The Impact of Two Different Dosing Courses of Acetazolamide on Ventilatory Sensitivity to Hypoxia and Hypercapnia in a Young and Old Cohort – A Comparison Study

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Abstract

Introduction – Acetazolamide (Az) is a carbonic anhydrase (CA) inhibitor used to treat acute mountain sickness (AMS). Current dose recommendations are to take 250mg of Az twice daily (BD) for 48 hours. However, evidence indicates that, due to impaired renal function, older people may require less to get the same protective effect. The present study aimed to assess this hypothesis by testing the hypoxic ventilatory response (HVR) and hypercapnic ventilatory response (HCVR) following the administration of Az at two different doses in young and older individuals.

Methods – 13 participants were recruited (7 young, M = 24.3 ± 3.1 and 6 old, M = 71 ± 2) and performed a HVR and HCVR using a steady state method on 3 occasions: a no drug control, after 125mg Az BD for 48 hours and after 250mg Az BD for 48 hours. Az-induced alterations in acid-base balance were confirmed via blood gas sampling on each visit.

Results – Both doses of Az caused significant reductions in bicarbonate (HCO_3^-), pH and base excess whilst also stimulating resting ventilation in both groups ($p<0.001$ for all). Hypoxic sensitivity was significantly blunted in the older group on Az ($p=0.031$). In contrast it was the young group that developed a blunted hypercapnic hypoxic response on Az, with a significant reduction in the slope ($-0.013x \pm 0.007x$ vs $-0.0098x \pm 0.006x$, $p=0.025$) and intercept (1.01 ± 0.5 vs 0.77 ± 0.4, $p=0.019$) of the ventilation line of best fit. Estimated glomerular filtration rate (eGFR) was significantly lower in the older group (125.2 ± 17.8ml/min/1.73m^2 vs 87.7 ± 8.1ml/min/1.73m^2, $p=0.001$).

Discussion – Alterations to acid-base balance were caused by 125mg Az BD in both cohorts, indicating the effectiveness of Az at inhibiting renal CA at 125mg BD. The lower eGFR recorded in the older participants would reduce the clearance of Az of the older participants. The impaired clearance of Az most likely caused the blunting effect of the HVR seen within

the older group, as Az would accumulate in the circulation and inhibit off-target CA isoforms within the peripheral chemoreceptors (PCR), reducing the ventilatory drive. In contrast, it was the young cohort who experienced a blunted HCVR after using Az. Inhibition of CA in the red blood cells (RBC) causes CO_2 retention and so may reduce the offloading of CO_2 at the blood brain barrier (BBB) causing this blunting effect. Compensation for this action may arise through a separate mechanism in the older participants.

Contents

ABBREVIATIONS

AMS	Acute Mountain Sickness
Az	Acetazolamide
BBB	Blood Brain Barrier
BD	Twice Daily
Bz	Benzolamide
CA	Carbonic Anhydrase
CCR	Central Chemoreceptor
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
eGFR	Estimated Glomerular Filtration Rate
f_R	Breathing Frequency
GFR	Glomerular Filtration Rate
HAI	High Altitude Illness
HACE	High Altitude Cerebral Oedema
HAPE	High Altitude Pulmonary Oedema
HCVR	Hypercapnic Ventilatory Response
HPV	Hypoxic Pulmonary Vasoconstriction
HVD	Hypoxic Ventilatory Decline
HVR	Hypoxic Ventilatory Response
ICP	Intracranial Pressure
Mz	Methazolamide
NO	Nitric Oxide
PAP	Pulmonary Arterial Pressure
PCR	Peripheral Chemoreceptor
PCO_2	Arterial Pressure of CO_2
PO_2	Arterial Pressure of O_2
$PetCO_2$	End tidal pressure of CO_2
$PetO_2$	End tidal pressure of O_2
RBC	Red Blood Cell
SaO_2	Arterial Oxygen Saturation
STD	Short Term Depression
VAH	Ventilatory Acclimatisation to Hypoxia
VEGF	Vascular Endothelin Growth Factors
V_T	Tidal Volume

1 LITERATURE REVIEW

High altitude illness (HAI) is a term that incorporates both cerebral and pulmonary syndromes that arise from unacclimatised individuals ascending rapidly to high altitudes that are significantly greater than the persons living altitude. These conditions are known as AMS, high altitude cerebral oedema (HACE), which lies on the same spectrum but is a more serious progression from AMS, and high altitude pulmonary oedema (HAPE) (Hackett & Roach, 2001). The following literature review will explore the epidemiology and pathophysiology of these disorders and focus on the current use of carbonic anhydrase (CA) inhibitors for prophylactic treatment. Following this there will be an overview of the current understanding of the ventilatory responses to hypoxia. The main body of this thesis will examine the negative effects of one particular CA inhibitor (Az) and explore whether older adults require a smaller dose to receive similar therapeutic effects as the young.

1.1 Epidemiology of HAI

AMS is a cerebral condition that, in most cases, develops after rapid ascent to high altitude that lacks a sufficient acclimatisation period. AMS symptoms are more common at altitudes exceeding 4000m, with incidence positively correlating with increasing altitude. Prevalence in a group of trekkers was 8.5% at 2850m and rose to 53% after continued ascent to 4559m (Maggiorini, et al., 1990).Yet the presence of AMS symptoms has similarly been noted in tourists visiting moderate altitudes as low as 1920m (Honigman, et al., 1993). It is a syndrome which is self-diagnosed through nonspecific symptoms that can lead to a subjective diagnosis. Which potentially explains why diagnosis is reported at the lower altitudes. Since 1993, the Lake Louise Questionnaire has been the universal method used during a sojourn to high altitude for diagnosing AMS, stating that it is the presence of a headache, plus one or more of the following: 1) gastrointestinal problems 2) fatigue &/or weakness 3) dizziness/light-headedness 4) difficulty sleeping. Symptoms are scored in the range of 0 to 3 for each category, with total scores of 3-5 indicating mild AMS, 6-9 suggesting moderate AMS and

10-12 proposing severe AMS (Roach, et al., 1993). Recently the Lake Louise consensus committee updated the questionnaire by removing difficulty sleeping from the symptom list in an attempt to improve the accuracy of diagnosis (Roach, et al., 2018). One of the main risk factors in developing AMS is the speed of ascent. Incidence increases when the ascent profile does not allow for an adequate acclimatisation period. Blosch and colleagues (2009) found that 50% of trekkers self-diagnosed AMS when taking 12 days to climb to 6265m whereas, in contrast, only 19% experienced any AMS symptoms when completing the same trek in 16 days. The current guidelines by the Wilderness Medical Society state that, above an altitude of 3000m, a person should not increase their sleeping elevation more than 500m per night, with rest days regularly incorporated (Luks, et al., 2010). Other predictors of AMS include: lower arterial oxygen saturation (SaO_2) under hypoxic conditions (Burtscher, et al., 2004), previous history of AMS and a previous history of migraines, the latter of which is supportive of the tight-fit hypothesis (discussed further in section 2.1) (Richalet, et al., 2012). Despite common assumptions, ageing does not influence a person's susceptibility for AMS. Whilst few studies have shown an inverse correlation between age and susceptibility (Gaillard, et al., 2005; McDevitt, et al., 2014), a recent meta-analysis looking at 17 studies involving 4824 participants (aged 10-76) travelling to high altitude (>3000m) found no association with age and the occurrence of AMS (Wu, et al., 2018). The alternate findings mentioned previously are likely due to a low completion rate within these studies and the physical limitations of the older people causing slower and less extreme climbs. In relation to this, fitness levels have also been shown to not be an indicator of a person's likelihood of developing AMS (Honigman, et al., 1995). This may be due to less fit people being less likely to attempt as severe climbs and are more likely to ascend at a slower rate. When AMS does develop, the symptoms usually resolve themselves after a few days of acclimatisation, or are easily

treatable through either medication, quick descent to a lower altitude or, if available, with the use of oxygen therapy.

If symptoms of AMS are left untreated or ignored, it can develop into the more serious and potentially fatal condition of HACE. HACE can cause a person to become confused, disoriented and ataxic. In very serious cases it can lead to the individual to become comatosed. This condition is often fatal in the extreme environments in which they occur if left untreated (Hackett & Roach, 2001). However, due to the effective treatment options for AMS, HACE is relatively uncommon in climbers, with its incidence reported as being between 0.5% - 4% of mountaineers, varying between people and from the maximum altitude reached (Rawal & Juan Pablo Cruz, 2019). It is extremely rare for HACE to develop without previous AMS symptoms which is why cases are much less frequent as prophylactic treatment or rapid descent is usually performed before HACE can develop.

HAPE is the second potentially lethal condition that can arise upon exposure to high altitude. The primary cause of HAPE is thought to be due to excessive hypoxic pulmonary vasoconstriction (HPV) causing raised pulmonary arterial pressure (PAP) (Mortimer, et al., 2004). This can lead to fluid leaking from pulmonary capillaries into the interstitial space, interfering with the lungs diffusing capacity. HAPE may be preceded by AMS although, unlike HACE, the two aren't inextricably linked and so it can occur in isolation. Risk factors for HAPE are similar to AMS, however the underlying cause is believed to be genetic and so repeat exposure to altitude does not reduce a person's chances of developing the condition. On the contrary people who develop HAPE once run a significant risk of recurrence (Mortimer, et al., 2004) primarily due to different pulmonary haemodynamics and a decreased capacity to regulate vessel tone in the pulmonary circulation. Dyspnoea (breathlessness) occurs during exertion and in worsening cases at rest too, alongside a dry cough and general weakness. This is exasperated if treatment is not sought which, like HACE, can become fatal.

HAPE is rare within the general mountaineering population, with incidence being reported at <0.2% (Hochstrasser, et al., 1986). However, in the same region, when ascending rapidly (>600m/day) the frequency rises to 4% in climbers (Maggiorini, et al., 1990). Hochstrasser (1986) noted that of the 50 climbers being evacuated by helicopter for HAPE, 20% of them had also developed HACE. It is believed this is in part due to worsening gaseous exchange within fluid filled lungs and a further reduction in SaO_2. Like HACE, treatment for HAPE involves prophylactic medication, immediate descent to lower altitudes, the use of oxygen therapy or recompression in a hyperbaric chamber if descent is not possible.

1.2 Pathophysiology of AMS and HACE

Despite extensive research within the field, the specific aetiology of AMS and HACE remain elusive. Hypoxemia is thought to play a central role in inducing AMS, however it cannot be considered the sole component based off two observations: 1) there is a delay upon exposure to high altitude and the onset of AMS symptoms and 2) symptoms are not immediately reversed when oxygen levels return to a normoxic levels. AMS more likely arises from a multifactorial origin as individuals who persistently suffer from AMS present a series of consistent maladaptive conditions in response to hypoxia that contrast to those who remain free from the condition (Imray, et al., 2010).

Elevated intracranial pressure (ICP) and increased cerebral volume are thought to be salient mechanisms in the development of AMS and HACE (Imray, et al., 2010). Both of which are determined by the balance between hypoxia induced vasodilation and hypocapnia induced vasoconstriction. An important expedition lead by Dr Brian Cummings in 1985 documented the contribution of elevated ICP towards AMS, whereby using telemetric ICP monitoring they took direct measurements of changes in ICP during a sojourn to high altitude. He observed a strong relationship between headache score and increased ICP (Wilson & Milledge, 2008). A further finding was that participants with the smallest cerebral ventricles had the largest AMS scores (Wilson & Milledge, 2008). The small ventricles cause the CSF to occupy a greater percentage of the ventricular volume, thus making them less responsive to volume changes in the brain. Upon publication this evidence supported a growing theory known as the ‘tight-fit hypothesis’, whereby people with less compliant cerebrovascular systems have increased susceptibility to AMS and/or HACE. The theory helps to explain why previous research has shown no relationship between increased cerebral blood volume and AMS development (Baumgartner, et al., 1999), as individual variability in ventricle size and compliance allows for rises in a pressure gradient within the cerebral circulation to be tolerated better in some

than others. Smaller increases in volume have an increased risk of maladaptive conditions in certain people and, as such, partly accounts for the large interindividual variability in AMS development. Less compliant cerebrovascular systems and thus, raised ICP, usually results in a headache which is one of the first signs that AMS is beginning to develop. This sensation of pain is caused by hypoxia stimulating the production of nitric oxide (NO) from endothelial nitric oxide synthase (Beleslin-Cokic, et al., 2004). NO accumulates along unmyelinated trigeminal fibres causing them to become sensitized (Sanchez del Rio & Moskowitz, 1999). Increased ICP causes pressure activation of the sensitized fibres onto the trigeminal ganglia, which project onto the cortex (Luks, et al., 2017) and acts as a primary contributor to the high-altitude headache common amongst AMS sufferers.

Progression to cerebrovascular oedema occurs via mechanical and cytotoxic factors. Circulating concentrations of free radicals and vascular endothelin growth factors (VEGF) increase in hypoxia which provides chemical stress that can act on the BBB and increase its permeability (Bailey, et al., 2009). This allows proteins and fluids to translocate through the BBB into the intracranial space in a process known as vasogenic oedema (Hackett, 1999). Mild vasogenic oedema is present in most people ascending to moderate or high altitude even in the absence of AMS and is related to increased cerebral perfusion (Kallenberg, et al., 2007). However, HACE is thought to develop through the extravasation of the RBC causing excessive oedema (Tokum, et al., 2016).

