduced time trial performance were also reported, however the responsible mechanisms remain unclear as the individual effects of each drug were not investigated.

To discern the direct effects of AZ and MZ on respiratory and locomotor muscle performance, Dominelli et al., 2018 conducted a study investigating muscle function after a fatiguing single-muscle exercise protocol in normoxia. Participants either completed a dorsiflexion protocol or inspiratory resistance protocol while taking placebo, MZ or AZ. The decline in baseline muscle function was attenuated in MZ treatment compared to AZ. After exercise, AZ treatment induced significantly greater fatigue in tibialis anterior and respiratory muscles compared to MZ and placebo (110). Since the comparison was made in single-muscle and in normoxia, whether these effects translate to whole-body exercise performance in hypoxia is unknown. Since MZ does not affect BK channels like AZ (101, 103), MZ may improve exercise performance in hypoxia by improving arterial oxygenation without inducing muscle fatigue (Figure 2b).

2.0 STUDY RATIONAL

To reduce the occurrence and severity of AMS symptoms, AZ is commonly prescribed for prophylaxis. By inhibiting the CA enzyme, AZ augments HVR and accelerates the acclimatization process to altitude. While it is unquestionably beneficial for AMS prophylaxis (1), AZ has negative effects on exercise performance (82, 84, 110). As mountaineering and trekking involves physical exertion in hypoxic environments, such undesirable effects of AZ warrants investigation of an alternative. Methazolamide (MZ) is also efficacious in AMS prophylaxis but may not hinder exercise performance to a similar degree. However, there is a lack of prospective studies comparing these two CA inhibitors head-to-head with respect to exercise performance at high-altitude, thus making definitive conclusions difficult.

3.0 RESEARCH QUESTIONS AND HYPOTHESIS

Research Questions:

1. Does MZ negatively impact time trial performance in hypoxia to the same extent as AZ?

Hypothesis:

1. Time trial performance in acute hypoxia will be better in MZ compared to AZ and PLA.
 - a. AZ will have the slowest time to completion, while placebo will have the fastest time to completion.
 - b. Peak quadriceps torque will be the least in the AZ condition.
 - c. Ventilation will be greater in both AZ and MZ compared to PLA.
 - d. Blood pH will be lowest on AZ and MZ compared to PLA.

4.0 METHODS

4.1 Ethics

The experimental procedures were approved by the Office of Research Ethics at the University of Waterloo (ORE #43720), which adhered to the recommendations outlined by the *Declaration of Helsinki* concerning the use of human participants. In addition, they were also registered on the clinicaltrial.gov registry (NCT05575180), and a no-objection letter was received from Health Canada. All participants were informed of the experimental procedures, the potential risks involved and provided written informed consent. Table 1 outlines the specific inclusion and exclusion criteria for the participants.

Table 1: Participant inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
<ul style="list-style-type: none">• Age: 18-40 years• Active: 3+ days per week for total of 150 minutes per week• Endurance-trained: competed in an endurance event within the last 12 months.• Normal pulmonary function	<ul style="list-style-type: none">• Current smoker or cannabis user• Weight <49kg• Obesity: BMI >30kg/m²• Females: Pregnant, suspect to be pregnant, planning a pregnancy, or nursing• Allergies or hypersensitivities: latex, sulfonamides, CA inhibitors• Cardiovascular disease• Respiratory disease• Gastrointestinal disease• Diabetes• Asthma (includes exercise-induced)• Hypertension• Arthritis• Current use of Ionafarnib, Methenamine, Topomax, or steroids/corticosteroids• Hepatic or renal insufficiency, electrolyte imbalance, history of seizures• Bleeding/clotting disorders

4.2 Participants

Fifteen young healthy participants (14 male, 1 female) between ages 18-40 years old were recruited for the study; the majority (n = 13) were experienced cyclists, had more than one year of endurance training experience and regularly engaged in at least 150 min per week of moderate to vigorous aerobic exercise. Participants were excluded if they had symptoms of cardiovascular, respiratory, or metabolic diseases or were taking any medications that would affect the participants' responses to exercise and hypoxia. If the participants were prescribed with medications that may have potential interactions of CA inhibitors, they were advised to avoid the intake for the duration of the study. If participants were not able to stop taking their prescriptions, they were excluded from the study. All participants were screened for pulmonary function status and were included in the study if they demonstrated normal pulmonary function. Specifically, according to the American Thoracic Society (ATS) guidelines, forced expiratory ratio (FEV₁/FVC) was to be >80% of their predicted values, which were calculated from their age, height and sex (111). Only females that were on a highly effective contraceptive were recruited for the study and were tested during the active phase of their contraceptive.

Table 2: Summary of demographics, pulmonary function, and maximal cardiorespiratory values.

Age (years)		25±4
Height (cm)		176.5±7.1
Weight (kg)		74.2±10.8
Pre-exercise spirometry	FVC (L)	4.9±0.7
	FVC (% predicted)	90±13
	FEV ₁ (L)	4.1±0.6
	FEV ₁ (% predicted)	90±14
	FEV ₁ /FVC	0.8±0.1
	FEV ₁ /FVC (% predicted)	100±7
Post-exercise spirometry	FVC (L)	5.3±1.1
	FVC (% predicted)	98±20
	FEV ₁ (L)	4.5±1.0
	FEV ₁ (% predicted)	101±22
	FEV ₁ /FVC	0.9±0.7

	FEV ₁ /FVC (% predicted)	103±7
Maximal exercise values	HR (beats·min ⁻¹)	190±8
	V _T (L)	2.6±0.5
	V _T (% FVC)	50±7
	Fb (breaths·min ⁻¹)	59±11
	\dot{V}_E (L·min ⁻¹)	171±37
	$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	56±7
	$\dot{V}O_2$ (L·min ⁻¹)	4.15±0.72
	VCO ₂ (L·min ⁻¹)	5.02±0.83
	$\dot{V}_E/\dot{V}O_2$	41±5
	$\dot{V}_E/\dot{V}CO_2$	34±4
	RER	1.21±0.06
	PO _{peak} (W)	356±63
	S _p O ₂ (%)	95±2

FVC, forced expired capacity; FEV₁, forced expired volume in 1 second; HR, heart rate; V_T, tidal volume; Fb, breathing frequency; \dot{V}_E , minute ventilation; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; PO_{peak}, peak power output; S_pO₂, oxygen saturation. Mean±SD

4.3 Drug Administration

An independent pharmacy provided the medications compounded in Coni-Snap Clear vegetable capsules and microcrystalline cellulose as filler. Individual blister packages were randomized and assembled by the principal investigator, but not the researcher involved in data collection to ensure the double-blinded nature. Acetazolamide (250 mg per dose) was orally administered 3 times per day (every 8 hours), with the last dose 1 hour prior to testing visit. The recommended dose of AZ to treat altitude illness is 750 mg per day (112) and the half-life is about 8 hours. The MZ dose to be as effective as a clinical dose of AZ is about 200 mg per day (113), but the half-life is 12-15 hours. Thus, methazolamide (100 mg per dose) was administered twice a day but separated by a placebo capsule to ensure identical dosing schedule as AZ and placebo. The placebo capsules were filled with microcrystalline cellulose and taken 3 times a day for 2 days, similar to AZ. Overall, all capsules looked identical and were orally administered in an identical manner to ensure double blinding. A washout period of 10 days between drug conditions was enforced during the scheduling of experimental testing visits.

4.4 Experimental Overview

All participants completed five testing days (Figure 5) in an indoor laboratory (21 ± 1 °C, $34\pm 20\%$ relative humidity, 759 ± 6 mmHg). Day one consisted of screening, pulmonary function testing, and a maximal exercise test to determine cardiorespiratory fitness. Day two involved a 5-km familiarization trial in hypoxia, in which participants were familiarized with the equipment set up, instrumentation, testing protocol and the hypoxic condition. Day three, four and five made up the experimental protocol, where participants completed 3 separate time trials following a dosing protocol of AZ, MZ or placebo. A description of the procedures conducted on each day is detailed in the following sections.

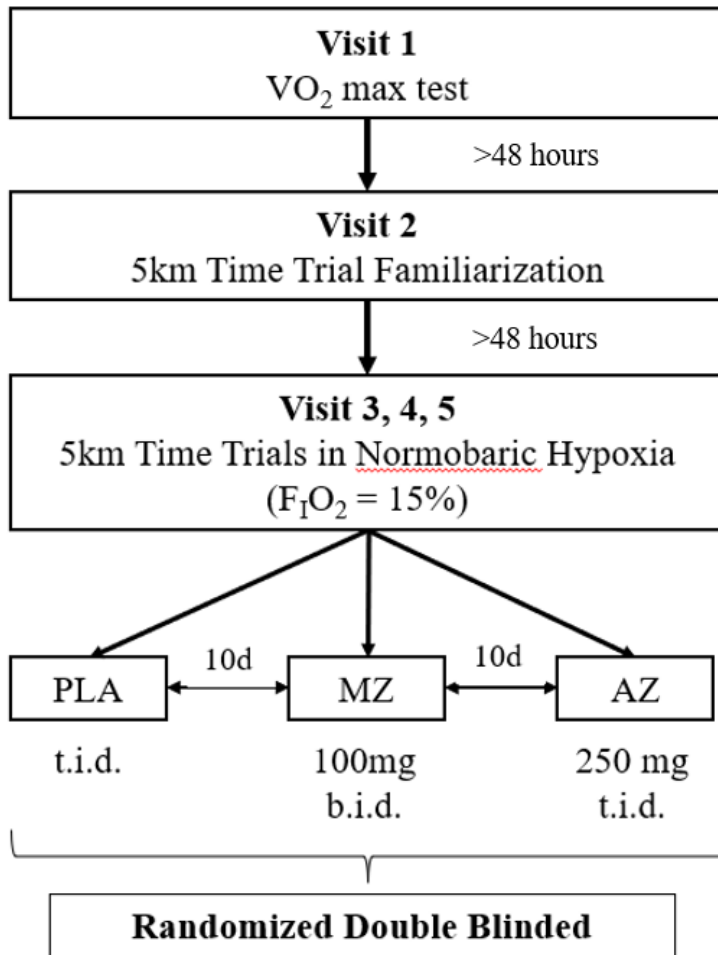


Figure 5: Schematic of overall study protocol. VO₂, oxygen uptake; TT, time trial; F_IO₂, fraction of inspired oxygen; AZ, acetazolamide; MZ, methazolamide; PLA, placebo; t.i.d, three times per day; b.i.d., twice per day.

4.4.1 Day One: Screening, and Maximal Exercise Test

Day one of testing involved a screening where informed consent was obtained. Prior to collection, bike seat and handle settings on the cycle ergometer (Monark, LC7TT) were adjusted according to the participants' preference, and noted for subsequent visits. Participants were then instrumented with a HR monitor, facemask, and pulse oximeter. After completing a 5-minute period of baseline rest on the cycle ergometer, participants performed a series of forced expired capacity

maneuvers to assess for pulmonary function, followed by a 5-minute self-selected warm up. Next, participants performed a ramp incremental exercise protocol, where the workrate increased by 30 watts (W) every minute; males began at 100W, and females at 80W. The testing protocol was terminated once the participants reached volitional fatigue and cannot maintain their cadence above 60 rpm.

