Regional Cerebral Blood Flow Circulation Differences Between the Middle Cerebral Artery and the Basilar Artery.

by

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A thesis
presented to the University of Waterloo
in fulfilment of the
thesis requirement for the degree of

Master of Science
in
Kinesiology

Waterloo, Ontario, Canada, 2017

Author's Declaration

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

I understand that my thesis may be made electronically available to the public

Abstract

Much of the research that has investigated cerebral circulation has used the MCA and has assumed that it is an indicator of what is happening in all regions of the brain. Yet little research exists to validate this notion. Recent research has suggested that the regulation of the circulation in the frontal region of the brain is different from that in the posterior region in that reactivity is higher in the front brain. To further explore this idea, transcranial Doppler ultrasound was used to measure cerebral blood flow velocity in the middle cerebral artery (MCA), which supplies much of the frontal region of the brain, and the basilar artery (BA), which supplies the posterior region. Conditions imposed alterations in end-tidal PCO_2 ($P_{ET}CO_2$) and in arterial blood pressure at the brain by sit-to-stand and tilt transitions. The hypotheses were that the MCA would be more reactive and have greater changes in relative velocity during changes in $P_{ET}CO_2$, the relative BA velocity would change less in both passive and active posture changes, and the cerebrovascular resistance would be higher in the BA during blood pressure changes.

Main findings include significantly different cerebrovascular reactivity between the two arteries which only existed when the data were expressed as absolute changes in velocity per change in mmHg of CO_2. When expressed as a relative change, there was a trend for the BA to have a higher reactivity ($p=0.082$), but this was not significant from the MCA, and this goes against the first hypothesis of this thesis, which was that the MCA would be more reactive than the BA.

In the trial which included a hyperventilation followed by a stand and breath hold, it was observed that there was a significantly different change in velocity at the stand nadir between the MCA and the BA (MCA: -41.37 ± 12.08% BA: -51.21 ± 18.35%, p=0.015). This suggests that the BA had greater changes in velocity than the MCA when there was both a change in blood pressure and a change in $P_{ET}CO_2$. Smaller changes in velocity in the MCA imply superior autoregulation, contrary to both the third and fourth hypotheses of this thesis which state that active and passive posture change will result in a greater change in the MCA relative velocity.

During hyperventilation trials when blood pressure decreased during hyperventilation, CVRi was higher in the BA than in the MCA. This was also evidenced in the interaction trial including both hyperventilation and stand. Although, the stand itself did not evoke this significant difference, the change in CO_2 combined with a stand resulted in the significant differences between the arteries. This suggested the downstream arterioles of the BA are more resistive than those of the MCA and this aligns with the fifth hypothesis of this thesis.

Acknowledgements

I want to express my most sincere gratitude to the many people who have help me throughout this degree. To Dr. Hughson for his understanding, support and patience. To Ikdip Brar and Laura Fitsgibbon-Collins for the hours spent helping me learn the numerous programs and equipment in the lab. To Danielle for her patience, expertise and general helpfulness during my data collection and analysis. To Kathryn for listening to my numerous questions and for reading and editing every document I produced. To Josie for helping me find participants and for always volunteering to help. To Travis, for keeping me sane in 1100B and for listening to my yell at my laptop on numerous occasions.

I would also like to thank several people who weren't associated with the lab. To Dr. Stobie and Dr. Snelgrove, for sticking with me at my lowest lows and helping me find a better, healthier me. To Monica and all my groups at CMHA for the innumerable pep talks and unfaltering encouragement while I battled with myself and school. To Hannah, Jolene, Adam and Peter for being my rocks even in my darkest hours.
Finally, I wish to thank my mom, Lisa, for putting up with seemingly endless amounts of phone calls, tears and frustrations, always with love and optimism.

With this army of wonderful people behind me I have been able to finish this degree. Thank you all for everything you do and have done for me. Without you, this would not have been possible.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ACA – Anterior cerebral artery
ACoA – Anterior communicating
Artery BA – Basilar artery
BAO – Basilar artery
occlusion BL - Baseline
CBF – Cerebral blood flow
CBV – Cerebral blood
velocity CCA – Common
carotid artery CO_2 – Carbon
dioxide
CR_{CO2} – Cerebrovascular reactivity
CVRi – Cerebrovascular resistance
index HUT – Head up tilt
ICA – Internal carotid artery
MCA – Middle cerebral
artery OH – Orthostatic
hypotension
P_aCO_2 – Partial pressure of arterial carbon dioxide
P_aO_2 – Partial pressure of arterial oxygen
PCA – Posterior cerebral artery
PCoA – Posterior communicating
Artery PI – Pulsatility index
PICA – Posterior inferior cerebellar artery

Introduction

The majority of cerebrovascular research has focused on the response of mean blood flow velocity in the middle cerebral artery (MCA) which greatly supplies the temporal, parietal and frontal lobes [2]. This artery has been extensively studied during challenges of CO_2 cerebrovascular reactivity (CR_{CO2}) and of autoregulation, and is commonly assumed to be an adequate measure of changes occurring across all regions of the brain[3,4]. Yet, little research has been done in this area to validate this assumption. More recent research is beginning to suggest regional blood flow differences exist in the brain, and that different arteries may react differently to stimuli, but the general understanding remains incomplete.