The diuretic and natriuretic effects of high altitude are less evident in those who are prone to AMS. Early evidence for the anti-diuretic effect was demonstrated by Peter Hackett (1982) who showed a weight gain of >2% in trekkers who were diagnosed with AMS after travelling to Pheriche, Nepal (4243m) despite suffering from loss of appetite and vomiting. Both hypoxia and physical activity have been shown to reduce glomerular filtration rate (GFR) whilst stimulating aldosterone and vasopressin release. The former being due to sympathetic

stimulation vasoconstricting the renal circulation causing renal hypoperfusion (Imray, et al., 2010) and the latter through activation of the renin-aldosterone system (Milledge, et al., 1982). Both mechanisms can, therefore, contribute to antidiuresis and anti-natriuresis at altitude (Bärtsch, et al., 1991). So, it's possible that in previous research conducted using an active ascent to altitude, the exertion required to arrive at altitude may be partly responsible for the fluid retention seen in mountaineers who develop AMS. However, research using simulated or passive ascent to altitude have also displayed significant fluid retention in people who develop AMS in the absence of any physical exertion. Under resting conditions, a 12-hour protocol within a hypobaric chamber at 426mmHg (~4880m) showed significant fluid retention within the first 3 hours of hypoxic exposure in participants who are AMS susceptible (Loeppky, et al., 2005); findings that indicate fluid and sodium retention are a presage to AMS. One study has reported that AMS symptoms act as a precursor to AMS (Westerterp, et al., 1996), however the majority of literature seems to indicate that fluid retention occurs before AMS symptoms develop (Loeppky, et al., 2005; Hackett, et al., 1982). The aforementioned high altitude fluid retention could be driven by an increased sympathetic drive. Epinephrine levels have been shown to increase in people who develop AMS (Kamimori, et al., 2009). Similarly, subjects who took the β-blocker propranolol had less pronounced AMS than those taking a placebo (Fulco, et al., 1989). These findings give evidence that sympathetic activation is the antecedent to fluid retention at high altitude Therefore hypoxia, in the absence of exercise, disrupts the relationship between fluid-regulating hormones which plays a role in the development of AMS. It must be noted that there is conflicting research with regards to the pathophysiology of AMS and HACE within the current literature, owing in part to large interindividual variability with the illness and the complexity of the multiple factors believed to be associated.

1.2.1 Pathophysiology of HAPE

Greatly elevated PAP due to exaggerated HPV is the most common feature of people who suffer with HAPE. In day to day life, the role of HPV is to shunt blood away from poorly oxygenated areas of the pulmonary circulation to maximise the ventilation/perfusion (V/Q) matching. The response originates from foetal life whereby blood bypasses the foetal lungs, instead moving through the foramen ovale. An excessive HPV response in hypoxia may be explained by an imbalance in biomechanical mediators. Reduced NO production (Duplain, et al., 2000) and elevated endothelin-1 levels (Sartori, et al., 1999) have been observed in HAPE susceptible individuals. However, exaggerated HPV (59±11mmHg) has been shown to not be enough by itself to trigger HAPE in individuals who are prone to the disease (Sartori, et al., 2000). An imbalance between the secretion and reabsorption of alveolar fluid, due to impaired sodium transport across alveolar epithelial cells, also contributes to the development of HAPE, specifically at the amiloride-sensitive sodium channel (ENaC). ENaC knockout mice die soon after birth due to an inability to clear their lungs of fluid (Hummler, et al., 1996). In humans, a significantly lower concentration gradient was shown in a group of HAPE susceptible people in comparison to HAPE resistant which is suggestive of impaired sodium clearance (Sartori, et al., 2004). Similarly, when using a β_2 agonist (promoting ENaC activity) HAPE incidence was reduced by 50% (Sartori, et al., 2002). HAPE therefore most likely arises from an imbalance between increased HPV (from raised PAP) and reduced fluid clearance.

1.3 Prophylactic Treatment of AMS – Carbonic Anhydrase Inhibitors

CA inhibitors are one of the more popular pharmacological methods for prophylactic treatment of AMS. Inhibition of CA isoforms in the renal, vascular endothelial, erythrocyte and CNS appear to be the most beneficial for AMS treatment (Joyce, et al., 2018). CA are metalloenzymes which contain one zinc atom in each molecule at the active site. The enzyme comes in 6 distinct families known as alpha (α), beta (β), gamma (γ), delta, èta and zèta. These families all perform the same function yet have very little structural similarities. αCA is the subset most represented in mammals, with βCA abundant in plants and γCA in methane producing bacteria. There over 12 active CA isoenzymes located throughout the human body (illustrated in table 1) which catalyse the interconversion of carbon dioxide (CO_2) and water (H_2O) to HCO_3^- and hydrogen (H^+), each with a slight sequence variation leading to small but specific differences in their activity (Shuchismita & Goodsell, 2004). Az is the most commonly used CA inhibitor for the treatment of AMS, however there are two other CA inhibitors (benzolamide and methazolamide) that have been studied for their capacity to attenuate AMS symptoms. The following section will review the pharmacodynamics of these three versions.

Table 1. Location and sensitivity of CA isoforms within the human body. Information in this table was sourced from Medical Physiology: a cellular and molecular approach by Walter Boron (2005) and from an article by Hilvo, et al., (2008).

Isoform	Subcellular Location	Tissue Location	Sensitivity to Acetazolamide, K_I (nM)
CA-I	Cytosol	RBC and GI tract	250
CA-II	Cytosol	Widespread	12
CA-III	Cytosol	Type 1 muscle	$2x10^5$
CA-IV	GPI-anchored	GI-tract, kidney, endothelium	74
CA-VA	Mitochondria	Liver	63
CA-VB	Mitochondria	Widespread	54
CA-VI	Secreted	Saliva	11
CA-VII	Cytosol	Widespread	2.5
CA-IX	Transmembrane	GI-tract	25
CA-XII	Transmembrane	Kidney	5.7
CA-XIII	Cytosol	Widespread	16
CA-XIV	GPI-anchored	Kidney, heart, skeletal muscle, brain	41

1.3.1 Acetazolamide

Acetazolamide (Az) is the most commonly used CA inhibitor amongst mountaineers for the prophylaxis against AMS (Luks, et al., 2016). It is a sulphonamide derivative with a molecular formula of $C_4H_6N_4O_3S_2$, and acts as a non-competitive inhibitor to CA by binding to the zinc ion via the nitrogen atom on their sulphonamide chemical group . It can be taken either via oral or intra-venous administration, with oral being the most preferred method due to the ease of administration. When taken chronically, the drugs primary target site is in the renal system. Inhibition of CA isoforms II & IV within the proximal and distal tubule disrupts

HCO_3- reabsorption and H^+ secretion, causing alkalotic diuresis. The resulting metabolic acidosis drives hyperventilation which helps to attenuate the drop in SaO_2. Acutely, at a dose of around 7mg/kg, Az inhibits RBC CA leading to an immediate retention of CO_2 that triggers hyperventilation through tissue acidosis (Swenson & Hughes, 1993). Tissue acidosis has been argued by some to be the primary stimulus by which SaO_2 is increased when taking Az, with renal CA inhibition offering no additive effect (Vovk, et al., 2000). Whereas others have argued this is only an additional stimulus on top of metabolic acidosis (Swenson, 1998). There is a case for both arguments when Az is used at altitude to counteract already present AMS symptoms due to their collective effects on stimulating respiration. However, it is recommended for people preparing for a sojourn to high altitude to begin taking the medication 1-2 days before leaving, thus pre-acclimatising their bodies to hypoxic environments. Under these circumstances, it is most likely that renal CA inhibition plays a predominant role. After 1-2 days at altitude, the renal system begins to reabsorb less HCO_3-, therefore restoring the hypoxic ventilatory drive which is initially reduced due to hypocapnia arising from hyperventilation (Taylor, 2011). Taking Az prophylactically before ascent allows an individual to undergo renal acclimatisation prior to a sojourn and therefore minimise the hypoxia induced respiratory alkalosis and maintain a hypoxic ventilatory drive.

The prophylactic effects of Az on AMS are robust and very well documented across the literature e.g. (Richalet, et al., 2012; Bradwell, et al., 2014; Low, et al., 2012). Conversely though, several adverse effects are also often observed after Az ingestion such as taste alteration, nausea, abdominal pain and paraesthesia. Perhaps of greater concern to mountaineers though is the reduction to performance which has become apparent with Az ingestion. Time to exhaustion at both submaximal and maximal exercise has been shown to be reduced at sea level whilst taking Az (Posch, et al., 2018). Under acute hypoxic conditions in a normobaric chamber, maximum power output was significantly less after Az ingestion

compared with placebo (Garske, et al., 2003). Similarly, after prolonged exposure at natural altitude, a reduced maximum power output and a lower VO_2 max have been observed (Bradwell, et al., 2014; Bradwell, et al., 2018). An important recent study quantified the decrements Az can have on muscle function by showing significant compromises of both diaphragm (18%) and locomotor (37%) muscles at baseline and after exercise in normoxia (Dominelli, et al., 2018). CA III is predominant in type 1 skeletal muscle, but this isoform is minimally inhibited by Az (Sly & Hu, 1995). Impairments in exercise performance most likely arise from alterations in cardiac functionality (Bradwell, et al., 2018). CA IV is abundant in the heart (Baird, et al., 1997), therefore, any accumulation of Az within the circulation may reduce cardiac functionality during dosing. Suppression of CA IV could cause intracellular acidosis within the heart which may slow the calcium ($Ca2^{+}$) transient, impairing left ventricular function and reducing cardiac output. This would reduce the already limited oxygen delivery to the working muscles further, leading to an earlier onset of fatigue.

In addition, Az has also been shown to impact cognitive performance (Wang, et al., 2013; Phillips, et al., 2017). While hypoxia alone is known to increase the incidence of neuropsychological impairment (see Phillips, et al., 2017), Az appears to have an additive effect to this. This is most likely caused from CA inhibition within the central nervous system (CNS) which can cause a number of different effects. CA plays an important role in long-term synaptic transformation, signal processing and attention gating (Sun & Alkon, 2002) and inhibition can nullify the acetylcholine-mediated theta activity in the hippocampus, an important process in information processing and memory consolidation (Sun, et al., 2001). A small dose of Az (125mg BD) has shown additive impairments in: cognitive performance, executive function, short-term memory and sustained attention within a young cohort in acute hypoxia (Wang, et al., 2013). In a separate study, the prevalence of cognitive impairment was far higher in people who were taking Az (47.8% vs 10.7%) (Phillips, et al., 2017). It is

integral for climbers, particularly at extreme altitudes, to maintain their cognitive capacity as small errors whilst climbing can prove to be fatal.

To lessen these adversities, identifying the lowest possible dose for prophylactic treatment of AMS is critical. One that counteracts any AMS symptoms whilst limiting superfluous doses which can cause negative side effects. Although there is no established consensus on a specific treatment plan for Az, current recommendations for prophylaxis of AMS is to take 250mg-500mg BD in capsule form (the higher dose recommended to those taking unavoidable fast ascents) taken 1-2 days before ascent or until symptoms are alleviated if beginning treatment whilst already at altitude (Multum, 2019). However current dose recommendations may need to be individualised based on the age of the individual who is travelling (Bradwell, et al., 2018), which is discussed in more detail within the introduction.

1.3.2 Methazolamide

Methazolamide (Mz) is a lipophilic methylated alteration of Az with much more specific target isoforms within the kidney. Oral administration of Mz shows SaO_2 rises similarly to Az with comparable reductions in AMS scores for both drugs (Wright, et al., 1994; Wright, et al., 1983). Mz penetrates better into tissues and also inhibits membrane-bound CA. This is demonstrated when comparing the HVR after Mz or Az administration. It has been shown that the magnitude of the HVR was reduced by Az (after intravenous application) but not with Mz, therefore indicating that CA inhibition is unlikely the cause of the reduction induced by Az (Teppema, et al., 2006a). Additionally, there are fewer side effects reported after using Mz orally. The reductions in muscle function and performance seen after oral Az ingestion are not shared by Mz (Dominelli, et al., 2018). Specifically, following a period of exercise to exhaustion, Az exaggerated diaphragm fatigue ($87 \pm 9\%$, $82 \pm 10\%$ of pre-exercise value) and dorsiflexor fatigue (twitch torque of $61 \pm 11\%$ vs. $57 \pm 13\%$ of baseline) compared with Mz. The differences in their effects are probably a result of differences in the pharmacodynamics

of the two drugs. For example, in vitro, a comparison study has shown Mz to be an activator of the transcription factor nuclear related factor 2 (NrF2) (Lisk, et al., 2013). NrF2 is activated by the body as a defence mechanism when oxidative stress is increased through increased reactive oxygen species production in the brain. Therefore, the mechanism by which Mz is reducing AMS symptoms may be more due to reducing cerebral vascular leak via activation of NrF2 and not hyperventilation/increased PaO2. Research on this drug for AMS treatment is still in an early phase and future work is required to see if these effects are pronounced in clinical trials.