4.4.2 Day Two: Familiarization to MVCs and Hypoxic Time Trial

Day 2 ensured that the participants were sufficiently familiarized with the equipment, neuromuscular tasks, time trial protocol and the hypoxic condition. All assessments performed on day 2 were repeated in an identical fashion on day 3,4 and 5. Participants were informed of the order of assessment, which were pre-exercise maximal voluntary contractions (MVCs), 5-km time trial, and post-exercise MVCs. For the MVCs, the participants were instrumented with electrodes for electromyography (EMG) on their left vastus lateralis (VL), which remained throughout the entire visit. Details of the MVC protocol are described within the “Neuromuscular Assessment” section.

Once the MVCs were completed, the participant moved onto the cycle ergometer. Except for an additional pulse oximeter, the same instrumentation and bike settings as day one were used. After instrumentation, the participant rested on the bike in normoxia for 5 minutes. Following baseline rest, participants performed a 2-minute self-paced warm up, where they were familiarized with the gear shift settings. Final adjustments to their seat height, handle height, and fan settings were made during this time, which were recorded to ensure all 3 subsequent visits were performed with identical settings.

Afterwards, the participants rested in hypoxia ($F_{I}O_2 = 15\%$) for 2 minutes, allowing end tidal PO_2 to stabilize, before commencing the time trial. Hypoxic gas from a compressed tank was passed through a humidification chamber before filling a 200L Douglas bag, which was connected to the

breathing circuit from the inspired side. During this time, the humidity of the gas was measured from the inspired end of the breathing circuit using a hygrometer (model 35519-050, VWR). After resting 2 minutes in hypoxia, participants began their time trial and were notified of when they completed each 1km increment; no other forms of active encouragement were given. More information of the time trial protocol is detailed in the “Time Trial Protocol” section.

Within 2.5-3 minutes post-exercise, the participants transitioned back to the MVC apparatus without an active cooldown and were instrumented to perform MVCs of the quadriceps muscle group and handgrip. Around 2.5 minutes after exercise, the participants performed their 3 sets of MVCs, in the same order as prior to the time trial. Once completed, participants were assessed for their perceived ratings of breathing discomfort (RPE-B) and leg discomfort (RPE-L) on the 0-10 Modified Borg scale; where 0 was “no discomfort” and 10 was “the most severe discomfort ever imagined or experienced” (114). Breathing discomfort was defined as “how heavy or laboured your breathing is”, and leg discomfort as “how tired your legs are”.

After completion of all the assessments, participants were given a self-report symptom checklist, a randomly assigned blister pack and a sports hydration drink. All were instructed when to consume the pills and recognize which symptoms were indicators to stop the intake.

4.4.3 Day Three, Four and Five: Experimental Protocol

Day three, four and five consisted of experimental trials, where a time trial visit followed completion of a drug arm, as summarized in Figure 6. Prior to each visit, participants would have taken seven oral capsules 8 hours apart with the last capsule taken 1 hour prior to reporting to the lab for testing. The participants also followed a standardized hydration protocol on testing day, which involved drinking a 250 mL sports drink at the same time as the last drug dose.

Prior to the MVCs, an arterialized capillary sample was collected from the participants to assess blood acid-base status; further details are provided in the “Arterialized Capillary Sample” sections. Participants then underwent an identical MVC and time trial protocol as day two. To ensure that the participant experienced the same hypoxic condition, a gas tank was designated for each participant and was used for all three experimental time trials.

Following the time trial and MVCs, a second arterialized capillary sample was taken, around 6-7 minutes post-exercise. After which, the rate of perceived exertion (RPE) was assessed, and other subjective information were recorded. Once participants were deinstrumented, they were given another assigned blister pack.

Between each time trial, there was a 10-day washout period to ensure that the effects of one drug arm would not affect results of subsequent trials. Since a minimum of 35 days was required to complete all the testing, the participants were instructed to maintain similar lifestyle patterns, specifically sleep duration, sleep quality and training volume especially during the last 3 visits. While these factors were not the main variables of the study, certain events can confound results of the experimental trial, thus some level of monitoring was warranted. Examples include travel stress and decrease in training load prior to a competition.

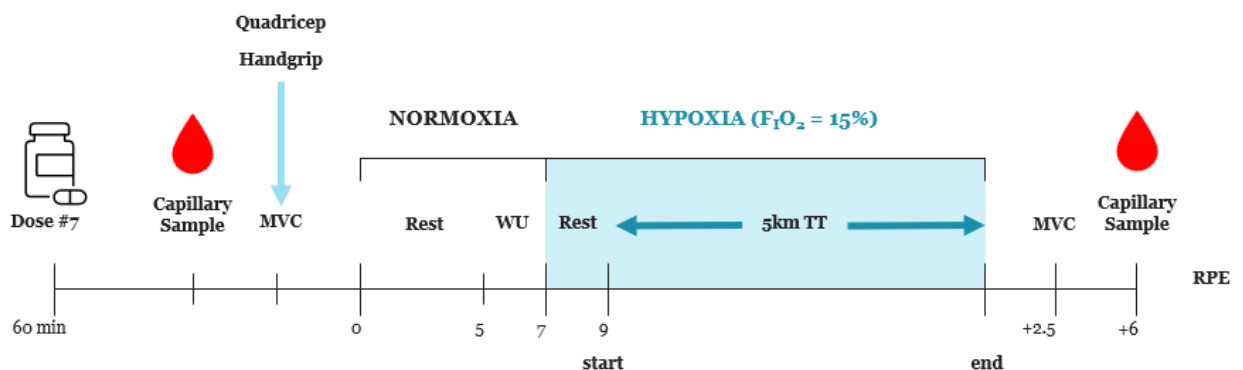


Figure 6: Summary of experimental time trial protocol. MVC, maximal voluntary contraction; WU, warm up; TT, time trial; F₁O₂, fraction of inspired oxygen; RPE, rate of perceived exertion.

4.5 Data Collection

4.5.1 Cardiorespiratory Responses

Raw data was recorded with a 16-channel analog-to-digital data acquisition system (PowerLab/16SP model ML 795; ADInstruments, Colorado Springs, CO); recording frequency for the maximal exercise test and time trials were 400 Hz and 2000 Hz respectively. Inspiratory and expiratory flows were continuously measured by having participants breathe through the mouthpiece, which was attached to two pneumotachographs (model 3813; Hans Rudolph, Shawnee, KS) located on either end of the breathing circuit. The expired pneumotachometer was heated to 37°C, while the inspired was kept at room temperature. Both pneumotachometers were calibrated using room air and a 3-L syringe. Two sets of calibrated O₂ and CO₂ analyzers (AEI Technologies S-3-A/I and CD-3Am, respectively; Applied Electrochemistry, Bastrop, TX) were used to sample mixed-expired gases and end-tidal gases during all testing visits. Mixed-expired gases were sampled from a mixing chamber to allow for the calculation of $\dot{V}O_2$ and $\dot{V}CO_2$. End-tidal gases were sampled at the mouth using a sample line connected to a second set of analyzers, similar to the first set, to determine end tidal O₂ and CO₂ tensions, P_{ET}O₂ and P_{ET}CO₂ respectively. A third O₂ analyzer (Gemini, 14-10000; CWE, Inc., Ardmore, PA, USA) was used to sample the O₂ content of the inspired gas (F_IO₂). All collected gases were dried using nafion tubing inside a sealed glass jar filled with Drierite prior to entering the gas analyzers. The humidity content of inspired gases was sampled via hygrometer (Traceable, model 35519-050; VWR, TX, USA) before it entered the breathing circuit, and was used to determine the water vapor pressure of inspired air.

Heart rate (HR) was measured using a telemetric sensor (Polar T34; Polar Electro, Kempele, Finland), and pulse oxygen saturation (S_pO₂) was estimated using two continuous patient monitors

(Radical-7 Pulse CO-Oximeter, Masimo, Irvine, CA, USA; model ePM 12M, Mindray, Mahwah, NJ, USA).

4.5.2 Arterialized Capillary Sample

Upon arrival for each experimental visit, the participants submerged their hand into warm water (45°C) for 5 minutes prior to blood sample collection. After their hand was dried and cleaned, and a single capillary sample (100 µl) was obtained from their 3rd digit. The sample was immediately analyzed using a blood gas analyzer (ABL80 FLEX CO-OX, Radiometer, Ontario, CA), then disposed in a biohazardous waste container. A sample was taken prior to pre-time trial MVCs, and another following completion of post-time trial MVCs, which was taken from the 3rd digit of the other hand. Both samples were taken while the participants were in normoxia. Both the researcher and participants were blinded to the results at the time of data collection.

4.5.3 Time Trial Protocol

A custom time trial protocol was conducted on a cycle ergometer (LC7TT novo, Monark). Power output in watts (W) was calculated from the cadence (revolutions per minute; rpm) and flywheel resistance (W), and the distance completed was calculated based on power. Unlike day one, the resistance was free to adjust according to participants' preference, to which they were familiarized on day 2. The handlebars on the right side allowed small adjustments ($\pm 10\text{W}$), and left side allowed larger adjustments ($\pm 30\text{W}$) to resistance. During the warm-up, participants were allowed to see their power output, and adjust their cadence and resistance to their preference. Once the participant completed the warm-up and began the time trial protocol, only the distance in kilometres was displayed. To ensure blinding, participants were not informed of their results at the time of completion.

4.5.4 Neuromuscular Assessment

A custom apparatus was used to perform isometric contractions of the vastus lateralis, where the participants were positioned at 90 degrees hip flexion and 90 degrees knee flexion. A load cell (Interface, SSM-Metric), with a range of 0-1000 Newtons, was positioned perpendicular to the shin, and attached to the participants' shin to the custom apparatus via an inelastic strap. Quadricep torque (qN) was measured in Newtons via load cell, which was calibrated after every MVC protocol with a 5-lbs weight. Further hip flexion was minimized via a belt that secured the participants' hip to the seat. EMG activity was measured using adhesive surface electrodes (H69P, Kendall); recording electrodes were placed on the muscle belly of the vastus lateralis, and ground electrode on the kneecap. Handgrip contractions were performed on a handgrip force transducer (ADInstruments, MLT004/ST Grip Force Transducer), which measured handgrip torque (hN). These contractions were performed while the participants were in normoxia.

EMG activity was sampled via a bioamplifier (CT-1000; CWE, Inc, Ardmore, PA, USA), where raw signals passed through a 12-bit D/A converter before being recorded at 2000 Hz through the same analog-to-digital converter used for cardiorespiratory metrics (PowerLab/16SP model ML 795, AD Instruments, Colorado Springs, CO, USA). The EMG signal was also band-pass filtered, with high cut-off frequency of 200 Hz and low cut-off frequency of 10 Hz. Quadricep (qN) torque and handgrip (hN) force were sampled at 1000 Hz using a separate analog to digital data acquisition system (PowerLab/4SP model ML 750, AD Instruments, Colorado Springs, CO, USA).

4.6 Data Analysis

4.6.1 Cardiorespiratory Responses

The measured flow from the inspiratory pneumotach was numerically integrated to calculate volume, which allowed for the determination of both tidal volume (V_T) and breathing frequency (F_b).

Inspiratory ventilation was calculated as the product of V_T and F_b , which was converted into expiratory ventilation using the Haldane transformation; both expressed in body temperature pressure saturated (BTPS). Oxygen consumption was determined by taking the difference between the inspired and expired oxygen using inspiratory ventilation and expiratory ventilation. Both $\dot{V}O_2$ and $\dot{V}CO_2$ were expressed in standard pressure temperature dry (STPD). All cardiorespiratory responses were analyzed in 1-km increments and expressed in absolute units or as percentage of the peak values obtained from the maximal exercise test on day 1.