In this area of research, researchers will often opt to use the posterior cerebral artery (PCA) as an indicator of posterior brain blood flow. This is because it is easily accessible through the transtemporal window at a depth of 60-70mm[5].
Although in 2013 Skow and collegues compared the CR_{CO2} of the BA to the PCA during a hyperoxic rebreathing test and found no significant difference between the two arteries, this thesis used the BA as it was the main posterior artery in the brain and supplies a greater posterior region than the PCA.

Cerebrovascular reactivity has been studied regionally, and the MCA velocity was found to have ~70% more reactivity than the PCA velocity reactivity in a task of end-inspiratory breath holding [6]. Different methods for challenging cerebrovascular reactivity have also found the MCA to be more reactive than the PCA [7,8]. In a breath holding task, research has suggested that both the VA and BA react similarly, and

both arteries are less reactive than the ICA and MCA [9]. The general consensus from these few studies is that the frontal brain is more reactive than the posterior brain.

Less research has looked at differences in autoregulation between the front and hind brain. The sole sit-to-stand study that looked at the frontal and posterior brain found no differences between PCA and MCA blood flow velocity or cerebrovascular resistance index (CVRi) throughout the transition or standing period [10]. In terms of passive posture changes, another group did both a HUT and a head down tilt combined with a modified rebreathing task and their findings suggested neither HUT nor head down tilt produced a significantly different effect between the MCA or the PCA [11]. Thus, it seems as though autoregulation may be preserved across all areas of the brain in a healthy population.

The data in this thesis suggests that regional differences may be observed when reactivity and autoregulation are challenged, which is contrary to the assumptions made in most research. A better understanding of the regional differences may allow us to better predict and prevent symptoms of orthostatic hypotension like syncope and falls.

Literature Review

Anatomy and Function of the Cerebrovascular System

The circle of Willis (Figure 1) is formed by the anastomotic union of the internal carotid arteries (ICAs) and the BA [12,13]. The ICA system is largely responsible for providing blood to the cerebrum [14]. The ICAs branch to form the right and left anterior cerebral artery (ACA) and MCA, and the BA splits to form the posterior cerebral arteries (PCAs), collectively known as the great 6 vessels, they form the basis for the circle of Willis. [2,14–16]. To complete the circle, the Posterior Communicating Arteries (PCoA) connect the PCA to the MCA on the left and right sides, and the Anterior Communicating Arteries (ACoA) connect the MCA to the ACA on both sides.

Even though the circle of Willis is in fact a circle, blood does not flow circularly. It has been shown that under physiologic conditions blood from the left and right ICA enters the brain and does not cross the midline of the brain [17]. In a healthy brain, the ACoA and the PCoA often have little to no flow [18]. For a complete Circle of Willis, some in-vivo work supports and shows no flow across the ACoA [19,20] and this is also supported by numerical simulations [21,22]. In a study which looked at pressure differences in the PCoA at the MCA end and the PCA end of a healthy brain, they found a pressure difference of zero, denoting no flow through the PCoA [23]. This is normal; the communicating arteries used to form the circle of WIllis are not needed to perfuse the brain adequately in healthy subjects [24].

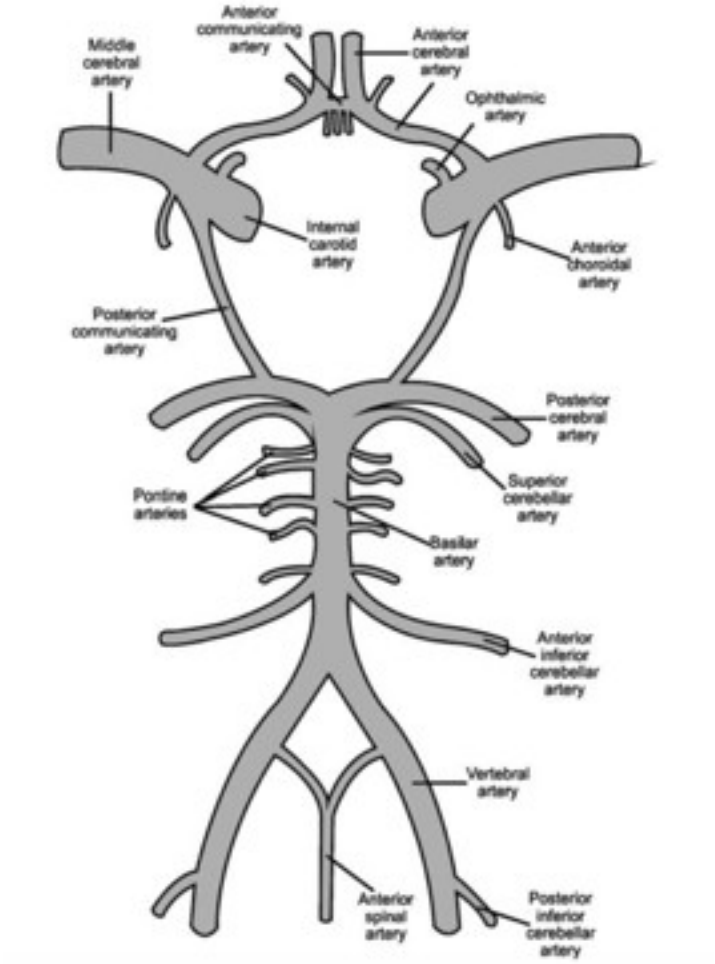

Figure 1: Anatomical representation of the Circle of Willis from: Vreselja et al. (2014) Anatomy of the Circle of Willis.