1.3.3 Benzolamide

Benzolamide (Bz) is a more hydrophilic inhibitor of CA than Az and is ten-fold more potent. Bz retains a strong renal action to induce metabolic acidosis but its limited membrane permeability restricts its uptake into the CNS, indicated by a reduction in psychomotor effects compared with Az (Collier, et al., 2016). To date only one study has analysed the effects of Bz on treatment for AMS, demonstrating that Bz reduced AMS scores compared with placebo (~50%) during a trek to Everest Base Camp (after ascent above 4240m). The Bz group also had lower pH, HCO_3- and $PaCO_2$ scores. Interestingly, despite a supposed reduction in CNS uptake, paraesthesia was still reported in 8/12 participants taking Bz at base camp (Collier, et al., 2016). Like Mz, Bz may become a more preferential choice for prophylaxis of AMS, however significant future research is required before recommendations can be made.

1.4 Ventilatory Responses to Hypoxia and Hypercapnia

The following section will review the current understanding of the different phases of the ventilatory response to hypoxia. It is a complex process with multiple stages ranging from an instant response to the long term ventilatory acclimatisation to hypoxia (VAH). Following this there will also be a section focusing on the HCVR and the role of the central chemoreceptors (CCR).

1.4.1 Acute Response

Immediately after exposure to hypoxia, the O_2 sensitive carotid chemoreceptors produce an immediate response to attenuate the reduction in PaO_2. The carotid body contains large sinusoids that have a very high perfusion rate which allow for this rapid response to changes in PO_2. Hypoxemia causes a Ca^{2+} influx into type 1 (glomus) cells which, when a threshold is reached, depolarizes the cell and releases neurotransmitters into the glossopharyngeal nerve. These axons enter the brain stems respiratory network and trigger hyperventilation through efferent responses at the diaphragm, inspiratory and expiratory muscle groups (Teppema & Dahan, 2010). Increases in respiratory frequency (f_R) and tidal volume (V_T) occur following immediate onset of a isocapnic hypoxic stimulus and terminate immediately after withdrawal (Powell, et al., 1998). Evidence indicates that poikilocapnic hypoxia alters only V_T which is indicative that hypocapnia (caused by the hyperventilation during poikilocapnic hypoxia) suppresses the peripheral drive to increase f_R (Steinbeck & Poulin, 2007). Due to the nature of the O_2-dissociation curve, hyperventilation is not usually triggered until PO_2 falls below a certain threshold (~70mmHg) after which limitations in O_2 diffusion at the alveolocapillary membrane arise (Powell, et al., 1998). Ventilation can continue to rise for up to 1 minute following a hypoxic stimulus in a process known as short term potentiation (STP) (Eldridge & Millhorn, 2011). This short stage of ventilation shares similar properties with the acute response and their time domains are closely linked. But STP occurs following a continued hypoxic stimulus of the carotid sinus nerve, which results in Ca^{2+} accumulation in presynaptic

areas of the ventilatory reflex loop. This increases the glutamate release from the carotid bodies causing ventilation to continue to slowly rise (Wagner & Eldridge, 1991). This could help to prevent a reflex activation of the short-term depression/HVD stages that follow and maintain an acute hypoxic ventilatory drive.

1.4.2 Short Term Depression

Acutely, the HVR is at its most sensitive straight after hypoxic exposure with sensitivity slowly declining following a sustained stimulus. Bascom and colleagues (1990) used hypoxic pulses within a sustained short-term hypoxic stimulus to test whether peripheral chemosensitivity is maintained and noticed a depressive effect (~4.2%.min) with a plateau of sensitivity at around 15 minutes into the stimulus. The depressive effect persists after re-institution to normoxia with full recovery of chemosensitivity taking up to 35 minutes (~1.9%.min for re-sensitisation. Recovery was accelerated if hyperoxic conditions were introduced (Vovk, et al., 2004). Despite being similar to the HVD, several key differences between this and the short-term depression (STD) stages are noted: Firstly, STD is primarily through a reduction in $f_{R,}$ whereas HVD arises mainly through a reduction in V_T (Pamenter & Powell, 2016). Secondly, the time courses of the 2 stages vary (~30s-3mins in STD vs >3mins with HVD). Thirdly, it is believed the neurochemical mechanisms behind each stage are different. Lesion of the ventrolateral pons in anesthetized rats abolish the STD response indicating the CNS circuitry of the HVR is critical in eliciting STD (Coles & Dick, 1996). STD is the first sign that hypoxia causes plasticity in the respiratory pathway. More specifically it produces metaplasticity, whereby the history of stimuli modulates the future response to forthcoming stimuli (Pamenter & Powell, 2016). Unlike the acute response, the timeline for the occurrence of STD is less established since differences in methodology such as experimental design or species tested can produce variable findings (Coles & Dick, 1996; Turner & Mitchell, 1997; Steinbeck & Poulin, 2007).

1.4.3 Hypoxic Ventilatory Decline

HVD usually begins to occur after ~3 minutes with the length of this stage varying slightly between individuals (Hupperets, et al., 2004). HVD causes ventilation to drop to a new plateau (but one that is still elevated above normoxic levels) after the initial peak response. Thus, HVD is what chains the temporal gap together between the acute response and VAH. The HVD slope is similar in both isocapnic and poikilocapnic conditions which points towards a hypoxic induced adaptation, independent of PCO_2 (Steinbeck & Poulin, 2007). However, a strong reduction in the V_T of HVD is observed in experiments on poikilocapnic hypoxia, with minimal effects on f_R (Pamenter & Powell, 2016). Conversely during isocapnia, although not as pronounced as V_T, decreases in f_R are observed (Steinbeck & Poulin, 2007). The physiological mechanisms of HVD are difficult to study due to variability between sexes, species, experimental procedure and genetic strains. However, current theories surrounding the physiological mechanisms responsible for HVD include: reduction in phrenic nerve activity (Soto-Arape, et al., 1995); peripheral de-sensitization (Dahan, et al., 1996); increases in cerebral blood flow (Teppema & Dahan, 2010); inhibitory neurotransmission between carotid body afferents & CNS respiratory circuits (Pamenter & Powell, 2016), and glutamic acid decarboxylase converting excessive glutamate into GABA within the CNS (Rowley, et al., 2012). It is most likely that a decline in ventilation occurs through several independent mechanisms acting within the same time domain. The true benefit for the HVD is still debated, however several theories have been put forward. Some of the more likely hypothesises for its development include: a reduced metabolic cost of breathing; minimising ventilation/perfusion (V/Q) mismatch, and to allow a ventilatory reserve should PO_2 be further reduced with continued ascent.

1.4.4 Ventilatory Acclimatisation

Despite the initial depressive effect on the PCR's, the magnitude of the HVR remains following periods of acclimatisation and average ventilation continues to rise gradually over

time that is dependent on the living altitude (Hupperets, et al., 2004). Initially it was believed that VAH occurred through changes in central chemoreceptor (CCR) sensitivity caused by adjustments in cerebrospinal fluid (CSF) and arterial pH from chronic respiratory alkalosis, re-setting the CCR's to a lower CO_2 threshold. However, it is now considered that these changes are more so the consequence of VAH and not the cause as compensation appears to lag rather than lead changes in ventilation (Dempsey, et al., 2014). Changes in peripheral chemosensitivity and how the afferent input is processed by the CNS is now generally considered the primary mechanism in contributing to VAH (Powell, et al., 2000). Strong evidence for this theory originates from denervation studies whereby removal of the bilateral carotid bodies abolishes the VAH (Smith, et al., 1986; Barnard, et al., 1987; Olson, et al., 1988). The two primary mechanisms by which the afferent signal of the carotid bodies are altered appear to be because of plastic changes within the O_2 sensing cells and an increase in the release of neurotransmitters. For example, the number of active ion channels (such as the potassium (K^+), Ca^{2+} and sodium (Na^+)) increases, which increases glomus cell excitability after chronic hypoxia (Teppema & Dahan, 2010; Kumar & Prabhakar, 2012). There are several neuromodulators that are thought to contribute to VAH, however substantial evidence points towards the role of dopamine, endothelin and cytokines in increasing peripheral chemosensitivity (Kumar & Prabhakar, 2012). Within the CNS, phrenic nerve activity appears to be elevated following sustained hypoxia (Powell, et al., 2000). The increased activity is likely induced by plasticity which is mediated by, at least in part, changes in neurotransmission, growth factors and inflammatory signals (Powell, et al., 2000). Both mechanisms are controlled by the O_2-sensitive gene expression of HIF-1α. Its concentration increases in both the carotid bodies and CNS after just one hour of hypoxia (Pascual, et al., 2001). The mechanisms by which HIF-1α contributes to VAH have been published in detail elsewhere and the reader is pointed in the direction of an excellent review by Powell & Fu

(2008) which covers the target sites of the transcription factor. In summary they include erythropoietin, vascular endothelial growth factor, endothelin 1, heme oxygenase, tyrosine hydroxylase and NO synthase.

1.4.5 Hypercapnic Ventilatory Response

Unlike the HVR, which is almost solely influenced by the PCR's, CO_2 accumulation during hypercapnia stimulates both the central (via H^+ ions when inside the BBB) and PCRs in order to trigger hyperventilation. Hypercapnia also induces vasodilation in the cerebral arterioles that causes cerebral blood flow to increase to washout any excess CO_2 from the brain tissues. Together both responses act to equilibrate pH via a negative feedback loop. The HCVR can be measured in the laboratory using either the steady-state method or via a rebreathing circuit, with participants claiming the steady-state method is a more comfortable experience due to a reduced sensation of dyspnoea (Mannée, et al., 2018). The carotid body accounts for ~20-30% of the response and is the cause of any immediate changes in ventilation when PCO_2 is increased. The CCR's take longer to activate; requiring between ~5-6 minutes to reach a steady state of ventilation following a hypercapnic stimulus (Vassilakopoulos, 2012). The differences in time constants arises from the location of the receptors within the body and possibly differences in neuronal dynamics. Like with hypoxia, changes in PCO_2 immediately trigger the carotid chemoreceptors as they are positioned close to the bifurcation of the carotid artery so they can sample the concentration of arterial blood H^+ almost immediately as it leaves the heart. However, the PCR influence can be minimised if the HCVR test is performed under hyperoxic conditions. As for the CCRs, it was initially believed that they were localized to the ventrolateral surface of the medulla. Within the medulla, the retrotrapezoid nucleus is considered the primary CCR site (Guyenet, et al., 2012), however, evidence has highlighted separate sites which also contribute to the CCR response located away from the ventral medulla. For example, separate lesions in the glutamatergic neurones in

the retrotrapezoid nucleus (Nattie & Li, 2002), serotonergic neurones in the medullary raphe (Dias, et al., 2007) and noradrenergic neurones in the locus coeruleus (Biancardi, et al., 2007) have all been shown to impair the CO_2-ventilatory response. This points to the HCVR being a complex system of chemoreception. Many types of neurones change their excitability alongside changes in PCO_2.Therefore, the mechanism is not as simplistic as the chemoreflex in the carotid bodies (Nattie & Li, 2009). Due to their location behind the BBB, the CCR's are not directly influenced by the arterialized H^+ concentration as H^+ ions cannot diffuse across the BBB readily (Morrell, et al., 2001). Instead a response is not triggered until CO_2 crosses the BBB, therefore raising the central acid base concentration surrounding the CCR neurones (Duffin, 2005).

2 INTRODUCTION

High altitude environments are becoming more accessible in the modern era. Between 10-25% of adults who travel to moderate altitudes (>2500m) develop AMS symptoms, such as headaches, nausea and fatigue (Honigman, et al., 1993). If left untreated and with continued ascent, have the potential to progress into the more serious conditions of HACE and HAPE. As greater numbers of the general public are travelling to high altitude locations, AMS is becoming an increasingly common public health issue. Furthermore, in line with a demographic switch towards an ageing society there are greater numbers of older people able to journey quickly to high altitude areas. Thus, it is important to consider how aging influences the body's mechanisms to cope with the hypoxic conditions, alongside assessing how the prophylactic effect of medications to attenuate AMS is impacted by age.