4.6.2 Maximal Exercise Capacity and Time Trial Performance

The work rate at termination of the incremental exercise protocol was determined as the peak power output (PO_{peak}). Absolute peak oxygen uptake ($\dot{V}O_{2peak}$) was an average of the last 20 seconds prior to termination. Relative $\dot{V}O_{2peak}$ was calculated by dividing the absolute value by participant's bodyweight in kilograms (kg), then converted to mL/kg/min.

In the time trial protocols, instantaneous power output, resistance, and cadence were outputted from the Monark software on a power-time graph, as well as the overall average power output and time to completion. At every 1-kilometre increment, the average power output, split time and $\dot{V}O_2$ were recorded. All power outputs and $\dot{V}O_2$ values were expressed in absolute values, W and L/min respectively, and relative values, % PO_{peak} and % $\dot{V}O_{2peak}$ of values obtained on day 1 respectively.

4.6.3 Electromyography

For EMG data, the root mean square (RMS) of the EMG signal was calculated with a 100-ms moving average. The average maximum RMS was extracted to determine peak EMG activity. In the pre-exercise MVCs, the highest of the 3 attempts was recorded for comparison. During the time trial,

the average of every kilometer was expressed as percentage of the pre-exercise MVC. For post-exercise MVC, the highest of the 3 attempts was expressed as a percentage of the pre-exercise MVC.

4.6.4 Blood gas data

Blood pH was converted into hydrogen ion (H^+) concentration using the equation $pH = -\log[H^+]$, and expressed as nanomolars (nmol). All other variables were reported as measured from the blood gas analyzer.

4.7 Statistical Analysis

Statistical analyses were conducted using Rstudio (version 4.3.2) (115). For inferential tests, a significance level of $p < 0.05$ was set and all tests were two-sided.

The primary endpoint was the time required to complete a stimulated 5-km time trial on a cycle ergometer. The value for time was continuous (interval) and measured once per visit. A one-way repeated measures ANOVA (116) was used to assess differences in time trial duration between all three conditions (AZ, MZ, PLA). Bonferroni pair-wise t-tests were conducted for post-hoc analysis. Similar tests were performed on other performance markers, such as power output. Categorical data, such as rate of perceived exertion and the frequency of symptoms reported, were analyzed using non-parametric Friedman test to assess for differences between 3 conditions, and Wilcoxon signed rank test was conducted for post-hoc analyses.

The secondary endpoints were cardiorespiratory, blood sample and neuromuscular variables. All three types of endpoints were interval data but measured differently: Cardiorespiratory endpoints were measured continuously during cycling; blood parameters and torque endpoints were discretely measured before and after exercise; and EMG was continuously measured throughout. For each cardiorespiratory endpoint, a 5 (20-, 40-, 60-, 80- and 100%-time trial) by 3 (AZ, MZ, PLA) two-way repeated measures ANOVA was used to assess for differences between distance and condition; each

timepoint was an average of the entire kilometer. One-way repeated measures ANOVAs were also performed for differences in overall average values across the 3 conditions. For blood sample measurements, a 2 (Pre and Post) by 3 (AZ, MZ, PLA) two-way repeated measures ANOVAs was conducted. To compare baseline and post-exercise blood data between the 3 conditions, a dependent samples t-test was conducted for each of the above variables to compare before and after exercise for each drug condition separately. Torque data were analyzed with a 2 (Pre and Post) by 3 (AZ, MZ, PLA) two-way repeated measures ANOVA, and EMG was assessed via a 5 (20-, 40-, 60-, 80- and 100%-time trial) by 3 (AZ, MZ, PLA) two-way repeated measures ANOVA. All dependent samples t-tests were conducted only if the assumption of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were met. If the aforementioned assumptions were violated, then the Wilcoxon Signed Rank test (non-parametric equivalent) was completed. When significant F ratios were detected, pairwise comparisons with Bonferroni correction were used to determine where the differences lay. In the case where the dataset was unbalanced or had missing data points, linear mixed models (117, 118) were used instead of repeated measures ANOVAs; similar post-hocs were conducted as the ANOVAs (119). Repeated measures correlations (120) were conducted to determine the relationship between raw endpoints and the magnitude of change with the conditions.

Summary statistics were used to describe baseline characteristics and other outcomes of interest. Categorical endpoints were presented as median and interquartile ranges. Continuous endpoints were summarized using the mean and standard deviations.

5.0 RESULTS

5.1 Pre-Exercise Rest

Hypoxic air significantly increased HR and \dot{V}_E , and decreased S_{pO_2} , but no significant differences were found in these variables ($p < 0.05$) between the 3 conditions during normoxic and hypoxic rest (Table 3). While \dot{V}_E was not statistically different, both AZ and MZ had higher $P_{ET}O_2$ and lower $P_{ET}CO_2$ than PLA during normoxic rest and hypoxic rest ($p < 0.05$), but there were no differences between AZ and MZ ($p > 0.05$). In addition, ventilatory efficiency and metabolic equivalents ($\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$) were higher on the drug conditions compared to placebo ($p < 0.05$). These results indicate that the drugs induced hyperventilation at rest, but no difference was found between AZ and MZ ($p > 0.05$) for all these ventilatory variables.

Table 3: Resting cardiorespiratory values in normoxia and hypoxia ($F_{I}O_2 = 1\%$) between placebo (PLA), acetazolamide (AZ) and methazolamide (MZ).

	Condition	Normoxic rest	Hypoxic rest	Notes
HR (bpm)**	PLA	78±8	92±14	
	AZ	78±11	89±17	
	MZ	79±9	96±14	
\dot{V}_E (L/min) **	PLA	15.4±4.4	24±6	
	AZ	16.7±4.5	27±6	
	MZ	16.2±3.1	26±7	
\dot{V}_A (L/min)	PLA	9.2±3.8	-	$\dot{V}CO_2$ during hypoxic rest was not available, thus cannot be calculated
	AZ	10.1±2.7	-	
	MZ	9.8±1.8	-	
V_T (L)**	PLA	1.2±0.4	1.4±0.4	
	AZ	1.3±0.4	1.5±0.4	
	MZ	1.2±0.5	1.4±0.4	
Fb (bpm)**	PLA	13±4	17±4	
	AZ	14±4	17±4	
	MZ	14±5	17±3	
$\dot{V}O_2$ (L/min)	PLA	0.47±0.14	-	Values were not calculated during hypoxic rest as the allocated time was not sufficient to achieve steady state
	AZ	0.41±0.11	-	
	MZ	0.39±0.07	-	
$\dot{V}CO_2$ (L/min)	PLA	0.41±0.13	-	
	AZ	0.36±0.08	-	
	MZ	0.36±0.06	-	
$P_{ET}O_2$ (mmHg)**	PLA	110±5	79±5	
	AZ	116±5*	83±5*	
	MZ	117±6*	84±4*	
$P_{ET}CO_2$ (mmHg)**	PLA	39±3	37±5	VA = 9.07
	AZ	32±3*	32±3*	9.71
	MZ	32±4*	31±3*	9.71
S_pO_2**	PLA	97±1	95±3	
	AZ	98±1	96±2	
	MZ	97±1	96±1	
$\dot{V}_E/\dot{V}O_2$	PLA	34±4	-	Values were not calculated during hypoxic rest as the allocated time was not sufficient to achieve steady state
	AZ	40±7*	-	
	MZ	42±7*	-	
$\dot{V}_E/\dot{V}CO_2$	PLA	37±4	-	
	AZ	45±6*	-	
	MZ	45±7*	-	

HR, heart rate; V_T , tidal volume; Fb, breathing frequency; \dot{V}_E , minute ventilation; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; S_pO_2 , oxygen saturation. $P_{ET}O_2$, end tidal PO_2 ; $P_{ET}CO_2$, end tidal PCO_2 . AZ, acetazolamide; MZ, methazolamide; PLA, placebo. *, significantly different from PLA, 0.05. **, effect of inspired gas, 0.05.

5.2 Performance Outcomes

The placebo condition demonstrated the fastest time to complete 5-km compared to AZ and MZ (556 ± 37 s, 569 ± 45 s and 575 ± 43 s respectively, $p < 0.01$), but no difference was found between AZ and MZ ($p = 0.47$) (Figure 7). Similarly, placebo had the highest average PO compared to AZ and MZ (224 ± 51 W, 212 ± 52 W, 207 ± 50 W respectively, $p < 0.01$), and no difference between AZ and MZ ($p = 0.24$). These work rates were equivalent to 63 ± 6 , 59 ± 6 and $58\pm 5\%$ of peak PO respectively from the participants' normoxic maximal exercise test. There was not a significant relationship between cardiorespiratory fitness and the % change in TT duration from PLA with AZ ($p = 0.12$) and MZ ($p = 0.37$).

Perceived dyspnea (RPE-B) was not significantly different between the 3 conditions ($p = 0.61$), but perceived leg discomfort (RPE-L) was significantly lower in MZ compared to PLA ($p < 0.05$). There was a significant correlation between time trial duration and RPE-L ($p = 0.04$, $r = -0.37$), which indicates that time trial performance was closely related to the exertion of locomotor muscles but not limited by it. In the case of MZ, participants' performance was less affected by their leg discomfort compared to PLA and AZ.

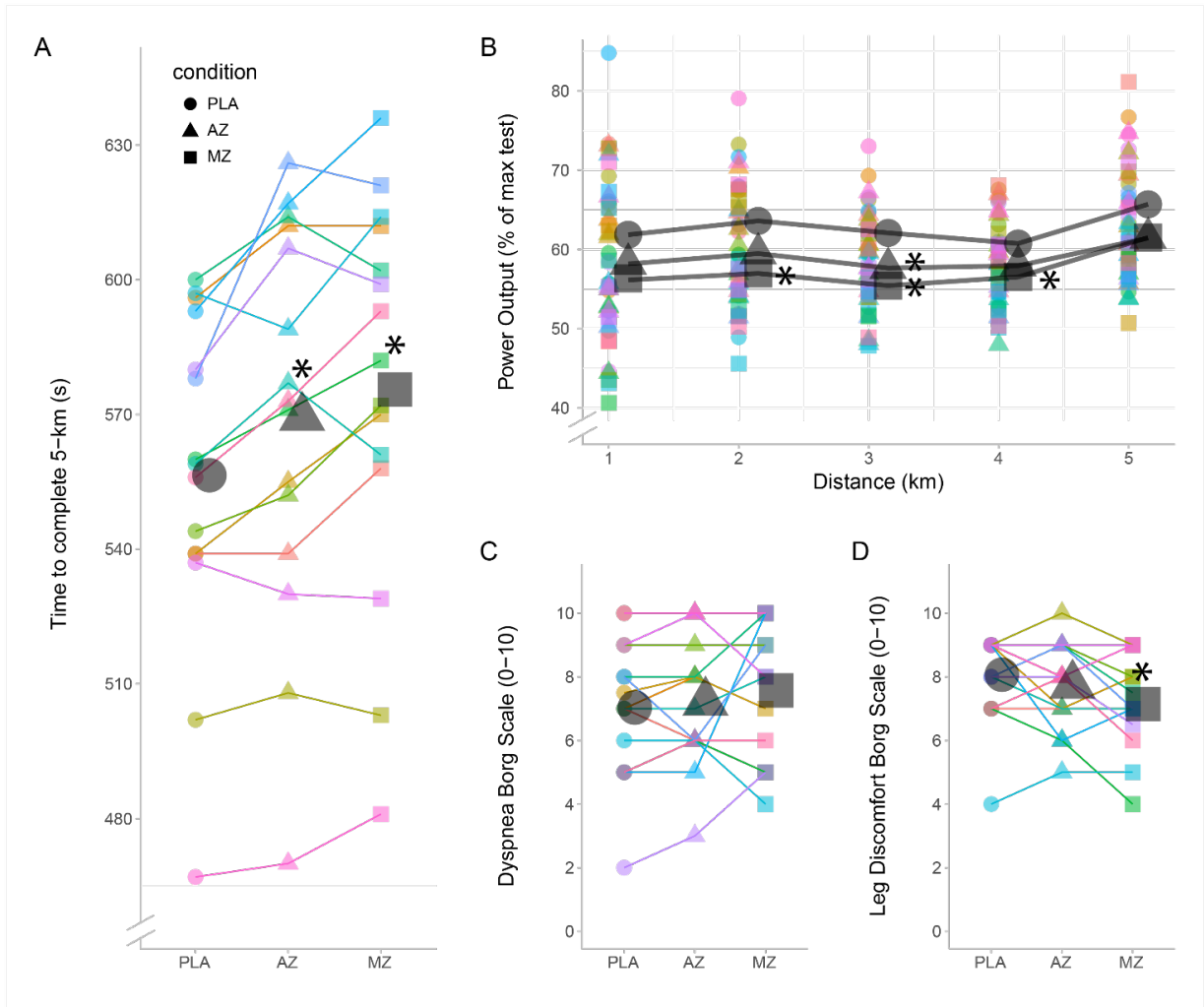


Figure 7. Individual and group mean data of time trial duration (TTs), power output (PO), ratings of perceived exertion for breathing discomfort (RPE-B) and leg discomfort (RPE-L) at end exercise. AZ, acetazolamide; MZ, methazolamide; PLA, placebo. *, significantly different from PLA, $p < 0.05$.