Aging is associated with alterations in several of the respiratory mechanisms responsible for mitigating the reduction in PaO_2 induced by hypoxia. Impairments have been seen in respiratory muscle strength, lung exchange surface area and compliance of the chest wall (Janssens, et al., 1999). The internal surface of the alveoli is flattened in older people leading to the reduction in surface areas of the lungs compared with the young (Crapo, 1993). Similarly, impairments in chest wall compliance are most likely related to calcification within the rib cage of older people. Age-relate osteoporosis can also weaken and change the shape of the thorax (Crapo, 1993) resulting in more frequent vertebral fractures. A combination of this, plus increased functional residual capacity in older people, can lead to impaired respiratory muscle strength. It has been shown previously using multiple diaphragm muscle strength tests that older people (mean age = 73) have significantly weaker diaphragm muscles (~18%) than a younger group (mean age = 29) (Polkey, et al., 1997). Furthermore, it is thought that the human carotid body morphology also likely deteriorates with age. Although there are no studies which directly quantify how ageing impacts human carotid body morphology, animal

models have shown that old age deteriorates the ultrastructure of the carotid bodies in rats. Specifically there is a reduction in the Golgi area, there are fewer neurotransmitters within the vesicles, an impaired morphology of the inner mitochondrial membranes and most importantly an increased number of necrotic cells (Pokorski, et al., 2004). As the morphological findings in rats have not been transposed directly onto human carotid bodies, we cannot say for certain that these impairments also occur in humans. However, it is known that there are similarities between the both the function and morphology of the carotid bodies between primates and rats (Clarke, et al., 1993). For example, the size of the microvascular bed, when expressed as a percentage of the total volume of the carotid body, is 5.4% and 5.7% in primates and rats respectively (Clarke, et al., 1993). In addition the two species share similar rates of blood flow to these areas ($0.59mlx10^{-3}/s$ vs $0.29mlx10^{-3}/s$ for primates and rats respectively) (Clarke, et al., 1993). Therefore, it is likely some of the findings in rats of morphological impairments in the carotid bodies can relate to human models. Despite the likely aforementioned impairments, the chemosensitivities (measured during HVR tests) of older people remains largely unaltered with increasing age (Paleczny, et al., 2014) with one meta-analysis showing increasing sensitivities with age (Lhuissier, et al., 2012). There are 2 main arguments as to why this may occur: 1) humans possess a surplus of chemoreceptor cells from birth and not all the cells are concurrently required to generate the HVR. Under this hypothesis, the viewed necrosis of chemoreceptor cells wouldn't influence their functionality. This is like how the brains neurones deteriorate with age, but this is not directly linked to impairments in the brains functionality (Peters, 2006). Or 2) Plastic changes occur within the brain that mitigate the morphological impairments within the carotid bodies. The brain displays a great degree of plasticity that allows it to adapt to changes in incoming information that maintain important reflex responses. Although the prior two hypotheses can be used to explore why ventilatory sensitivity to hypoxia remains intact in the elderly, it has not been

fully elucidated as to where the exact mechanism behind this preservation lies. However, the maintenance of the functional integrity of both the PCRs and CCRs helps explain why susceptibility to AMS does not appear to be influenced by age (Wu, et al., 2018) Older people are still able to hyperventilate effectively from a hypoxic stimulus and maintain their PaO_2. As there is not a greater number of cases of AMS in the older population, prescriptions of prophylactic medication prior to a high-altitude sojourn are not currently individualised based on the age of the person who is travelling. However an emerging hypothesis is that due to impairments in renal function in the older population, they may need to be receiving less than the current dose-recommendations of Az, the most commonly used medication used by people travelling to high altitude destinations (Luks, et al., 2016).

As outlined in the literature review, the primary target site of Az is to inhibit CA isoforms II and IV within the renal proximal and distal tubule causing a greater loss of HCO_3^-, Na^+ and potassium (K^+) in the urine whilst retaining more H^+ and chloride (Cl^-). Ultimately, this lowers serum pH which induces a state of metabolic acidosis that is counteracted by respiratory alkalosis in an attempt to equilibrate pH. In doing so, the hyperventilation increases PaO2. However, CA isoforms are also present in: the CNS; erythrocytes; PCRs and CCRs; within the muscles, and in the vascular endothelium of capillaries supplying the muscles, brain and lungs (Swenson, 1998). Thus, superfluous doses of Az can result in local inhibition of CA isoforms in the peripheral tissues, heart and erythrocytes from an increased concentration within the circulation (Hilvo, et al., 2008). As mentioned previously this effect is apparent in exercise and cognition studies (Dominelli, et al., 2018; Bradwell, et al., 2018; Wang, et al., 2013) whilst also having an effect on the HVR depending on the level of local CA inhibition (Swenson & Hughes, 1993). As CA-II is common amongst glomus cells within the CB, superfluous doses of Az that accumulate within the circulation can diffuse into the CB and inhibit the CA isoforms within the CB. This will reduce the excitability of the cells

causing them to fire less, resulting in a more supressed ventilatory response to hypoxia. Unpleasant side-effects effects are also commonly reported with Az ingestion such as nausea, sickness and fatigue which may increase with an excessive dose.

Surprisingly, little research has been directed towards how the older population respond to current dose recommendations of Az despite the primary target site of the renal system being impaired with age. Impairment occurs at a number of sites across the kidneys such as: a loss in number and size of functional nephrons; reduced renal parenchyma (Gourtsoyiannis, et al., 1990); increased glomerulosclerosis (Esposito, et al., 2007), and a reduction in GFR (Lindeman, et al., 1985). Collectively, these decrements are known as nephrosclerosis. Research examining healthy kidney donors noted that nephrosclerosis occurred in only 2.7% of donors aged <30, whereas this number grew to 58% from donors aged between 60-69 and finally 73% from donors aged >70 (Rule, et al., 2010). Az is a diuretic, and thus eliminated from the body via renal excretion, therefore an ageing renal system and impaired GFR may lead to excessive Az accumulation. This effect has been demonstrated in single dose studies of Az on glaucoma patients showing that renal clearance of free Az is reduced in the old compared with the young (8.88 vs 15.7ml.min^{-1}.kg^{-1}) resulting in excessive accumulation of free Az within erythrocytes (Chapron, et al., 1985). Furthermore, the renal clearance of Az showed a strong correlation with creatinine clearance (r=0.846) (Chapron, et al., 1985). An effect which has also been mirrored after chronic dosing (Chapron, et al., 1989). When examining the effects of the drug for the purpose of AMS treatment, it has recently been demonstrated that when taking current dose recommendations (250mg BD) of Az at an altitude of 4559m, maximum power output was reduced further with an increasing age (r = -0.83) (Bradwell, et al., 2018). These findings support the notion that, for the current dose recommendations of Az, impaired pharmacokinetics can cause excess accumulation within the circulation of the older individuals, resulting in several adverse side effects. Recent

evidence has highlighted that doses of Az as low as 62.5mg BD are enough to attenuate symptoms of AMS (McIntosh, et al., 2019) These lower doses may be more beneficial to the older population if the current dose recommendations are indeed superfluous within this population. Excessive Az accumulation within the circulation will decrease the output of the PCRs during a hypoxic stimulus that will reduce an individual's drive to hyperventilate, resulting in lower SaO_2 levels that will potentially increase the incidence of AMS under long term exposure to a hypoxic environment.

To examine this hypothesis, the present study aimed to assess how both a small and moderate dose of Az influences the ventilatory sensitivity to hypoxia and hypercapnia in the young and the old. Resting ventilation is universally increased in all people using the Az, shown by substantial reductions in end tidal PCO_2 ($PetCO_2$) at rest (Bradwell, et al., 2018; Teppema & Dahan, 1999). However, the actions of Az on the isocapnic HVR and HCVR are diverse. Under isocapnic conditions, driven by peripheral chemosensitivity, ventilation has been reported to increase (Tojima, et al., 1986), decrease (Teppema, et al., 2006a; Scheuermann, et al., 1999) and stay the same (Teppema, et al., 2007; Bashir, et al., 1990) following Az use. During acute exposures, increases in the ventilatory responses to hypoxia are normally observed when PetCO2 levels are held at pre-drug levels. As previously mentioned, when Az is used chronically $PetCO_2$ is decreased. Therefore, any pre-drug levels for $PetCO_2$ can be considered hypercapnic if that value is used in an attempt to achieve isocapnic hypoxia post-dosing. Thus, stimulating a greater ventilatory response due to the contribution of central components of chemosensitivity as well as the CO_2 interaction within the carotid bodies increasing the CCRs output. Reduced ventilatory responses to hypoxia following Az ingestion are more common due to inhibition of local CA in the carotid bodies at clinical oral doses, rather than from inhibition of the targeted renal CA. This mechanism is highlighted in experiments using Mz (a much more potent inhibitor of renal CA in a much greater quantity

than Az (33mg/kg vs 3mg/kg). The use of Mz had no effect on the HVR, whereas after Az ingestion there was a reduction of 44% (Teppema, et al., 2006a). This suggests that the reduction of the ventilatory response was caused by Az's effects on the PCRs. Alternatively a reduction to the HVR may be due to the effect Az has on skeletal muscle, with the diaphragm significantly decreasing its contractility strength after chronic dosing (Dominelli, et al., 2018). As for the HCVR, a leftward shift is commonly reported in the ventilation-CO_2 response curve after Az is ingested (Teppema & Dahan, 1999; Swenson & Hughes, 1993) but the slope sees similar variability as with the isocapnic HVR. Often disparate findings are caused by variations in experimental procedure such as the quantity of dose, time of testing (acute vs chronic use) and the method used to administer Az (oral vs intravenous). With that being said, to date no study has examined specifically how ageing influences the actions of Az on peripheral and central chemosensitivities. If current dose recommendations are indeed superfluous in older people, the hypothesis would be that hypoxic sensitivity could be decreased in the older participants due to a larger inhibitory effect on local CA in the peripheral and possibly central components as the excess Az is transported through the circulation to these areas. Furthermore, the difference of the effect between two doses of Az on ventilation may be diminished in older people due to complete inhibition of CA provided by the lower dose. This would give an indication that larger doses are unnecessary in older people, rather giving rise to the detrimental side effects mentioned above through excessive accumulation within the body. In order to test this theory, young and old participants were recruited and performed steady state isocapnic and hypercapnic hypoxic test after being prescribed both a small (125mg BD) and moderate (250mg BD) course of Az for 48 hours.

3 METHODS

3.1 Ethics

Ethical approval was granted by the University of Birmingham (ERN_15-0181B) and was conducted in accordance with the 7th Version of the Declaration of Helsinki. Written informed consent was received from all participants and participants were aware they were free to withdraw at any point during the study without giving reason.

3.2 Participants

Participants consisted of both males and females and were split into 2 cohorts, a young (male = 4, female = 3, age range = 22-31, M = 24.3 ± 3.2, *n*=7) and an old (male = 5, female = 1, age range = 68-74, M = 71 ± 2, *n*=6). All participants were considered generally healthy, were non-smokers and were free from any cardiorespiratory, kidney or liver problems. Participants were excluded if they had diabetes, any allergies towards sulphonamide containing products or had an estimated GFR (eGFR) <30ml/min/1.73m^2. The testing was carried out on females during the first 14 days of their menstrual cycle, or at any time if they were post-menopausal or using the contraceptive pill. Prior to each testing session, participants were asked to abstain from eating for 2 hours before the procedure, consuming caffeine and alcohol for at least12 hours before and participating in vigorous exercise for at least 24 hours before.

3.3 Apparatus

Hypoxic mixtures were administered by the dynamic end-tidal forcing system (DEF) in the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham (~160m elevation). The system is described in detail elsewhere (Robbins, et al., 1982) but briefly, the DEF uses breath by breath gas analysis, with computer feedback control, to achieve the desired levels of end-tidal gases at a given level regardless of fluctuations in respiratory patterns. This is achieved by the controlling computer software (Breath DP Version 1, University Laboratory of Physiology, Oxford, UK) adjusting the composition of

inspired PO_2 and inspired PCO_2 through a rapid gas mixing (MKS Instruments 1559A; Munich, Germany) and humidifying system (Paykel & Fisher Healthcare HC150, Fisher & Paykel Healthcare, Auckland, New Zealand) which is calculated from the expired gas composition. Respiratory volumes are measured with a bidirectional turbine (Cardiokinetics Ltd, Lancashire, UK) and a volume transducer (VMM-400, Interface Associations, CA, USA) with continuous gas sampling every 0.5s from a side arm of the mouthpiece which is analysed using a mass spectrometer (MOXAR Respiratory System, AEI Technologies, PA, USA). A pulse oximeter (3900 Pulse Oximeter, Datex-Ohmeda, Louisville, CO, USA) was used during all periods of gas control to continually monitor heart rate and SaO_2.

3.4 Participant Screening

Participants were screened by an independent GP (Dr Brian Johnson – British Association of Sport & Exercise Medicine) before beginning the experimental sessions to assess their capacity to take Az. Each participant completed this pre-screening check which consisted of a general health questionnaire alongside checks on blood pressure and both pulmonary and cardiac function. Upon completion of screening individual prescriptions were provided for each participant who then collected their Az from a local pharmacy prior to the first experimental session (see below).

3.5 Familiarisation

Participants underwent a familiarisation session prior to testing in order to become accustomed with the laboratory and the equipment. During this session participants eGFR was assessed to confirm a value of >30mL/min/1.73m^2. Serum creatinine (Scr) levels were measured to estimate GFR in participants from a capillary blood sample obtained from a finger prick. The sample was placed into Crea i-Stat cartridges which was analysed using an i-STAT handheld device (ABBOTT Laboratories Ltd, xx). Once a Scr value was obtained this was inserted into the CKD-EPI equation derived from Levey et al., (2009), which is

considered to be the most accurate current method of obtaining eGFR from a measurement of serum creatinine. The equation is as follows:

$$\text{eGFR}\ (\text{mL/min/1.73m}^2) = 141 \times \min(\text{Scr/k}, 1)^{\alpha} \times \max(\text{Scr/k}, 1)^{-1.209}$$
$$\times\ 0.993^{\text{age}} \times 1.018\ (\text{if female}) \times 1.159\ (\text{if black})$$

Whereby: Scr is serum creatinine in (mg/dL), α is -0.329 for females and -0.411 for males, *k* is 0.7 for females and 0.9 for males, min indicates the minimum of Scr/k or 1 and max indicates the maximum of Scr/k or 1.