5.3 Cardiorespiratory Data

The summary of all the cardiorespiratory data during exercise is presented in Table 4. As the time trial progresses, \dot{V}_E increases and S_pO_2 decreases ($p < 0.01$) (Figure 8). At the second kilometer, S_pO_2 was statistically higher in AZ compared to PLA ($p < 0.05$). Otherwise, there were no differences in \dot{V}_E nor S_pO_2 between the three conditions ($p > 0.05$). There was a difference in V_T during the last 3 kilometers ($p < 0.01$), where both AZ and MZ were greater than PLA ($p < 0.05$); such changes from

PLA were equivalent to an increase of 4% V_T/FVC . Breathing frequency (Fb) was lower in AZ and MZ compared to PLA during the last 2 kilometers ($p < 0.05$).

End tidal PO_2 was only greater in AZ and MZ compared to PLA during the first kilometer ($p < 0.05$), but no differences thereafter. Absolute $\dot{V}O_2$ and relative $\dot{V}O_2$ (% of normoxic max test) were lower in MZ compared to PLA for most of the time trial ($p < 0.05$), except in the 3rd kilometer (Table 4). Methazolamide had consistently greater $\dot{V}_E/\dot{V}O_2$ compared to PLA throughout ($p < 0.05$). There were no differences in $\dot{V}O_2$ between AZ and PLA, but $\dot{V}_E/\dot{V}O_2$ was greater than PLA in the first 3 kilometers ($p < 0.05$). Throughout the time trial, no significant differences were present between AZ and MZ in terms of $\dot{V}O_2$ or $\dot{V}_E/\dot{V}O_2$ ($p > 0.05$). Furthermore, no correlation was found between the average S_pO_2 and time trial duration (Figure 9) ($p = 0.10$). In terms of the ratio of $\dot{V}O_2/PO$, no significant differences were observed between the three conditions ($p > 0.05$) (Figure 10B).

Carbon dioxide production was significantly lower in AZ and MZ compared to PLA ($p < 0.05$) throughout the entire time trial. While there was no statistical difference in the PO between AZ and MZ ($p = 0.24$), $\dot{V}CO_2$ was even lower on MZ compared to AZ during the first 2 kilometers ($p < 0.05$) (Table 3). Since $\dot{V}CO_2$ was significantly lower for a given \dot{V}_E , arterial PCO_2 would decrease due to hyperventilation. Indeed, $P_{ET}CO_2$ was consistently lower on AZ and MZ compared to PLA ($p < 0.05$) throughout the time trial. Methazolamide had lower $P_{ET}CO_2$ than AZ only in the first 2km ($p < 0.05$) but was not different afterwards, which aligns with the differences in $\dot{V}CO_2$. Likewise, $\dot{V}_E/\dot{V}CO_2$ was consistently higher in MZ and AZ compared to PLA ($p < 0.05$), with MZ being greater than AZ except in the last kilometer. On average, the ratio of $\dot{V}CO_2/PO$ was significantly lower in the AZ and MZ condition compared to PLA ($p < 0.05$) (Figure 10A). These differences were only observed within the first 2km, which closely followed the findings in $\dot{V}CO_2$ and $P_{ET}CO_2$ between AZ and MZ.

Respiratory exchange ratio was significantly lower in AZ and MZ compared to PLA ($p < 0.05$), but no differences were found between AZ and MZ ($p > 0.05$). The difference in RER between PLA and the CA inhibitors would be indicative of exercise intensity as participants would reach a greater power output on PLA, possibly higher than their ventilatory threshold where $\dot{V}CO_2$ exceeded $\dot{V}O_2$. The lack of difference in RER between AZ and MZ suggested that participants maintained a relatively lower intensity than PLA, which was true considering the lower PO values (Figure 7).

Table 4. Cardiorespiratory variables during 5-km time trial between placebo (PLA), acetazolamide (AZ), and methazolamide (MZ).

	Condition	1k	2k	3k	4k	5k	Average
HR (bpm)	PLA	151±11	167±11	173±9	176±11	181±10	171±9
	AZ	150±11	169±8	174±7	179±7	183±8	171±8
	MZ	153±8	169±10	175±6	179±6	184±6	173±6
HR (%max)	PLA	79±7	88±6	91±5	93±5	95±4	89±5
	AZ	79±6	89±5	92±4	94±4	96±4	90±4
	MZ	80±4	89±3	92±3	94±3	97±3	91±3
\dot{V}_E (%max)	PLA	49±7	72±11	77±11	82±11	88±13	75±8
	AZ	51±6	76±11	80±11	83±12	88±11	76±9
	MZ	51±7	76±10	80±11	82±12	89±12	76±9
V_T (L)	PLA	2.7±0.8	2.7±0.6	2.6±0.6	2.5±0.5	2.5±0.4	2.5±0.5
	AZ	2.6±0.4	2.9±0.5	2.8±0.5*	2.7±0.5*	2.6±0.4*	2.7±0.4*
	MZ	2.6±0.4	2.9±0.5	2.8±0.5*	2.7±0.5*	2.6±0.4*	2.7±0.4*
V_T (%FVC)	PLA	48±8	52±9	50±8	48±8	48±8	48±9
	AZ	50±8	55±8	54±8*	52±8*	51±8*	52±8*
	MZ	50±10	56±9	53±8*	52±8*	51±7*	52±8*
Fb (bpm)	PLA	30±6	41±7	46±5	50±5	54±6	45±5
	AZ	30±5	41±7	44±6	47±5*	51±6*	43±5
	MZ	30±6	41±5	44±5	47±5*	51±6*	43±4
$\dot{V}O_2$ (L/min)	PLA	2.3±0.4	2.9±0.5	3.0±0.5	3.1±0.5	3.2±0.5	2.9±0.4
	AZ	2.2±0.3	2.8±0.4	2.9±0.4	3.0±0.4	3.0±0.5	2.8±0.4
	MZ	2.1±0.5*	2.7±0.5*	2.8±0.5	2.8±0.5*	2.9±0.5*	2.7±0.5*
$\dot{V}O_2$ (%max)	PLA	56±7	71±7	73±7	74±7	77±8	71±6
	AZ	53±6	69±8	72±6	72±7	73±7	68±7
	MZ	50±4*	65±4*	68±4	69±3*	71±4	65±3*
$\dot{V}_E/\dot{V}O_2$	PLA	36±5	42±4	44±4	46±3	48±3	43±3
	AZ	40±4*	46±5*	47±4*	48±4	49±4	46±3*
	MZ	42±4*	49±5*	49±4*	50±4*	51±4*	48±4*
$P_{Et}O_2$ (mmHg)	PLA	81±4	89±3	91±3	92±3	93±2	89±3
	AZ	85±5*	91±5	92±5	93±4	94±4	91±5
	MZ	86±3*	92±4	93±4	93±4	95±3	92±6

P_{ET}CO₂ (mmHg)	PLA	37±2	33±3	30±3	28±3	26±3	30±3
	AZ	30±3*	27±3*	26±3*	25±3*	24±2*	27±2*
	MZ	27±4*†	25±4*†	25±3*	25±3*	23±3*	25±3*
ṠCO₂ (L/min)	PLA	2.5±0.6	3.6±0.8	3.5±0.7	3.4±0.5	3.4±0.5	3.3±0.6
	AZ	2.1±0.5*	3.0±0.6*	3.1±0.6*	3.1±0.6*	3.1±0.7*	2.9±0.6*
	MZ	1.9±0.5*†	2.8±0.6*†	2.9±0.5*	2.9±0.5*	3.1±0.6*	2.8±0.6*
Ṡ_E/ṠCO₂	PLA	33±3	35±3	39±4	42±3	48±3	38±3
	AZ	41±3*	43±4*	45±4*	46±4*	49±4*	44±3*
	MZ	46±5*†	48±5*†	47±5*†	48±4*†	51±4*	47±5*†
RER	PLA	1.10±0.10	1.21±0.09	1.15±0.06	1.11±0.05	1.09±0.07	1.14±0.06
	AZ	1.02±0.09*	1.07±0.09*	1.05±0.07*	1.03±0.07*	1.03±0.07	1.04±0.07*
	MZ	0.96±0.07*	1.03±0.08*	1.05±0.05*	1.04±0.03*	1.05±0.04	1.03±0.05*

HR, heart rate; V_T, tidal volume; F_b, breathing frequency; Ṡ_E, minute ventilation; ṠO₂, oxygen uptake; RER, respiratory exchange ratio; S_pO₂, oxygen saturation; P_{ET}O₂, end tidal PO₂; P_{ET}CO₂, end tidal PCO₂; AZ, acetazolamide; MZ, methazolamide; PLA, placebo. *, significantly different from PLA, 0.05. †, significantly different from AZ, 0.05.

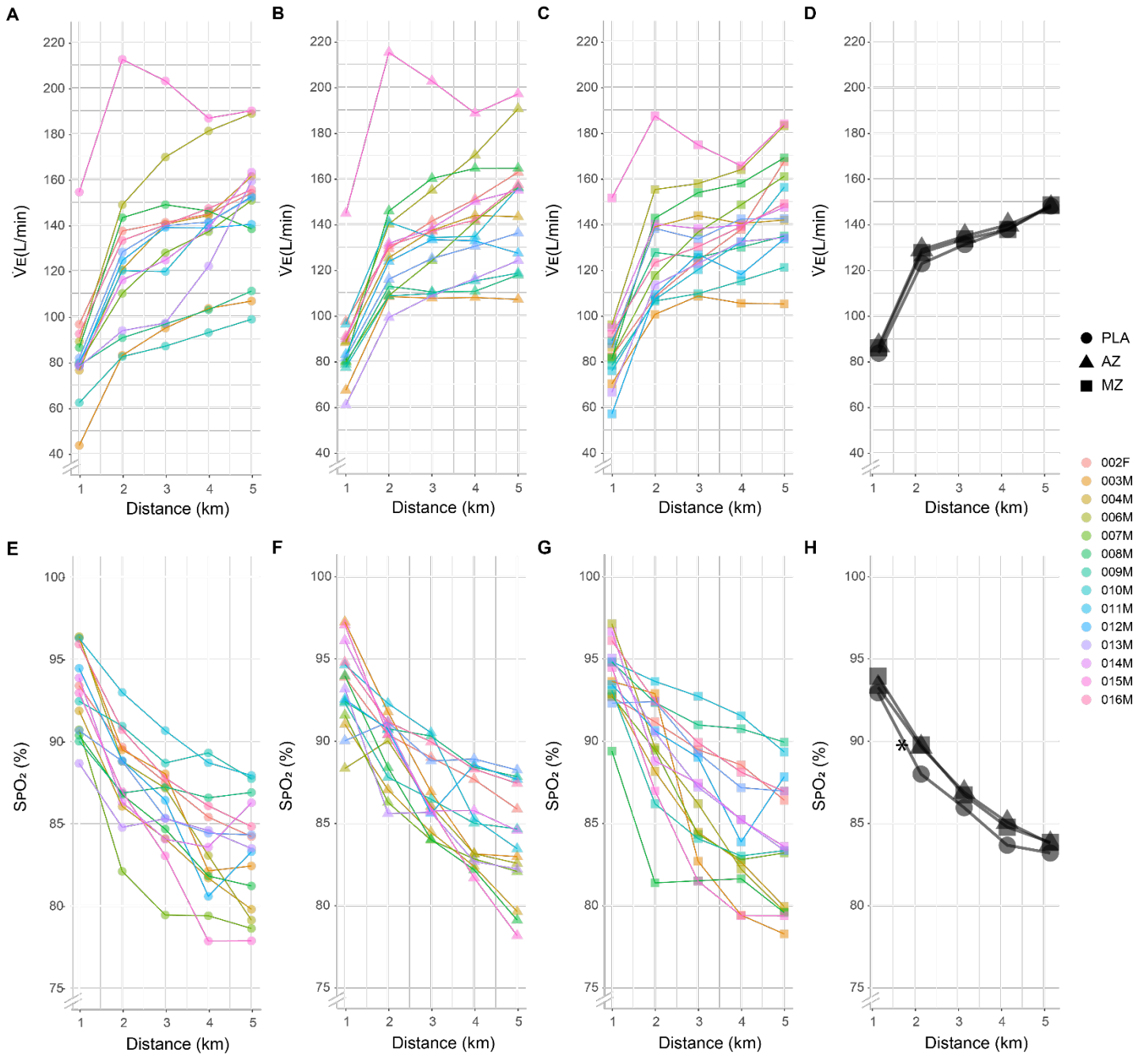


Figure 8. Individual and group mean data for ventilation (\dot{V}_E) and oxygen saturation (S_{pO_2}) during hypoxic time trial, panel A-D and E-H respectively. \dot{V}_E , minute ventilation; S_{pO_2} , oxygen saturation; AZ, acetazolamide; MZ, methazolamide; PLA, placebo. *, significantly different from PLA, 0.05.

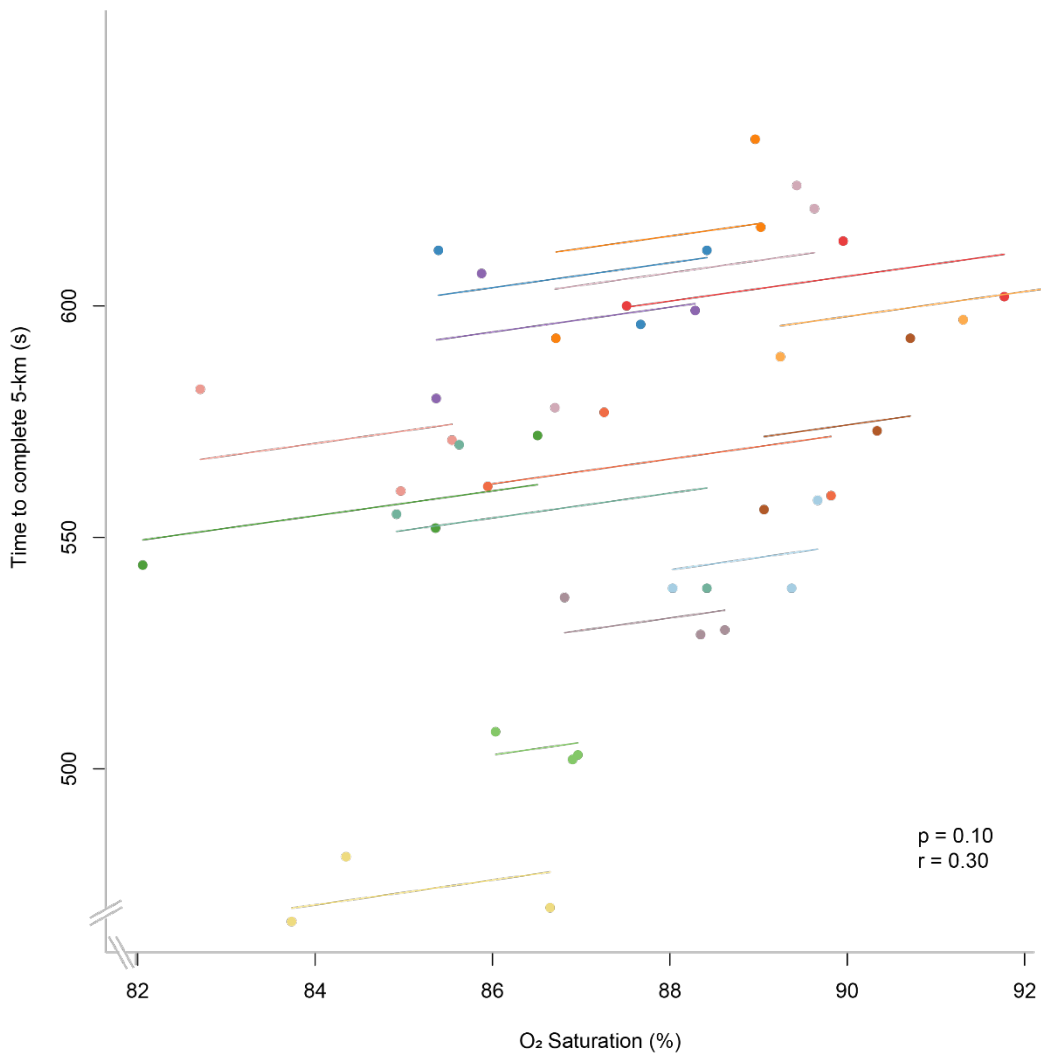


Figure 9. Relationship between time trial duration and average S_pO₂ across all three drug conditions (placebo, acetazolamide, methazolamide). S_pO₂, oxygen saturation.

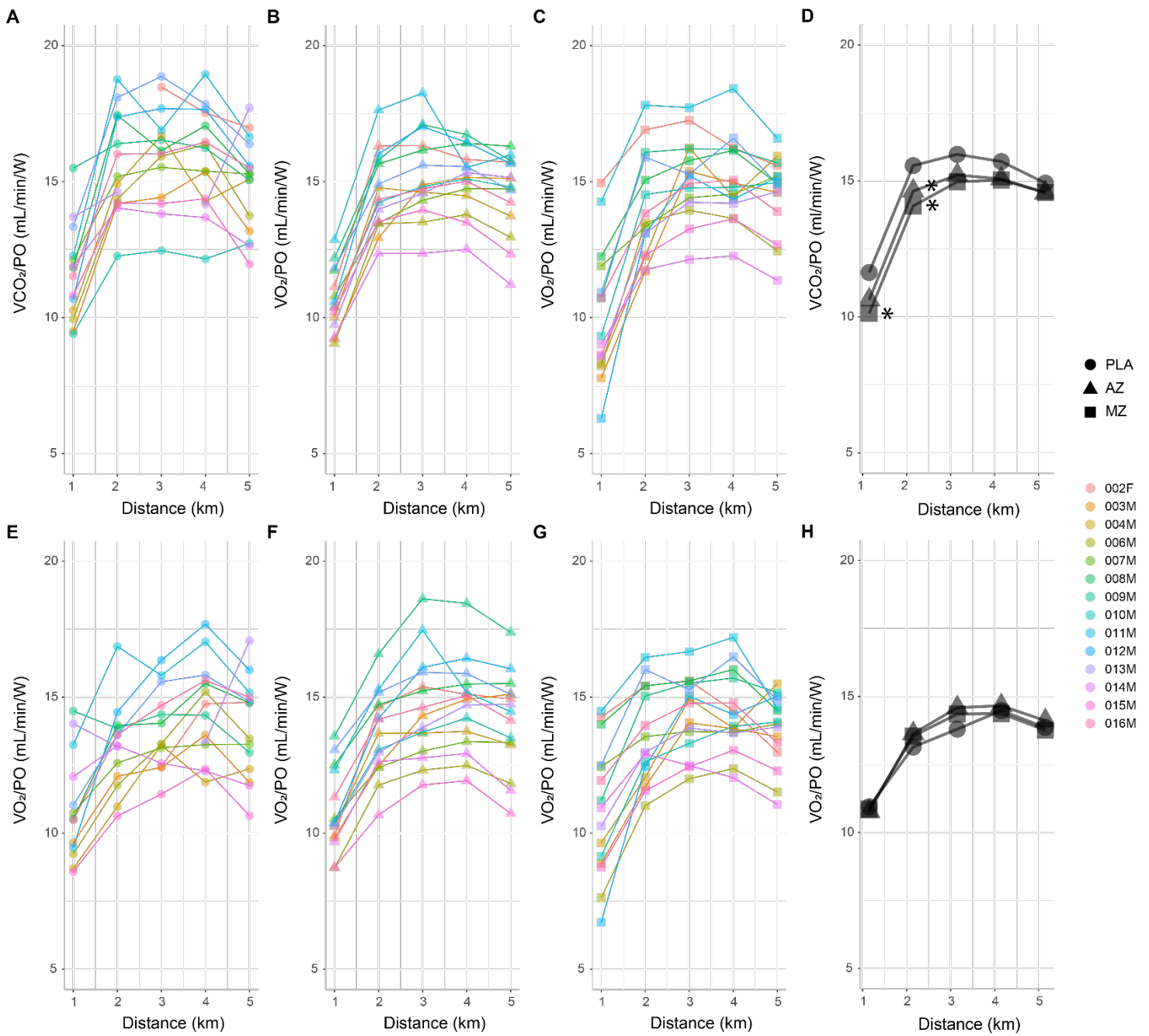


Figure 10: Individual and group mean data for the proportion of carbon dioxide production ($\dot{V}CO_2$) and oxygen uptake ($\dot{V}O_2$) to power output (PO), panel A-D and E-H respectively. PLA, placebo; AZ, acetazolamide; MZ, methazolamide; *, significantly difference from PLA, $p < 0.05$.

5.2 Neuromuscular Fatigue

Pre-exercise quadriceps torque was significantly lower in AZ compared to PLA and MZ ($p < 0.05$), but no differences post exercise (Table 5). The magnitude of change in quadriceps torque was also not different between conditions ($p = 0.65$). These findings were also true when expressed as % of pre-exercise MVC ($p > 0.05$). Handgrip torque was no different between conditions before or after exercise ($p > 0.05$). No differences found in EMG activity throughout the TT ($p > 0.05$) (Table 6).