3.6 Experimental procedure

In total, 3 experimental sessions were performed: a baseline, following 125mg Az BD for 48 hours and after 250mg Az BD for 48 hours. The experimental sessions were always conducted in that order for each participant, as the pharmacodynamics of the drug are already well understood (Joyce, et al., 2018). Visits 2 and 3 (dosing visits) were separated by 48 hours, so participants began dosing with the larger dose immediately after being tested with the smaller dose. The control visit was conducted prior to the dosing visits, typically in the week before. Az was administered orally to the participants for the duration of the study. Az induced changes in acid base balance was assessed prior to each session via a finger prick capillary blood sample. The sample was transferred into G3+ i-Stat cartridges which was analysed using the i-STAT handheld device (ABBOTS Laboratory).

During each experimental session both a HVR and HCVR test was performed with a 30-minute washout period separating the two tests. To begin with participants lay supine on a bed with their back propped up and nose occluded. They were then positioned on the mouthpiece of the DEF. Participants inhaled normoxic air for the initial 5 minutes on the DEF to become accustomed to breathing through the mouthpiece and to establish resting values of $PetCO_2$. During isocapnic hypoxia $PetCO_2$ was clamped at ~1mmHg above resting levels, whereas

$PetCO_2$ was clamped ~7mmHg above resting levels for the hypercapnic test. Once the participant signalled they were happy to continue, the isocapnic HVR test began with a stepwise decrease of end tidal PO_2 ($PetO_2$). Three 3-minute steps were used to progressively decrease PetO2 (100mmHg, 65mmHg, 55mmHg) to the final value of 45mmHg (~5500m) that was then held for 20 minutes (a length chosen in order to assess the effects of Az on the HVD). A 30-minute washout period was then introduced in which participants came off the mouthpiece and breathed room air to remove any residual effects of hypoxia from the body. The HCVR test began immediately after the washout period. The test started with 10 minutes of normoxic hypercapnia to activate the central component of chemosensitivity. Following this $PetO_2$ was again decreased to mimic the initial HVR test whilst maintaining the hypercapnic stimulus, only with the last stage of 45mmHg being held for only 5 minutes instead of 20 minutes due to the severe dyspnoea experienced by the participants. A graph illustrating the experimental procedure can be seen in figure 1.

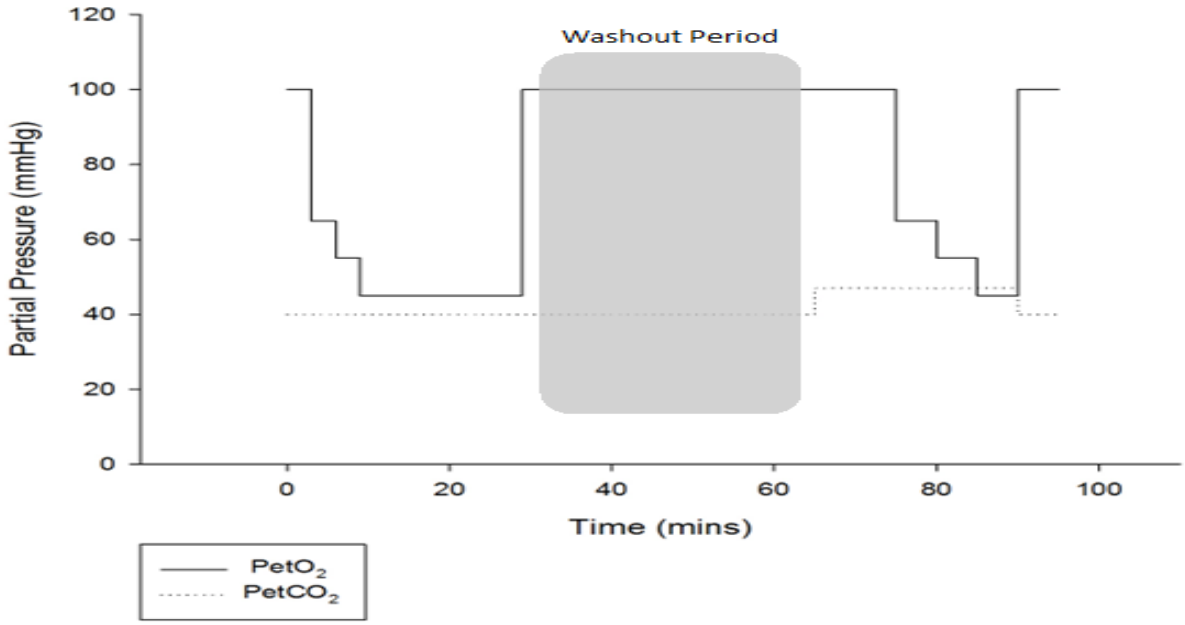


Fig 1. Manipulation of end tidal gases during the isocapnic and hypercapnic hypoxic tests. PetCO2 values are estimated based off an average normal PetCO2 (~40mmHg)

3.7 Data Analysis

Minute Ventilation (V_E) was provided by the DEF as gas volume ATPS which was converted to gas volume BTPS before analysis using the following equation.

$$\text{V.BTPS} = \text{V.ATPS} \times \frac{(P_{atm} - P_{water}) \times 310)}{(P_{atm} - 47\text{mmHg}) \times (273 + T)}$$

Whereby: P_{atm} is barometric pressure, P_{water} is partial pressure of water vapour (47mmHg) and *T* is environmental temperature (21°C).

Gas volumes were continuously provided after each individual breath. Upon completion these values were averaged into 30s blocks using the programme Average.exe (University Laboratory of Physiology, Oxford University) with the peak 30s block of a step used to measure chemosensitivity. Hypoxic sensitivity was evaluated as the relationship between ventilation ($L.min^{-1}$) and oxygen saturation (SaO_2). The HVD was calculated as the percentage decline from the peak V_i to the final 30s of ventilation in the last stage (45mmHg) of hypoxia (V_f).

3.8 Statistical Analysis

Statistical analysis was performed using IBM SPSS (Version 26) software. A (2x3) mixed design ANOVA was used to compare the blood gases and ventilatory responses pre and post dosing. Mauchly's Test of Sphericity was used to examine the variance of the data and main effects of estimated marginal means were compared using Bonferroni confidence interval adjustment. Where significant main effects were found, Bonferroni post hoc tests were used to identify where the differences fell. The significance value was pre-set as $p<0.05$. All data are expressed as means with ± denoting standard deviation.

4 RESULTS

4.1 Blood Gases

Table 1 displays the blood gas values for old and young participants during the study. There were no differences between the old and young group before the participants began taking Az. Following the treatment course, significant main effects within the groups are seen in pH ($F(2,22) = 54.509$, $p<0.001$, $\eta_p^2=0.832$), PCO_2 ($F(2,22) = 19.083$, $p<0.001$, $\eta_p^2 =0.634$), TCO_2 ($F(1.23,13.531) = 47.773$, $p<0.001$, $\eta_p^2=0.931$), HCO_3^- ($F(1.204,13.248)=137.895$, $p<0.001$, $\eta_p^2=0.926$) and base excess ($F(2,22)=122.319$, $p<0.001$, $\eta_p^2=0.917$). The only significant difference between young and old at any time points was that the young cohort had a significantly higher base excess after taking 250mg BD of Az for 48 hours ($p=0.023$) which surprisingly didn't translate to a lower pH at that point ($p=0.496$). Despite a change in the mean values of PO_2, due to high variability of the results this did not reach significance ($p=0.125$). The closest difference of PO_2 to reach significance was between young and old after using Az 250mg BD for 48 hours ($p=0.070$) and this may have reached the significance if a larger sample was available to test.

Table 1: Blood gas volumes given by the I-Stat handheld from a sample of arterialized capillary blood. * shows significant difference from control, ** shows significantly different from both control and Az 125mg BD, # shows significantly different between young and old, $p<0.05$.

	Control		Az 125mg BD		Az 250mg BD	
	Young	Old	Young	Old	Young	Old
pH	7.43±0.02	7.45±0.03	7.37±0.04*	7.38±0.01*	7.33±0.03*	7.34±0.02*
PO_2 (mmHg)	79.13±21.9	74.88±10.5	92.9±24.57	82.93±12.39	84.79±10	73.16±10.9
PCO_2 (mmHg)	35.39±3.27	34.09±1.78	28.66±3.2*	30.82±2.91	29.7±4.34*	31.13±1.93
TCO_2 (mmol/L)	24.83±2.5	24.67±1.21	17.14±1.46*	18.83±1.72*	16.43±1.62*	17.67±1.63**
HCO_3^- (mmol/L)	23.67±2.4	23.58±1.21	16.4±1.334*	18.15±1.85*	15.5±1.4**	16.8±1.6**
Base Excess (mEq/L)	-0.67±2.58	-0.33±1.75	-8.29±0.95*	-7.17±2.04*	-10.71±1.11**	-8.5±1.87*#

4.2 Normoxic Ventilation

Figure 2 shows the resting $PetO_2$ and $PetCO_2$ during the different stages of the study. There was a significant main effect within groups for both $PetO_2$ ($F(2,22)=23.754$, $p<0.001$, $\eta_p^2=0.683$) and for $PetCO_2$ ($F(2,22)=95.196$, $p<0.001$, $\eta_p^2=0.896$), whereas there was only a significant effect between the two groups for $PetCO_2$ ($F(2,22)=5.544$, $p=0.011$, $\eta_p^2=0.335$). The young group had significantly lower $PetO_2$ at baseline (104.31 ± 3.89mmHg vs 112.33 ± 1.74mmHg, p=0.001) and after using 125mg BD of Az for 48 hours (112.37 ± 3.71mmHg vs 117.54 ± 2.23mmHg, p=0.013) whilst they also had significantly higher PetCO2 at all time points (*control* = 38.49. ± 2.3mmHg vs 33.43 ± 1.71mmHg, *Az 125mg* BD= 32.42 ±1.84mmHg vs 30.26 ± 1.59mmHg, *Az 250mg BD* = 30.83 ±2.14mmHg vs 28.34 ± 1.49mmHg, $p<0.05$). This is in part due to older participants having a significantly higher average resting ventilations at baseline (8.06 ± 2.3L.min^{-1} vs 12.04 ± 2.41L.min^{-1}, $p=0.011$) in addition to the pharmacokinetics of Az. Higher resting ventilation was consistent in the older group after dosing on 125mg BD for 48 hours (9.62 ± 2.48L.min^{-1} vs 12.04L.min^{-1}, $p=0.050$). But this affect disappeared on the higher dose ($p=0.081$). As displayed in figure 2 both the young and the old participants had significantly higher/lower resting PetO2/PetCO2 respectively after using Az. Furthermore, both groups significantly reduced their normal PetCO2 between the two doses. Table 2 shows the value that $PetCO_2$ was clamped at for the duration of each test. This was +1mmHg above resting in the isocpnic test and + 7mmHg in the hypercapnic test.

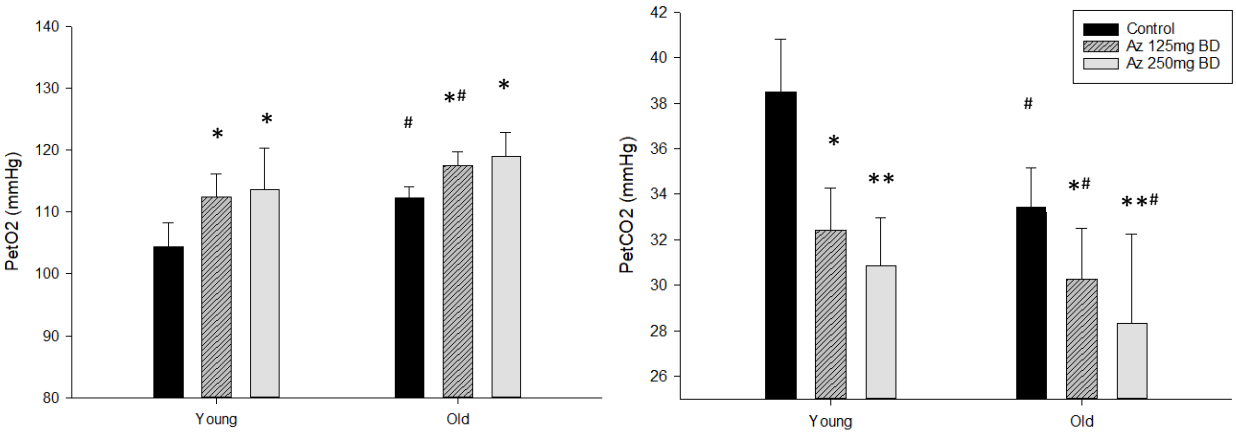


Fig 2: Average resting PetO2 and PetCO2 during each stage of testing derived from participants breathing regular normoxic air on the DEF. * shows significant from control, ** is significant from Az 125mg BD and # signifies difference from the young group. Error bars are standard deviation.