Table 5. Neuromuscular and torque variables before and after hypoxic 5-km time trial.

	Condition	Pre	Post	Pre-Post Diff
Quadriceps Torque** (N; %MVC pre)	PLA	574±76	498±90; 87±11	-76±65, -13±11%
	AZ	543±77*	477±84; 88±12	-67±64, -12±13%
	MZ	552±69	492±106; 89±14	-65±80, -11±14%
Handgrip Torque (N; %MVC pre)	PLA	439±77	429±81; 98±11	-14±47, -3±11%
	AZ	415±47	407±98; 98±10	-10±39, -2±10%
	MZ	429±47	424±88; 99±10	-11±34, -2±9%

MVC, maximal voluntary contraction; AZ, acetazolamide; MZ, methazolamide; PLA, placebo. *, significantly different from PLA, 0.05. **, effect of exercise, 0.05.

Table 6. Summary of electromyograph activity throughout the hypoxic 5-km time trial and after.

	Condition	Pre	1k	2k	3k	4k	5k	Post TT MVC
EMG (V)	PLA	1.56±0.57	1.08±0.61	1.10±0.57	1.09±0.52	1.03±0.49	1.00±0.46	1.57±0.77
	AZ	1.57±0.48	1.07±0.61	1.04±0.57	0.95±0.48	0.83±0.41	0.83±0.43	1.54±0.60
	MZ	1.81±0.82	1.21±0.7	1.17±0.56	1.09±0.45	1.00±0.36	0.99±0.36	1.61±0.58
EMG (%MVC pre)	PLA	-	79±24	80±20	80±18	76±18	72±20	99±38
	AZ	-	78±32	78±34	72±33	64±31	64±33	98±26
	MZ	-	85±71	80±53	72±40	66±33	65±37	97±25

EMG, electromyography; TT, time trial; MVC, maximal voluntary contraction.

5.3 Capillary Blood Samples

Resting H^+ concentrations, HCO_3^- concentrations and base excess were significantly different between all conditions ($p < 0.05$). Both AZ and MZ induced a partially compensated metabolic acidosis compared to PLA, as demonstrated with higher H^+ concentrations and lower HCO_3^- concentrations, but AZ had a greater magnitude of acidosis than MZ ($p < 0.05$). All variables had an effect of exercise ($p < 0.01$), but not every variable was different between conditions post-exercise.

Hydrogen ion concentrations were not different between conditions ($p > 0.05$), but standard HCO_3^- concentrations and base excess was lower in AZ than PLA and MZ ($p < 0.05$). Independent of CO_2 levels, HCO_3^- levels were not different between MZ and PLA ($p = 0.19$). Since time trial performance was not different between AZ and MZ, this suggested that impaired performance was not attributed to metabolic status. However, the change in HCO_3^- and blood PCO_2 levels (PCO_2) from pre to post exercise were significantly less in AZ and MZ compared to PLA ($p < 0.05$). Hematocrit and hemoglobin concentrations were greater after time trial ($p < 0.05$), which indicated some fluid loss. However, no significant difference was found between conditions before or after time trial ($p > 0.05$).

Table 7. Results of capillary blood samples before and after hypoxic 5-km time trial.

	Condition	Pre	Post	Pre-Post Diff
H^+ (nmol) **	PLA	39.9±2.3	61.8±13.4	23.5±11.8
	AZ	48.9±3.3*	67.0±10.7	16.3±10.1*
	MZ	44.0±2.3*†	62.7±7.9	18.9±8.8
PCO_2 (mmHg) **	PLA	37.8±2.7	25.7±7.2	-12.0±7.3
	AZ	34.0±3.3	28.9±2.8	-4.9±2.7*
	MZ	35.1±3.7	31.1±4.9*	-3.6±3.8*
Hct (%) **	PLA	47.7±3.9	50.5±3.4	3.1±2.4
	AZ	49.0±3.4	49.3±7.8	1.3±4.1
	MZ	48.7±4.2	51.9±3.3	3.0±2.9
HCO_3^- (P) (mmol/L) **	PLA	22.9±6.3	11.2±3.2	-11.7±3.4
	AZ	16.5±1.5*	10.2±1.8	-6.4±2.2*
	MZ	19.2±2.3*†	12.3±2.1†	-7.0±3.3*
HCO_3^- (standard) (mmol/L) **	PLA	23.5±6.4	13.9±3.3	-9.6±3.4
	AZ	17.7±1.1*	12.0±1.7*	-5.9±2.2*
	MZ	20.3±1.7*†	13.9±1.9†	-6.5±3.0*
ctHb (g/dL) **	PLA	15.6±1.3	16.5±1.1	1.0±0.8
	AZ	15.9±1.2	16.1±2.6	-0.1±2.4
	MZ	15.9±1.4	17.0±1.1	1.0±1.0
Actual Base Excess (mmol/L) **	PLA	-1.0±1.4	-14.7±5.8	-13.6±5.4
	AZ	-8.6±1.5*	-17.7±3.1*	-9.4±3.7*
	MZ	-5.1±2.2*†	-14.7±3.3†	-9.7±4.7*

H^+ , hydrogen ion; Hct, hematocrit; HCO_3^- (P, st), standard bicarbonate; ctHb, concentration of total hemoglobin in blood; PCO_2 , partial pressure of CO_2 ; AZ, acetazolamide; MZ, methazolamide; PLA, placebo. *, significantly different from PLA, $p = 0.05$. †, significantly different from AZ, $p = 0.05$. **, effect of exercise, $p = 0.05$.

5.4 Symptom Reporting

The AZ condition demonstrated the most frequent reporting of symptoms compared to PLA ($p < 0.05$), but not MZ ($p = 0.35$) (Table 8). The most reported symptom was fatigue, which accounted for 27% of total symptoms. When asked which drug arm was the most difficult, 6 out of 15 participants reported AZ was the most difficult, while another 5 reported MZ. The remainder thought all conditions were equally difficult. There was no relationship between the change in symptoms to change in TT from PLA ($p > 0.05$).

Table 8: Summary of the frequency of reported symptoms.

ID	PLA	AZ	MZ	Subjective Notes	Change in TT from PLA
001M	0	0	0	All similar	AZ 0%; MZ +3.5%
002F	0	4	4	AZ was hardest	AZ +2.7%; MZ +2.7%
003M	2	3	1	PLA and MZ were similar	AZ +3.0%, MZ +5.8%
004M	0	2	0	AZ was hardest	AZ +1.2%, MZ +0.2%
006M	0	0	1	All similar	AZ +1.5%, MZ +5.2%
007M	0	0	0	AZ was hardest	AZ +2.0%, MZ +3.9%
008M	3	1	0	All similar	AZ +2.3%, MZ +0.3%
009M	0	0	0	MZ was hardest; AZ was easiest	AZ +3.2%, MZ +0.4%
010M	0	1	4	AZ was hardest	AZ -1.3%, MZ +2.9%
011M	0	8	4	MZ was hardest; PLA was easiest	AZ +4.1%, MZ +7.3%
012M	0	7	2	MZ was hardest; PLA was easiest	AZ +8.3%, MZ +7.4%
013M	1	2	0	MZ was hardest; PLA was easiest	AZ +4.7%, MZ +3.3%
014M	0	2	0	AZ was hardest; PLA was easiest	AZ +1.3%, MZ +1.5%
015M	0	0	0	All similar	AZ +0.6%, MZ +3.0%
016M	2	4	3	AZ was hardest; MZ was easiest	AZ +3.1%, MZ +6.7%
Total	8	34	19		

PLA, placebo; AZ, acetazolamide; MZ, methazolamide; TT, time trial.

6.0 DISCUSSION

The primary novel finding was that both AZ and MZ impaired whole-body exercise performance in acute hypoxia; both conditions demonstrated a slower TT duration than PLA but were not different from each other. Unlike the original hypothesis, MZ was also detrimental to hypoxic exercise performance. Our secondary finding was that AZ significantly decreased peripheral muscle function, as evidenced by lower peak quadricep torque compared to PLA, while there was no effect of MZ in this regard. The lack of effect on baseline peak quadriceps torque demonstrated that MZ potentially had a different mechanism of hindering performance than AZ. The third finding was that CA inhibition did not improve arterial oxygenation during intense hypoxic exercise, but had a significant effect on arterial CO₂ tensions. Specifically, there were no significant differences in \dot{V}_E or S_pO_2 between the conditions, but $\dot{V}CO_2$ and $P_{ET}CO_2$ were significantly lower in AZ and MZ compared to PLA. In summary, MZ may not be a better alternative for exercise performance in acute hypoxia compared to AZ, but there was no clear physiological explanation for its negative influence.

6.1 Performance Outcomes

Whole-body exercise performance can be affected by multiple factors, such as the ambient conditions, mode of exercise, and intensity of exercise. In the context of hypoxic exercise with CA inhibitors, severity and duration of hypoxia, symptomology and perceived effort also have an influence on exercise performance. The main objective of the present study was to compare AZ and MZ and their effects on exercise performance in acute hypoxia. Due to the differences in lipophilicity and other physiological mechanisms independent of CA inhibition, it was hypothesized that MZ would have resulted in better performance outcomes than AZ. Although the level of peripheral fatigue and metabolic acidosis were different, symptomology and perceptual factors might have masked how much those physiological differences matter in whole-body exercise performance.

Compared to previous studies done on CA inhibitors, the study's present work had several methodological differences. A shorter time trial was implemented, thus involving a higher exercise intensity and a shorter hypoxic exposure. Although with the standard doses of each respective CA inhibitor, these methodological variations would present a distinct set of results from previous findings.

First, heavy aerobic exercise imposes a greater physiological demand on the cardiorespiratory system as there is a greater need for oxygen. Participants operated at or close to their sustainable limits of cardiac output and ventilatory output, as indicated by $>85\% \dot{V}_E$ and $>90\%$ HR values (Table 5). Due to greater perfusion rates and \dot{V}_E , gas exchange would be compromised with greater exercise intensity; specifically A-aDO₂ would widen, thus exacerbating arterial hypoxemia (88). Furthermore, such intensity of aerobic exercise would induce more peripheral fatigue due to these greater physiological demands. Therefore, unlike at lower intensities, performance would be more dependent on how fatigue resistant the participants are. If either AZ or MZ exacerbates fatigue, then they may have a greater negative impact on the resulting performance outcome. Previous time trial studies found no differences in time to completion with AZ or MZ when compared to PLA (106, 121) and both used a duration that was twice as long. The average HR was around 20 bpm lower in the 12.5km time trial compared to 5-km (159 bpm vs 180 bpm, respectively) (106). Likewise, average power output was about 60W lower (158W in 12.5km vs 220W in 5km, respectively) and RER was drastically lower (0.86 vs 1.02, respectively) (109). The difference in HR and metabolic demand demonstrated that 12.5-km time trials elicited a lower exercise intensity than 5-km time trials, which meant that performance in the longer protocol may be relatively less affected by how peripherally fatigued the participants are. Thus, the disparity in exercise intensity might offer insight as to why

there would be performance changes with CA inhibitors in a shorter time trial, but none in a longer protocol.

Second, a rapid ascent to 3000m ($P_{iO_2} \sim 106$ mmHg) would put an unacclimatized individual at a moderate risk of AMS (122). Past research were mostly done at lower P_{iO_2} (96 mmHg and below) (83), so the risk of AMS would be greater and thus CA inhibitors would provide more benefit than PLA during those ascents. Hypothetically, there would be a ‘threshold’ altitude at which performance can be improved with a standard dose of CA inhibitors rather than be hindered, but it currently stands undetermined as there are many other confounding factors to performance (Figure 11). The current study simply looks at the minimum elevation that one needs to rapidly ascend to be prescribed CA inhibitors.

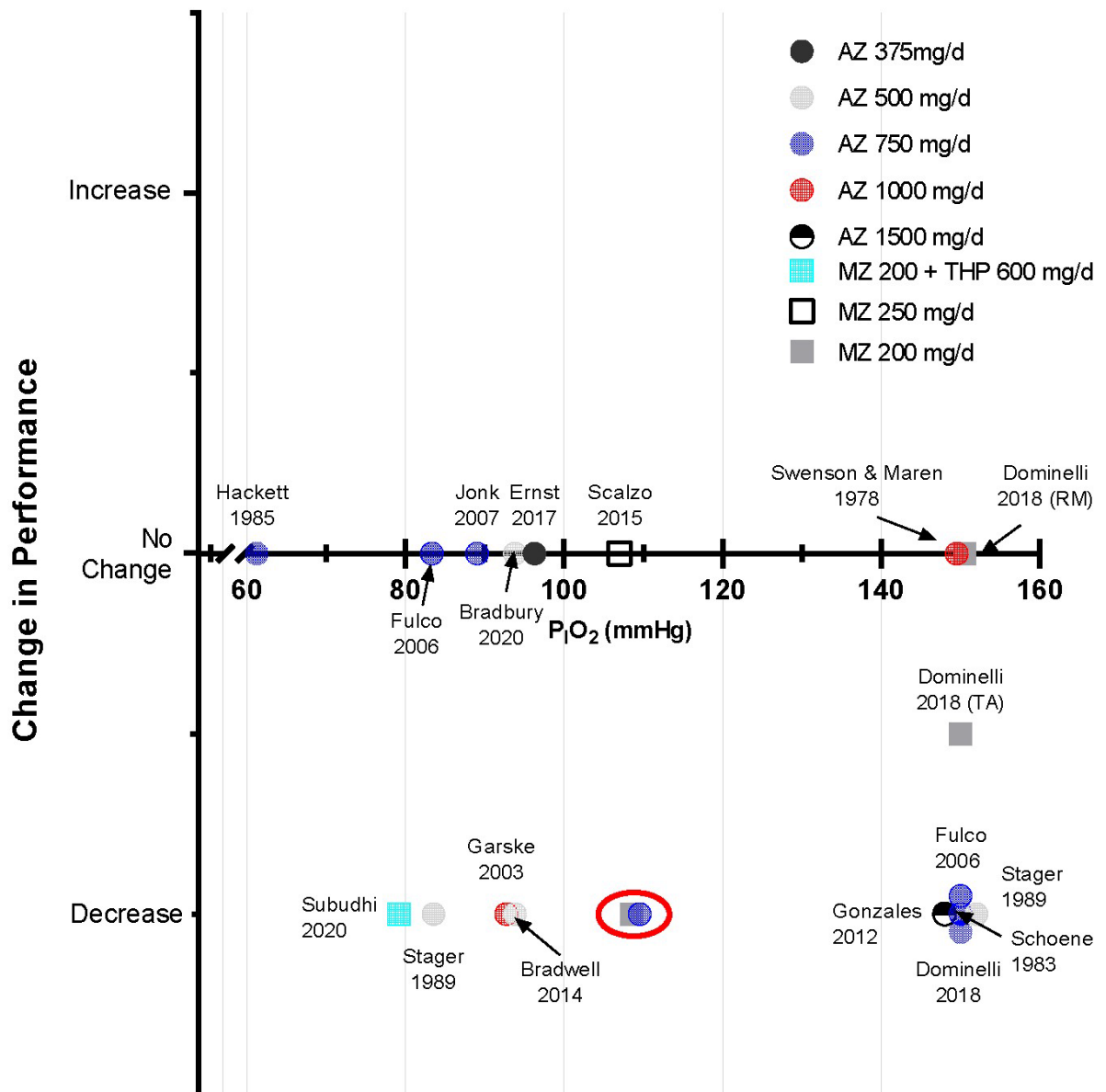


Figure 11: Findings of studies assessing the effects of AZ and MZ on performance outcomes. Results of the current study are highlighted by a red circle. P_IO₂, partial pressure of inspired oxygen; AZ, acetazolamide; MZ, methazolamide; THP, theophylline. Adopted from 2023 review paper (83).

Third, the dosage of AZ and MZ used in this study was the recommended doses for AMS prophylaxis (110, 112, 113). Previous studies on AZ have used higher and lower doses, which would have varying degrees of the drug's influence on performance as some physiological effects are dose

dependent (91, 96). Although many studies have evaluated the effect of different doses of AZ on exercise performance, it must be noted that the outcomes were likely confounded with the level of altitude (Figure 11). Differences in P_{iO_2} would have a major influence on whether AZ was beneficial, detrimental or had no effect on performance (83). The findings of this study determine how taking standard doses of AZ and MZ, at an elevation that one will most likely be prescribed, affects whole-body exercise performance. The decrease in performance was due to an inability to reach higher work rates, and the primary contributors may not be what was originally hypothesized.

6.2 Physiological Mechanisms of Performance Decrement

6.2.1 Oxygenation

Hypoxia impairs performance primarily through decreased inspired O_2 , which consequently reduces arterial PO_2 and O_2 saturation, and arterial O_2 content. Increasing \dot{V}_E can improve arterial O_2 content by increasing arterial PO_2 . CA inhibitors were anticipated to reduce the impairment caused by hypoxia, because they would stimulate ventilatory drive by means of metabolic acidosis. Greater H^+ concentrations would stimulate central chemoreceptors and increase \dot{V}_E , and consequently increase P_aO_2 and potentially improve S_pO_2 . In this study, AZ and MZ did not show any significant differences in \dot{V}_E during rest and exercise compared to PLA (Table 4 and Figure 8). The participants saw a significant drop in PO with CA inhibitors, especially on MZ, and the difference was reflected in $\dot{V}CO_2$ where AZ and MZ were significantly lower than PLA. As \dot{V}_E increased for a given $\dot{V}CO_2$, $P_{ET}CO_2$ consequently decreased, indicating hyperventilation. In instances where $\dot{V}CO_2$ and $\dot{V}_E/\dot{V}CO_2$ was drastically greater, as seen in kilometer 2, S_pO_2 was statistically higher in MZ than PLA ($p < 0.05$). Based on the Bohr effect, decreasing P_aCO_2 would cause a left shift of the O_2 -hemoglobin dissociation curve, thus improving O_2 loading onto hemoglobin, and increasing S_pO_2 for a given PO_2 . In addition, \dot{V}_E was more efficient on AZ and MZ, as demonstrated with a greater V_T and lower Fb

than PLA (Table 5), which would relatively decrease the amount of alveolar dead space. However, such changes in $P_{ET}CO_2$ and ventilatory patterns were not enough to increase O_2 saturation significantly and consistently throughout the exercise bout, and positively influence performance.

Increases in \dot{V}_E can be partially explained by the extent of metabolic acidosis. While resting \dot{V}_E was not different, AZ elicited a greater level of metabolic acidosis compared to MZ, as evidenced by a greater plasma H^+ and base deficit (Table 7). Conversely, this meant MZ induced a similar \dot{V}_E in a less acidotic state; it can be attributed to its greater lipophilicity which allows it to cross the blood brain barrier more readily. Furthermore, the magnitude of plasma H^+ increase mildly correlated with the magnitude of hyperventilation; weak to modest correlation was found between the resting H^+ and change in $P_{ET}CO_2$ from hypoxic rest to end-exercise ($p = 0.04$, $r = 0.37$) and end-exercise $\dot{V}_E/\dot{V}CO_2$ ($p < 0.01$, $r = 0.56$). Therefore, the magnitude of CA inhibition did not lead to a proportional increase in \dot{V}_E output, thus CA inhibitors had limited positive effects on oxygenation during intense hypoxic exercise.

6.2.2 Carbon Dioxide Production

According to the alveolar ventilation equation (Equation 2), alveolar PCO_2 is tightly linked to how much CO_2 is produced ($\dot{V}CO_2$) and how high the ventilation is (\dot{V}_E). Considering that alveolar and arterial PCO_2 are very similar, it can be further expanded that arterial CO_2 content is tightly related to CO_2 production and ventilatory output.

During normoxic rest, no difference in \dot{V}_E or $\dot{V}CO_2$ were observed between the 3 conditions, but $P_{ET}CO_2$ was significantly lower in AZ and MZ (Table 5). The difference might be a result of metabolic changes with CA inhibition, where excretion of bicarbonate was also reflected as a decrease in end tidal PCO_2 (Table 5). There was indeed a strong correlation between resting HCO_3^- levels and resting $P_{ET}CO_2$ ($p < 0.01$, $r = 0.79$). However, PCO_2 in the capillary blood samples were not significantly different between the three conditions ($p > 0.05$) (Table 7).

At the onset of exercise, all these variables started to change; specifically, \dot{V}_E and $\dot{V}CO_2$ would increase proportionally, thus P_aCO_2 remained unchanged. However, unlike PLA, \dot{V}_E increased disproportionately with $\dot{V}CO_2$ in AZ and MZ. The more hyperventilated the participants were, the lower the $P_{ET}CO_2$ as seen in Table 5. While there was no statistical difference in PO between AZ and MZ in the first 3 kilometers, $\dot{V}CO_2$ was significantly lower in MZ during this period ($p < 0.05$).

Following exercise, HCO_3^- was lower in AZ compared to PLA and MZ ($p < 0.01$), but not between MZ and PLA ($p = 0.94$). Blood PCO_2 was also significantly lower in PLA compared to MZ ($p < 0.05$), but AZ was not different from PLA ($p = 0.14$) nor MZ ($p = 0.21$). The smaller change in HCO_3^- and PCO_2 with AZ and MZ might be a result of lesser CO_2 production from lower metabolic demand. A strong correlation existed between PO and $\dot{V}CO_2$ ($p < 0.01$, $r = 0.77$), however, there might be mechanisms attributing to the decrease in $\dot{V}CO_2$ other than simply a reduced PO. Specifically, with MZ, there was an interesting observation with $\dot{V}CO_2$ changes during exercise. While $\dot{V}CO_2$ appeared to stabilize within 2 kilometers of the time trial in PLA and AZ, $\dot{V}CO_2$ took longer to stabilize in MZ. Between kilometer 2, 3 and 4, PO was not different within each condition, so supposedly $\dot{V}CO_2$ should not differ as well. But $\dot{V}CO_2$ was statistically lower in kilometer 2 than kilometer 3 and 4 in the MZ condition ($p < 0.05$). Furthermore, the ratio of $\dot{V}CO_2$ to PO was significantly lower in MZ compared to PLA ($p < 0.05$) (Figure 10); this was unlikely due to an increase in efficiency but rather reflected how MZ affected the amount of CO_2 expired. These findings suggested that $\dot{V}CO_2$ kinetics may be affected, but the current exercise protocol was not designed to examine and quantify this as it would require a constant workload (123, 124). Previous literature reported delayed $\dot{V}CO_2$ kinetics with administration of AZ, potentially due to CO_2 retention (125); some evidence of this were also observed in the current time trial protocol, to be discussed in the following section.