Table 2. $PetCO_2$ values during the HVR and HCVR tests. * shows significant from control, ** is significant from Az 125mg BD and # signifies difference from the young group. ± shows standard deviation.

	Young		Old	
	Isocapnic	Hypercapnia	Isocapnic	Hypercapnia
Control	39.49 ± 2.3	45.49 ± 2.3	34.43 ± 1.71#	40.43 ± 1.71#
Az 125mg BD	33.42 ± 1.84*	39.42 ± 1.84*	31.26 ± 2.23*#	36726 ± 2.23*#
Az 250mg BD	31.83 ± 2.14**	37.83 ± 2.14**	29.34 ± 3.9**#	35.34 ± 3.9**#

4.3 Estimated Glomerular Filtration Rate

Figure 3 shows that the older participants had a significantly lower eGFR than the younger group (125.2 ± 17.8ml/min/1.73m^2 vs 87.7 ± 8.1ml/min/1.73m^2, p=0.001) which in turn lead to the older participants having significantly higher serum creatinine levels than the young (0.69 ± 0.07μmol.l vs 0.87 ± 0.14μmol.l, p=0.010). Regression analysis showed a strong negative correlation between age and eGFR (y = -0.794x + 144.54, r = -0.803).

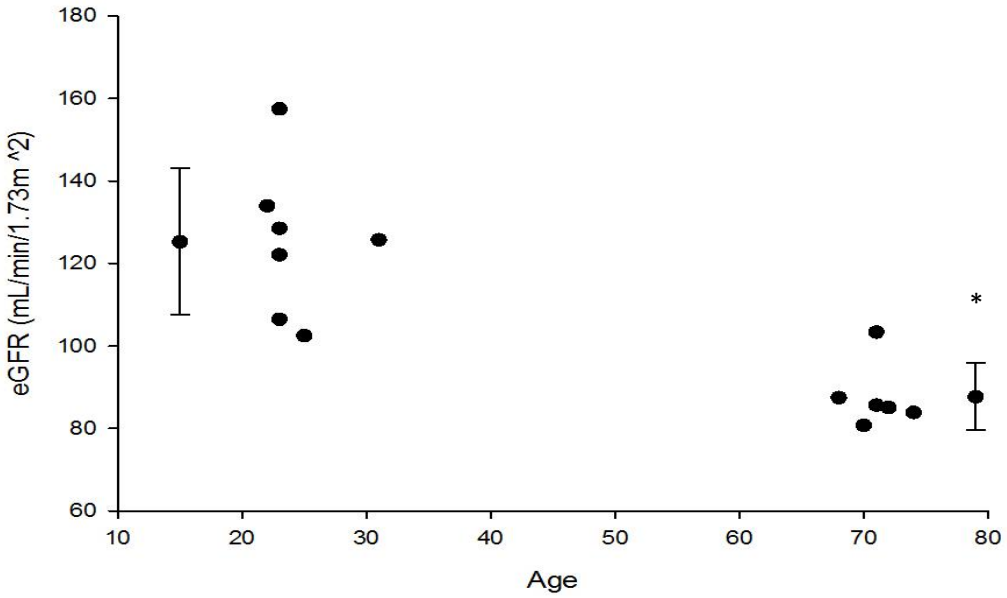


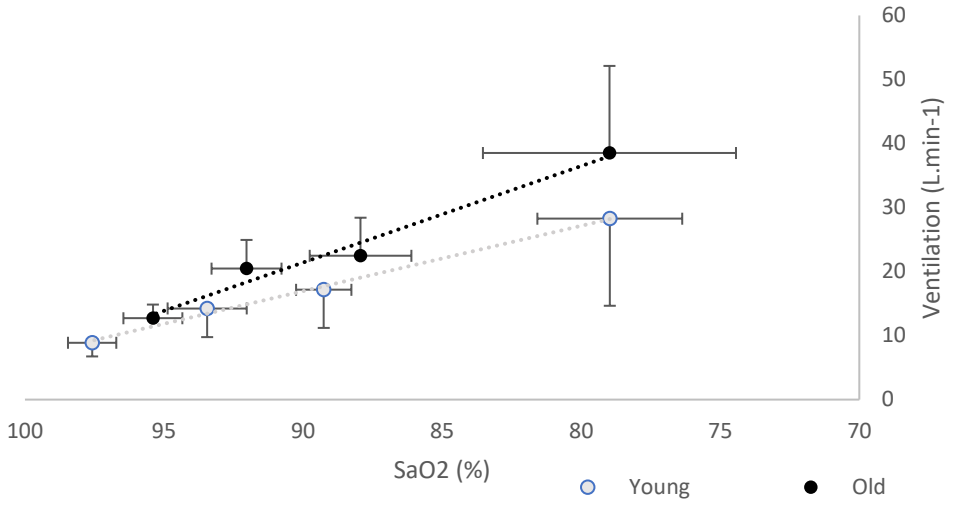
Fig 3: Scatterplot displaying how kidney function (eGFR) decreases with increasing age. eGFR calculated from the CKD-EPI equation. * shows significant value, p<0.01. Error bars show standard deviation.

*

4.4 Hypoxic Sensitivity

Hypoxic sensitivity is here defined as the relationship between ventilation ($L.min^{-1}$) and oxygen saturation (SaO_2). Figure 4 (next page) shows the ventilation ($L.min^{-1}$) - oxygen saturation (SaO_2) relationship. The older group showed a blunted HVR following 125mg of Az BD (illustrated by the significant reduction of the slope of the regression line, p=0.031) that is almost shared when taking 250mg of Az BD (p=0.059). This blunting effect is not shared by the younger group (p=0.744). Similarly, during the HCVR tests (figure 5), there remained no change in the older groups ventilatory responses from control after both treatment courses of Az. There was a reduction in the slope for the young group between the control and 250mg Az BD but with the low numbers tested this failed to reach significance (p=0.132).

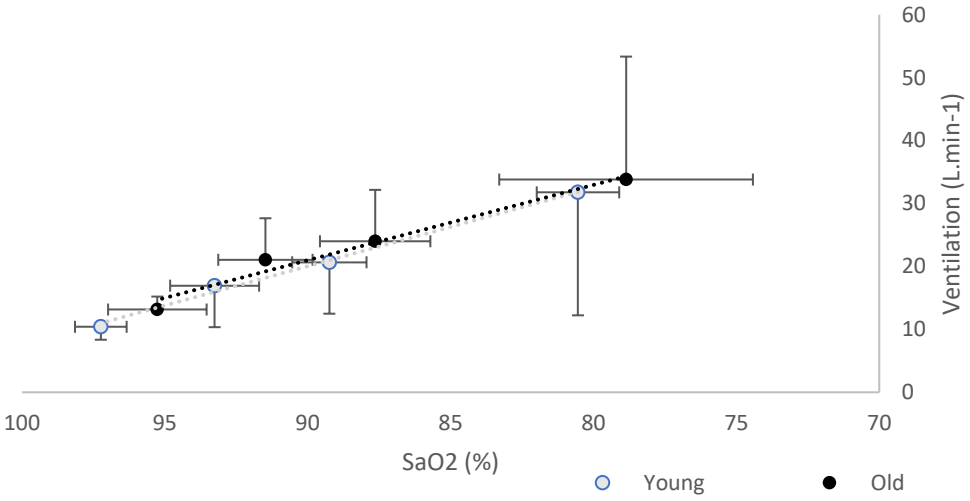
Control



Young, $y = -1.02x + 108.7$

Old, $y = -1.51x + 157.1$

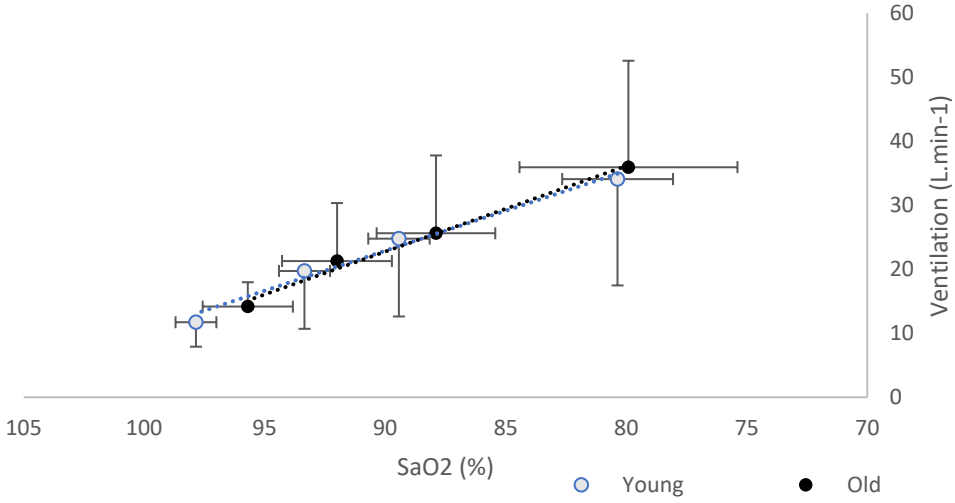
Az 125mg BD



Young, $y = -1.25x + 132.5$

Old, $y = -1.12x + 128.8$ *

Az 250mg BD



Young, $y = -1.25x + 135.2$

Old, $y = -1.28x + 143.2$

Fig 4: Relationship between ventilation and SaO_2 during the HVR test in old and young participants during a control and after a treatment block of Az. * represents significant reduction in the slope, $p<0.05$

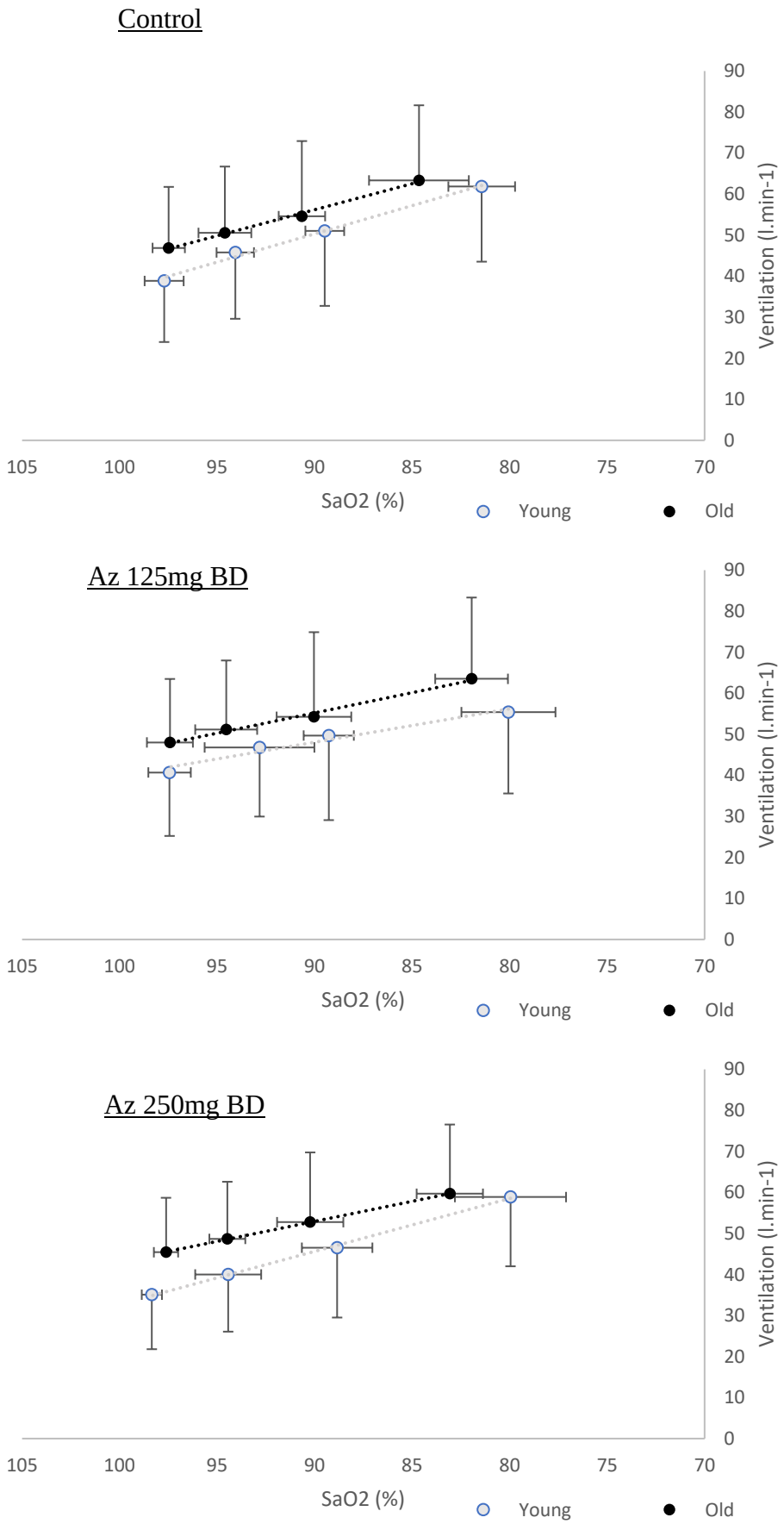


Fig 5: Relationship between ventilation and SaO_2 during the HCVR test in old and young participants during a control and after a treatment block of Az.