6.2.3 Transport of Carbon Dioxide

Inhibiting CA will have direct effects on blood acid-base status, as well as the regulation of pH changes. Transportation of CO₂ from peripheral tissue to the alveoli occurs through three different pathways: majority (60%) through the conversion to HCO₃⁻ in plasma, 30% by direct binding to hemoglobin within erythrocytes, and 10% dissolved with plasma (126). With complete inhibition of CA in the red blood cells, CO₂ transport will be significantly impaired, as seen with a high dose of CA inhibitors (96). A rationale for the current study's prescribed dose was to not cause significant inhibition of CA in erythrocytes, but enough to inhibit CA in the kidneys to induce metabolic acidosis (75). Although CO₂ transport was not significantly impaired, it was not guaranteed that there was absolutely no impact on CO₂ transport. In fact, impairment of CO₂ transport by CA inhibitors were reported to be dose-dependent (96), thus it can be postulated that there was some level of impaired CO₂ transport in the current study, given the changes and differences seen with $\dot{V}CO_2$. The lesser change in HCO₃⁻ and PCO₂ pre-post exercise in AZ and MZ ($p < 0.01$, Table 7) implied that there was less of CO₂ carrying capacity, where less CO₂ was being catalyzed and transported in the plasma as HCO₃⁻. During situations of high CO₂ production and perfusion, such as intense exercise, there would be a larger CO₂ gradient between arterial and venous blood (96). Despite a potentially "intact" reserve of CA enzymes in the blood, the turnover of CO₂ to HCO₃⁻ would not be efficient to expel CO₂ from the periphery. While internal CO₂ production would be lower in AZ and MZ due to lower PO, some level of increased CO₂ gradient may explain why PCO₂ did not drastically change from pre to post exercise. In conclusion, CO₂ transport might have some possible influence on exercise performance.

6.2.4 Peripheral Muscle Fatigue

Independent of CA inhibition and changes in blood acid-base status, there were other mechanisms that could impair exercise performance. Consistent with previous studies, pre-exercise neuromuscular fatigue was more pronounced on AZ compared to PLA and MZ, as evidenced by a lower peak quadriceps torque at rest (Table 6). Extrapolating this to whole body exercise, this most likely contributed to the lower PO during the time trial. Handgrip torque was not statistically significant between AZ and PLA ($p = 0.4$); the lack of difference may be due to fiber type composition. The distribution of fast twitch fibers are relatively greater in quadriceps muscle group than the forearm muscles (~45-52% vs ~40-45% respectively) (127, 128). Acetazolamide was reported to affect BK channels in fast twitch fibers more than those of slow twitch (110, 129), which could offer an explanation for why the quadricep muscle group was more affected than the forearm muscles. Furthermore, the assessment of handgrip torque was mainly to assess changes in central fatigue. Since the handgrip was not one of the primary muscle groups used during the cycling exercise, it helped confirm that the change seen in quadriceps torque was due to peripheral fatigue in the exercising muscles and not centrally driven.

As previously mentioned, AZ was a potent opener of BK channels, and the inability to regulate intramuscular K^+ levels contribute to fatigue. While not measured in this study, AZ and MZ were reported to decrease plasma K^+ concentration (110), but were not significantly different from each other. Since MZ did not induce fatigue despite lower plasma K^+ concentrations, it provided evidence that the magnitude of plasma K^+ loss did not correspond to peripheral muscle fatigue. In addition, it has been suggested that H^+ influences muscle function by altering K^+ concentrations, where lower pH led to increased K^+ (130). With the different pH between AZ and MZ, it further adds to the evidence against the notion that a decrease in performance correlates with plasma K^+ changes.

However, earlier literature postulated that intracellular K^+ changes were more representative of skeletal muscle function than plasma K^+ ; both variables were beyond the current study's focus. As a potent BK opener, AZ hindered performance by affecting the function of skeletal myocytes and inducing greater muscle fatigue than PLA and MZ.

6.2.5 Perceptive Effort

Changes at the level of the skeletal muscle might further explain the decrease in PO as seen with AZ, but does not offer a plausible explanation for MZ. While peripheral muscle function was relatively intact and metabolic acidosis was to a lesser magnitude compared to AZ, MZ still demonstrated a significantly lower PO. Consistent with the previous study (110), MZ showed no changes in resting peripheral muscle function, where peak quadriceps torque was not different from PLA ($p = 0.32$). However, isolated single muscle exercise in normoxia omitted critical factors involved in whole-body exercise in hypoxia; the metabolic demand of recruiting more muscle mass and the challenge of reduced $F_{I}O_2$ would significantly increase \dot{V}_E . In the placebo condition, participants ventilated at $\sim 88\%$ of their peak \dot{V}_E while exercising at $\sim 65\%$ of peak PO. In the case of MZ, a significantly lower PO ($62 \pm 8\% PO_{peak}$) achieved similar \dot{V}_E . A potential explanation would be that the lipophilic nature of MZ resulted in greater localization within the central nervous system compared to AZ (105, 131). Consequently, localization of MZ within the brain would stimulate chemoreceptors more directly than if it were localized within the cerebral vasculature. Numerous participants reported that they were breathing more on MZ, despite no different \dot{V}_E than AZ and PLA, and experienced more central symptoms such as headache. These findings suggested that there was a greater ventilatory drive for a given \dot{V}_E output with MZ. Additionally, RPE-B was not different between the 3 conditions, but RPE-L was significantly lower on MZ; which indicated that participants were more relatively limited by RPE-B than RPE-L on MZ. As performance consists of both

subjective and objective factors, the negative performance effects of MZ might be more subjective in nature, specifically by imposing a dyspneic limitation. Otherwise, there was no clear physiological cause for the decrease in power output with MZ compared to PLA.

6.3 Implications

An earlier study had used lower doses of AZ (250 mg per day vs 750 mg per day) and found no differences in \dot{V}_E at a given intensity and at maximal exercise compared to PLA. Although the description for RPE assessment was not specified, there were no reported differences in RPE between AZ and PLA (132). A separate study also reported that AMS prophylaxis was similar between a lower dose and standard dose (133). It can be postulated that a lower dose of CA inhibitors may provide benefits compared to the standard dose. Specifically, for MZ, a lesser stimulation of ventilatory drive could potentially diminish dyspneic limitation. The practical implications of the present study would advise that, during rapid ascents to altitude, taking a standard dose of CA inhibitors would not be beneficial, especially during heavy physical exertion, as the ability to exercise would be compromised. Future interventions may consider implementing a lower dose of CA inhibitors to determine if they are indeed beneficial to exercise performance at this specific altitude. On the other hand, the standard dose of MZ may produce different outcomes at a higher elevation, in that the induced hyperventilation could sufficiently increase S_pO_2 enough compared to PLA.

6.6 Limitations and Future Considerations

Numerous limitations were present in this study that needed to be considered for interpretation and formulating future research questions. An equal distribution of male and female participants was not possible due to the eligibility criteria. Many female participants were not on a highly effective birth control; this reduced the pool of female participants that can be recruited.

The duration of the hypoxic exposure was standardized to 12 minutes at most. However, the specific duration varied between participants based on how long it took for them to complete the time trial. Hypoxia is known to have a time-dependent factor, so it is possible that participants with slower time trial times experienced greater effects of hypoxia, compared to participants with the fastest times. The severity of hypoxia, however, was controlled within the participant in that they are breathing in same fraction of O₂ for all three time-trials.

The level of CA inhibition may vary due to differences in body mass. To standardize the relative dose, it will require making individual prescriptions according to bodyweight, which was not possible for this study. In addition, the dosages of AZ and MZ might not produce similar levels of CA inhibition. It was not the focus of this study to confirm that the magnitude and distribution of CA inhibition was similar between the two drugs. For our current sample size, body mass did not have a significant relationship with the change in TT performance from AZ and MZ ($p > 0.05$). Participants were only ensured to not fall in the high dose range, where it would result in complete impairment of CO₂ transport due to inhibition of CA enzymes in erythrocytes.

Neuromuscular assessments were done in normoxia, rather than hypoxia. Participants in this study were immediately switched to normoxia upon completion of their time trial. Previous literature observed that the recovery period after exercise was found to be shorter in normoxia compared to acute hypoxia (134). If the participants were to remain in hypoxia for the entire study, including both the neuromuscular tests and time trial, the fatiguing effects of AZ and MZ might be more exaggerated. In addition, voluntary contractions were not a true objective measure of neuromuscular function and fatigue as it can be influenced by subjective factors. The results of MVCs could also be affected by central fatigue, but it was found that there were no differences in central fatigue between conditions, as indicated by the handgrip torque values (Table 6). A future study assessing fatigue

should incorporate more objective neuromuscular assessments, such as twitch torque from electrical or magnetic stimulation.

Hypobaric hypoxia and normobaric hypoxia may be physiologically different, but there was no clear consensus (135–138). Within a time course of a 40-minute hypoxic exposure, \dot{V}_E in standard temperature pressure dry (STPD) was found to be lower in hypobaric hypoxia compared to normobaric hypoxia (135). When expressed as body temperature pressure saturated (BTPS), \dot{V}_E was no significantly different between normobaric and hypobaric hypoxia; similar results were observed in a 6-hr hypoxic bout (139). However, it was observed that F_b was significantly higher during the initial 10 minutes, which led to the postulation that hypobaric hypoxia increases ventilatory dead space for a given \dot{V}_E compared to normobaric hypoxia (135). Since the duration of 5-km TT falls within that time course, hypobaric hypoxia may have different performance outcomes with hypobaric hypoxia, as V_T was greater in AZ and MZ compared to PLA (Table 5). Hypobaric conditions may also reduce the work of breathing due to the lower atmospheric pressure, thus affecting the magnitude of respiratory metaboreflex on exercise performance. Furthermore, our hypoxia exposure involved no amount of inspired CO_2 . So, the results were limited to the sole effect of lower F_{IO_2} , excluding the effects of hypobaria and changes in CO_2 . However, the effect of CO_2 in hypobaria might be negligible as the partial pressure of CO_2 will be also lower than that of sea level, thus further decreasing the effects of ambient CO_2 on ventilatory drive during exercise.

Age was previously observed to increase the prevalence of side effects and symptoms with administration of CA inhibitors (140, 141). Within this study, time trial performance was found to be greatly affected by the drugs in older participants (>30 years), as evidence by more reported symptoms and more drastic impairments in performance (+30s compared to PLA). On the contrary, in some younger participants, time trial performance was not greatly affected by the drugs and no

symptoms were reported (Table 8). There was not a statistically significant relationship between age and the change in TT performance with AZ ($p = 0.09$) and MZ ($p = 0.33$), given our current sample size. A future study discerning age differences may be warranted as certain occupations involving altitude ascents comprise largely of individuals above 40 years of age.

Analyzing blood gas data from capillary blood had some limitations, as it could neither be explicitly arterial or venous blood if not addressed properly. In other words, the sampling conditions before and after exercise might not be equivalent. The water bath was used to sufficiently vasodilate the capillaries so the PCO_2 values obtained will match that of arterial blood (142, 143). Following 6 minutes of exercise cessation, perfusion rate, blood pressure and body temperature may already be reduced from that at termination of exercise. It cannot be assumed that the capillary blood sample was like arterial blood, thus PCO_2 values obtained after exercise should be interpreted with caution. To confirm the presence and magnitudes of CO_2 gradients, arterial and venous catheters will be required.

7.0 CONCLUSION

Carbonic anhydrase inhibitors act negatively on exercise performance in acute hypoxia beyond arterial oxygenation. AZ and MZ also had unique mechanisms that hindered exercise performance. Acetazolamide induced peripheral muscle function, while MZ with an unclear mechanism. Whether AZ and MZ will have similar effects with longer duration exercise, chronic hypoxic exposure or lower dose remains to be determined.

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