4.5 Absolute Ventilatory Response

Az at 125mg BD changed peak ventilation ($l.min^{-1}$) in 2 out of 7 of the young cohort during the isocapnic hypoxic test as opposed to 5 out of 6 in the older group. Az at 250mg BD affected 5 out of 7 of the younger group when compared with 125mg BD whereas the old remained mostly unchanged when comparing these 2 doses (5 out of 6). Of the changes that occurred from 48 hours of Az use, 5 participants (4 young, 1 old) developed a stimulatory ventilatory response from Az, 5 participants (1 young, 4 old) developed an inhibitory ventilatory response to Az whilst 3 were unnafected by the medication (2 young, 1 old). Individual peak ventilation data from the HVR is displayed in table 3.

Table 3. Peak ventilation ($L.min^{-1}$) for each participant calculated using the programme average.exe.

Participant ID	Age	Peak Ventilation ($L.min^{-1}$)		
		Control	Az 125mg BD	Az 250mg BD
01	23	15.67 ± 1.93	24.56 ± 1.26	28.95 ± 1.94
02	23	15.41 ± 2.43	17.53 ± 2.76	23.97 ± 2.54
03	22	34.01 ± 2.07	33.73 ± 2.25	32.04 ± 1
04	23	28.03 ± 1.63	26.06 ± 4.28	31.75 ± 2.5
05	23	19.99 ± 2.39	23.64 ± 2.89	34.63 ± 3.79
06	25	37.24 ± 1.48	37.64 ± 2.88	34.12 ± 1.37
07	72	31.13 ± 1.58	23.46 ± 1.05	22.77 ± 3.75
08	70	53.69 ± 2.95	35.47 ± 0.82	54.72 ± 3.3
09	74	55.21 ± 4.74	38.5 ± 1.84	33.58 ± 5.87
10	71	16.64 ± 2.12	25.12 ± 1.58	26.4 ± 1.92
11	71	71.02 ± 5.67	70.28 ± 5.77	75.68 ± 5.9
12	68	37.9 ± 3.61	30.97 ± 3.54	31.79 ± 2.74
13	31	57.17 ± 7.06	75.12 ± 2.2	73.84 ± 4.48

4.6 HVD

HVD was quantified as the percentage decrease from peak ventilation at 45mmHg $PetO_2$compared to the level of ventilation at the end of the 20-minute hypoxic exposure at 45mmHg $PetO_2$. There were no significant effects of Az on the HVD for either the young or older group. However, there was a significant difference between the HVD of the young and older group at baseline between (22.6 ± 8.6% vs 32.2 ± 3.7%, p=0.045).

4.7 SaO_2

The only change in SaO_2 was that the young group increased their mean SaO_2 when taking 125mg Az BD whilst being exposed to the hypoxia at 45mmHg $PetO_2$, but this was not enough to reach significance (78.97 ± 2.6% vs 80.54 ± 1.4%, p=0.127). There were no differences between the two groups SaO_2 levels at any point during the testing period.

5 DISCUSSION

5.1 Main Findings

The present study was the first to attempt to identify whether Az dose-response is affected by ageing, by measuring the ventilatory sensitivities to hypoxia and hypercapnia; with the hypothesis being that current dose recommendations may be excessive in the older population. The main findings of this study were: 1. That ventilatory sensitivity decreased in the older group following 125mg of Az BD; an effect that was not shared by the younger group. 2. Ventilation – saturation relationship was also blunted in the older group only following 125mg Az BD 3. That increasing the dose of Az to 250mg BD did not have any additive effects on the ventilatory responses to hypoxia in the older participants, indicating that the lower dose was satisfactory in inhibiting the available CA a within the renal system. 4. Individual changes to the HVR varied in the young participants when dosed with Az (125mg BD *and* 250mg BD). Whilst some participants ventilatory responses to hypoxia were stimulated by the drug, others were inhibited, whilst some experienced no change at all. 5. That 125mg BD of Az was effective in stimulating resting hyperventilation in both cohorts based on the alterations in blood acid base balance and subsequent changes in normal $PetO_2$ and $PetCO_2$.

The argument around which quantity of Az is most beneficial for AMS treatment is longstanding. In 2000 a meta-analysis by Lionel Dumont concluded that 750mg of Az per day was the optimum dose for treatment of AMS and that anything lower did not attenuate symptoms (Dumont, et al., 2000). This conclusion was drawn primarily due to a lack of evidence at the time for the efficacy of lower doses. More recently, a second meta-analysis identified that the risk of AMS decreases with an increasing dose of Az (55% at 250mg per day, 50% at 500mg per day and 45% at 750mg per day. Note that this review included studies that used both once daily and twice daily doses). However, when a slow ascent was

incorporated, there was no improved prophylactic effect of taking 750mg per day over 250mg per day whereas side effects such as paraesthesia and polyuria did increase (Kayser, et al., 2012). Note that the age range within these 2 meta-analyses were not reported. One finding of the present study was that the smaller dose of 125mg BD of Az effectively triggered metabolic acidosis which, in turn, stimulated hyperventilation (shown by a reduction in base excess in table 1). As this is the primary mechanism by which Az counteracts AMS, it can be inferred that Az at 125mg BD would most likely have been effective in treating AMS in the studied population had prolonged exposure to hypoxia been introduced. This finding however is not a unique proposal within the literature. On the contrary, it adds to a growing collection of work which advocates that a lower dose should be preferential for climbers ascending in a slow and controlled manner to avoid both AMS and the unwanted side effects of Az. Similarly, other authors have reported that Az can inhibit the ventilatory sensitivity to hypoxia (aged 19-24, Az at 4mg/kg) (Teppema, et al., 2006) but this work is the first to present that inhibition of hypoxic sensitivities after Az use is predominant in older people, whereas the young are more unpredictable.. Due to this unpredictability, when analysing collectively, the differences can cancel out and there appears to be no change in hypoxic sensitivities of the young. However, table 3 shows clear stimulatory and inhibitory effects after Az use on the isocapnic HVR and thus, assessing individuals as opposed to the group for this test reduces the likelihood of a type 1 error occurring. This may be the reason why some authors conclude that there is no effect of Az on the ventilatory response to hypoxia (Bashir, et al., 1990; Teppema, et al., 2006) (Bashir (1990) study involved participants aged 25-35 and used 500mg BD for 3 days). This dataset is one of the few that shows Az can have a stimulatory effect on the magnitude of the HVR in some individuals. Interestingly, this variability was not present during the HCVR which could indicate the differential effect of the HVR is related to the carotid bodies.

5.2 Az Concentrations in the Older Cohort

As presented in this paper, eGFR is negatively correlated with age (fig. 3). This result was formulated from a measure of serum creatinine which showed that the older adults in this study had higher levels of creatinine within their circulation. It has been demonstrated that free Az clearance is correlated with creatinine clearance (r=0.846) (Chapron, et al., 1985) (Chaprons study involved 4 young (27-44) and 4 old (76-82) participants using 5mg/kg Az) hence an assumption can be made that in the older population in the current study Az renal clearance was lower. Furthermore it has been reported that Az plasma protein binding is reduced in older people (aged 76-82) leading to a greater than 50% increase in the free fraction of Az (the active concentration of the drug) (Wallace & Verbeek, 1987). One reason for the decline of Az plasma protein binding is due to a decline in serum albumin that comes with age. Albumin is one of the primary plasma proteins that Az binds to in the body but it has been shown that there is a negative correlation between age and levels of serum albumin in the circulation (population tested was aged between 65-90, r=-0.716 in males, r=0.794 in females) (Gom, et al., 2007). A case study investigating an 84 year old hypoalbuminemic patient noted that Az plasma protein binding increased with albumin infusion (r=0.91) which also resulted in the free fraction of Az falling (r=0.88) (Gomolin & Chapron, 1992). In a healthy population, an increase in the concentration of a free drug would normally be counteracted by increased clearance of that drug. But as Az is eliminated almost solely through the renal system, an impaired GFR in older people (average age 76.9±7.6) means the kidneys cannot accommodate this increase to maintain homeostasis, and so the free fraction of Az remains high (Chapron, et al., 1989). These points may help explain why only the older group experienced a reduction in hypoxic sensitivity following their prescribed course of Az. Complete inhibition of CA within the kidney's was most likely provided by the lower dose due to the greater free fraction of Az within their circulation, thus causing the remaining free

Az to shift to different tissues/receptor sites such as the PCR's, which is discussed further below.

5.3 Isocapnic HVR & Acetazolamide

The reduction of hypoxic sensitivity (figure 4) indicates that Az reduced the HVR in the older group which is most likely due to Az inhibiting the PCRs of this group only. Reductions in $PetCO_2$ (an effect of Az) can also cause blunted HVRs, however the difference between the young and the old never changed throughout the study, and both groups reduced their $PetCO_2$ throughout the study indicating it was the pharmacokinetics of Az that caused this change. CA isoforms are present within the carotid body PCR's and are inhibited by Az. The inhibitory effect of Az on the PCR's has been demonstrated in feline studies whereby the hypoxic sensitivity that was displayed before Az infusion was abolished after using the drug (Teppema, et al., 1988). Similarly, this finding is supported by studies looking at the ventilatory responses to CO_2 before and after Az infusion. These studies have shown that Az (4mg/kg) significantly reduces the carotid sinus nerve output in response to a CO_2 stimulus (Hayes, et al., 1976; McCloskey, 1968). It has been proposed that direct inhibition of the carotid body CA (Az at 10mg/kg) impedes the rise in H^+ following a hypoxic or hypercapnic stimulus that reduces the chemosensory output (Vovk, et al., 2000), therefore decreasing the peripheral sensitivities; a mechanism which is complimented by this study's findings. The aforementioned mechanism may only be partly responsible for the viewed reduction however, as decrements in contractility strength of the diaphragm after taking 250mg Az three times daily (TD) (Dominelli, et al., 2018) and from Az (at the same dose) attenuating hypoxic pulmonary vasoconstriction (Swenson, 2006; Teppema, et al., 2007) have both been proposed as mechanisms which elucidate Az's effects on the HVR. The reduced drive to hyperventilate did not cause any reductions in SaO_2 levels within the present study under acute hypoxic conditions, but this has not been tested following chronic hypoxic exposure. A

long-term reduction in ventilation at altitude could lead to increased hypoxemia and lower SaO_2 levels. This would potentially increase an older person's susceptibility to AMS as a reduced SaO_2 correlates with increased AMS scores (Roach, et al., 1998).

It should be noted that the older group within this study had a higher average starting hypoxic sensitivity (illustrated via a greater slope of ventilation – saturation relationship although this did not reach significance), which may be an indication that they possessed more excitable PCR's which were more susceptible to the inhibitory actions of Az. It may be argued that with a larger sample size this baseline difference may dissipate. However, cross-sectional analysis of 4675 participants revealed that an increased hypoxic sensitivity is commonly associated with ageing and so baseline differences are unlikely to change with a greater sample size (Lhuissier, et al., 2012).

Although it cannot be directly interpreted from the present study, the results of superfluous doses of Az can have further adverse effects than simply an inhibitory effect on the PCR's. On the contrary, inhibition of the PCR's can sometimes have benefits for high altitude visitors. Trekkers who experience periodic breathing at altitude can have this effect attenuated by Az (at a dose of 250mg BD for this study) and lead to a better night's sleep (Lombardi, et al., 2013). However, most alternate effects of Az remain negative. Within this study, older participants reported more adverse side effects associated from metabolic acidosis such as fatigue, nausea and taste alteration (4 out of 6 old vs 1 out of 7 young) indicating age-related alterations in the pharmacodynamics of the medication. Per contra, paraesthesia was noted by almost all participants (12 out of 13) when completing the dosing course of Az. From what has been previously reported in the literature after Az ingestion, the evidence from this study may support emerging work that older people experience greater reductions in both cognitive and exercise performance than those of a younger age due to the superfluous amount of the drug in their circulation, the latter of which (exercise) has already

been demonstrated at altitude (age range from 21-77 taking Az 250mg BD) (Bradwell, et al., 2018).

Interestingly, some of the young participants (and one old) experienced significant increases in their HVRs. This is a rather abnormal finding within the literature as most authors either report no change (Teppema, et al., 2007; Bashir, et al., 1990) or a blunting (Teppema, et al., 1988) of the HVR. An increase has been reported before after taking 500mg of Az once per day for 4 days (Tojima, et al., 1986), but within this protocol PetCO2 levels were held at pre-drug values and so the stimulus can be considered hypercapnic rather than isocapnic as there is a greater contribution from the CCRs. Within these participants, it is unlikely Az had any direct effect on the CA isoforms within the PCRs due to the inhibitory effect of Az on CA isoforms on the PCRs. It is most likely that participants increased their HVR due to intra-individual variability which arises from between-day repeated measurements of HVR's. Early evidence using the rebreathing technique indicates that intra-individual variability when performing the HVR is between 7.6%-63.8% (Sahn, et al., 1977) showing that repeating HVR tests on the same individual on different days can produce a significantly different HVR. More recently, using the more accurate steady state method of HVR assessment, evidence has indicated intra-individual HVR variability to be 26% (Zhang & Robbins, 2000). This variability may explain why the younger group had much more variability in their isocapnic HVR than the older group. If Az was not inhibiting their PCRs, as it was in the older group, then it may be expected to see a degree of variability within the younger groups HVR results. Repeated tests on a larger sample size is required to see if the results displayed within this study translate into a larger population.

5.4 Hypercapnic HVR & Az

Surprisingly, this study found that it was the young group, rather than the old, who started to develop a blunted HCVR following Az ingestion. In fact, using an alternative form of

analysis (peak ventilation at each stage of the HCVR test using $\Delta Vi/\Delta PetCO_2$, illustrated in Appendix figure 7) this reduction did reach significance (p=0.025). If more participants had been recruited the former analysis method may have eventually reached significance which would indicate a powerful central component of Az within the youth. CA plays an important role in the control of ventilation in the CCRs. The fluctuating concentration of H^+ ions in the CSF are what drives changes in ventilation following either a hypocapnic or hypercapnic stimulus. Due to their positive charge, H^+ ions cross the BBB relatively slowly, thus for H^+ ions to increase in the CSF, CO_2 molecules must diffuse across and then undergo the hydration reaction to form HCO_3^- and H^+ which then alters the buffering capacity of the CSF to stimulate a change in ventilation. In clinical doses, due to it's physical-chemical properties, Az does not easily cross the BBB and so direct inhibition of the CA within the CSF is unlikely (Maren, 1967). In fact, direct inhibition of CNS CA causes an increase in central CO_2 sensitivity (Adams & Johnson, 1990) highlighting that the doses provided in this study were not sufficient to cross the BBB. Blunting potentially arises from greater CA I inhibition within the erythrocytes, thus causing greater CO_2 retention (Swenson & Hughes, 1993; Tufts, et al., 1996). This may impact the usual offloading of CO_2 in the brain capillaries that would, in turn, decrease the H^+ and HCO_3^- within the CSF following a hypercapnic stimulus, lowering the ventilatory sensitivity to CO_2. Due to the clear decreases in renal clearance of Az in older people (Chapron, et al., 1985), one may expect that the old experience similar, if not greater, blunting of their HCVR due to the greater concentrations of Az within their circulation enhancing their CO_2 retention. However, this was not the case in this study as the older group did not show any blunting effect on their HCVR after both dosing courses of Az, whereas the younger group were more receptive during the HCVR and did. One compensatory mechanism may be that the old develop a much larger increase in brain blood flow following a hypercapnic stimulus which counteracts the CO_2 retention they experience.

Az is a potent vasodilator of the cerebral circulation and it has been shown that with advancing age (aged 27 – 70 within this study) the magnitude of cerebral vasodilation following Az ingestion (1g of Az in a single dose) remains intact (Hojer-Pedersen, 2005) despite a clear reduction in baseline CBF levels (Ances, et al., 2009). Similarly, it has been reported that as you increase the dose of Az, the rate of CBF also increases. A study conducted by Grossmann and Koeberle (2000) found that increasing the dose of Az (at 5, 10, 13, 15 and 18mg/kg) significantly increased CBF in participants aged between 18 and 79 in normoxia (R^2=0.307, $p<0.05$). This study also found that with every dose tested (besides 13mg/kg) increases in CBF did not correlate with age following Az administration, demonstrating older people maintain the cerebral vasodilatory response to Az. It is possible that within our study, if the older group had a greater quantity of free Az in their circulation, that this resulted in greater cerebral vasodilation and therefore, a greater increase in CBF. It must be noted that his hypothesis has never been accurately tested. To directly measure this hypothesis, one would have to use a transcranial doppler whilst performing a hyperoxic hypercapnic breathing test (hyperoxic to silence the PCR's) in the old whilst taking Az. It must be considered that the smaller sample size from this part of the study especially (2 older participants could not complete the experiment) leads there to be a higher possibility that a type II error occurred.

5.5 Optimum Az Dose

From the presented data in table 1, 125mg Az BD was successful in stimulating the targeted effects in both the age groups of HCO_3^- secretion, that lowered pH and base excess. Resting ventilation was also clearly stimulated based from significant increases in $PetO_2$ and decreases in $PetCO_2$. What is not clear is whether the larger dose had any additive effects on these variables. Despite the magnitude of this effect increasing on 250mg Az BD, it may be that this was simply due to continued Az ingestion, rather than an increasing dose. The

former evidence suggests that a dose of 125mg BD will be effective in treating AMS, which compliments work done previously within the area (Basnyat, et al., 2003; Basnyat, et al., 2006). Therefore, due to reduced renal clearance, it may be beneficial for older people to decrease their dose further to 62.5mg BD with recent evidence highlighting the effectiveness of this prescription for the treatment of AMS (Mcintosh, et al., 2017; McIntosh, et al., 2019). Only upon unexpected rapid ascent to high altitude should taking a larger dose be recommended, as a slow ascent profile remains the best natural remedy to reduce the risk of AMS (Kayser, et al., 2012).

5.6 Clinical Relevance & Future work

With more older people able to journey quickly to high altitude it remains important to identify how the pharmacodynamics of Az are altered within the older population. The following work presents that a lower dose of 125mg Az BD may be beneficial in older age, however future work is required to validate this claim. Research examining how exercise and cognition is affected by different doses of Az in older people will help to build a better understanding of the negative consequences current dose recommendations of Az can have, if prescribed to the older person. Furthermore, a protocol similar to Chapron's (1985) work may be necessary in order to accurately quantify how concentrations of Az differ within the circulation between the young and the old when taking Az over a prolonged period. In this study, they took several blood and urine samples in a small cohort of 4 younger and 4 older participants, following a single intravenous dose of Az (5mg/kg). This allowed them to quantify the concentration of Az within the circulation of the participants and the excretion rate of Az. Furthermore, using ultrafiltration, they also measured the plasma protein binding of Az allowing them to see the level of free Az within the circulation following administration of the drug.

Use of alternative AMS medications (i.e. Mz) appear to have fewer side effects than Az when tested in a young population (Dominelli, et al., 2018) and so future work may need to address whether the effect of this medication is consistent within an older population whilst still protecting against AMS.

5.7 Limitations

One obvious limitation within this study was the small sample size that was tested. For the results to be considered more robust, a much larger sample must be tested, with the scope to include participants who are between 30-60 in order to correlate all the results with age. This would have improved the reliability of the HCVR results as, in the present study, one third of participants were unable to complete the test due to severe dyspnoea.

This study was not a double blind, randomised placebo-controlled trial. The lack of a placebo control was down to the powerful known side effects of Az (i.e. taste alteration & fatigue) meaning participants are often aware whether they are on the drug or not and can easily become unblinded. Similarly, the non-randomised method was implemented as the pharmacokinetics of the drug are already well understood.

5.8 Conclusion

In conclusion, the following study reported that the old experience a blunting of their HVR after a 48-hour course of Az which is not shared by the younger group. As both groups experienced a similar reduction in pH and $PetCO_2$, which would reduce and increase the HVR respectively, the blunting has been attributed to a reduced renal function, which consequently impairs the renal clearance of Az, causing a higher concentration of Az to accumulate in the circulation and in non-targeted areas of the body. This inhibitory effect on the HVR arises from Az inhibiting the CA isoforms within the PCR's which reduces their output. This study is one of the first to report that Az can have a stimulatory effect on the HVR in certain individuals. Due to the variable effects the drug can have on the younger

person, this may have been missed in previous papers due to analysing the results collectively rather than individually.

Furthermore, it was reported that the young experience a blunting of their HCVR which was not shared in the older group. This is most likely is due to Az causing CO_2 retention within the erythrocytes and therefore reducing offloading of CO_2 at the BBB. In the older group the lack of blunting may be due to a greater rise in brain blood flow, but due to the smaller numbers for this test a type II error cannot be ruled out as one would expect a similar blunting effect within this population due to their reduced renal clearance of Az and consequent increased concentration within the circulation.

Finally, the data from the blood gases show that the 125mg Az BD was an efficient stimulus to lower HCO_3^- which triggered metabolic acidosis in both the young and the old. It could be said then that a 125mg Az BD is enoughto prevent AMS at altitude if a slow ascent profile is also incorporated. Even though this study was conducted at sea level with only acute hypoxic exposure, this conclusion has been shared by other authors who assessed AMS scores at altitude (Basnyat, et al., 2003; Basnyat, et al., 2006).

These results highlight that Az has altered pharmacodynamics based on the age of the person who is travelling to altitude and this requires consideration into the optimum dose of Az that is prescribed. It is recommended that older people take a smaller dose of Az compared with a younger person travelling to the same altitude at the same rate. From these results this dose could be 125mg BD, but future work needs to explore whether 62.5mg BD is just as beneficial to stimulating ventilation whilst limiting any negative side effects of Az.

6 REFERENCES

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7 APPENDIX

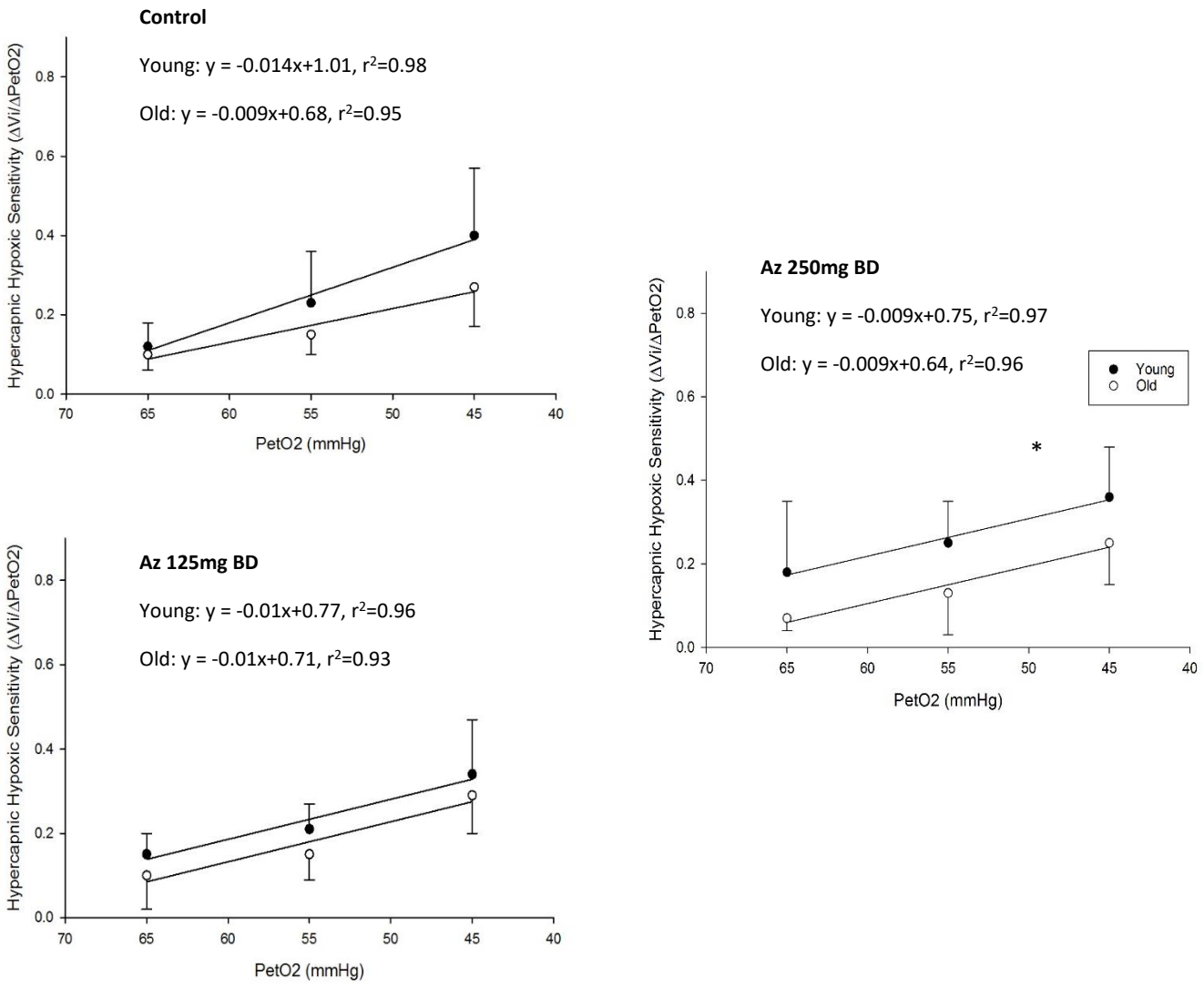

Fig 7: Hypercapnic hypoxic Sensitivity lines for $\Delta Vi/\Delta PetCO_2$. Data points are means for each group and error bars are standard deviation. * shows a significant reduction in the slope (p=0.025) and intercept (p=0.019) of the regression line