Ultimately, if there is insignificant blood flow in the ACoA or the PCoA, this divides the circulation of the circle of Willis into two: the front brain and the posterior brain. This fact had led researchers to conclude the blood flow from the ICAs and the blood flow from the VAs supply their own perfusion area and no blood transfers between the MCA and the PCA [23]. Stenosis research also supports this notion. When the ICA was occluded, the most notable velocity decrease is observed in the MCA and the ACA, suggesting front brain is most affected by ICA stenosis [23]. Flow rates in PCA are less affected by unilateral ICA stenosis. This is probably due to

the fact that blood flow of posterior circulation is mainly provided by VA-BA system. [23]. This is further supported by the fact that if a vertebral artery (VA) is occluded, the PCoAs are more important to maintaining perfusion in the circle of Willis than the ACoAs. [24]

The MCA is the largest cerebral artery, running horizontally through the sylvian fissures, vertically on the surface of the insula, as well as laterally and exiting the insular cistern [25]. MCA is important as it is the most prevalent cerebral artery in the existing research. The MCA is responsible for supplying blood to the temporal, parietal and frontal lobes [2]. These areas contain the primary motor cortex, Broca's area, the prefrontal cortex as well as auditory and olfactory areas [2].

The vasculature of the posterior brain is slightly less complex. At the base of the skull, the VA penetrate the foramen magnum and the dura, running through the subarachnoid space, ventrolateral to the medulla oblongata before reaching the bulbopontine junction where the BA is formed [2,25]. The BA has numerous branches which supply various parts of the posterior brain including the pons, the brainstem and the cerebellum [2,13,25]. Its branches supply blood to cranial nerve VI (abducens), VII (facial) and VIII (vestibulocochlear) [13,25]. It also supplies areas important for planning and programing movements and muscular coordination, regulating muscle tone and maintaining equilibrium and posture by influencing the trunk musculature[26].

Symptoms of Decreased Blood Flow

Data from stroke research can be used to help understand what happens when certain areas of the brain are not receiving enough blood. During a stroke, blood circulation to an area of the brain is blocked causing ischemia, a of lack and nutrition as well as a build-up of waste[2]. A decrease in the frontal brain flow is often caused by occlusion of the ICA. Those with ICA occlusion have diminished blood flow through the artery, but collateral flow occurs in the circle of Willis to preserve blood flow to meet metabolic demands [27–29]. Due to this, sometimes the diminished blood flow can be asymptomatic. Symptomatic ICA occlusion is characterized by transient ischemic attack, minor stroke, minor-disabling neurological deficits [28]. Ischemia in the carotids has also been observed to cause limb and facial numbness and paresis, visual impairments, aphasia, syncope, dysgraphia, and dysarthria [30,31]. This makes sense; occluding blood flow to the front brain affects the output of Broca's area, the area involved in speech. It also affects the frontal eye fields as well as the primary motor cortex, causing an effect on the outputs of these areas as well resulting in seeing spots of blacked out vision.

In the posterior brain, recanalization is less likely to occur and often basilar artery occlusion (BAO) results in stroke and a fatal outcome [32,33]. In a study of 10 cases of PCA infarction, prodromal symptoms included confusion, visual impairment, and amnesia [34]. If stroke does not occur, vertebrobasilar insufficiency or BAO results. Vertebrobasilar insufficiency is a complex clinical picture, characterized by a lack of blood flow to the vertebrobasilar system most often causing symptoms like motor and oculomotor deficits, vertigo, nausea, headache, confusion, weakness, sudden loss of consciousness and tinnitus [35–39]. Some research also suggests up to 15% of

patients with vertigo also have hearing loss [35]. Bearing in mind what the vertebrobasilar system supplies, these numerous symptoms come as no surprise. The vertebrobasilar circulation supplies the vestibular pathways as well as the auditory systems like the cochlea; the decreased blood flow has been shown to produce vertigo and deafness [35]. The area of the brain critical to consciousness in humans is the median pontine tegmental grey matter which is immediately ventral to the ventricular system and is supplied by the penetrating branches of the BA [38]. The lack of consciousness and confusion experienced by patients is most likely due to insufficient blood flow to this area. Additionally, amnesia results from damage to the limbic structures, another area supplied by the vertebrobasilar system [34].

The stroke data suggest that the area of the brain not receiving blood flow, for whatever reason, produces symptoms related to that area. Thus, because the symptoms of orthostatic hypotension and presyncope have striking similarities to that of BAO it seems rational to assume the same area of the brain is affected. This may in fact be a sign that the posterior brain is experiencing a different CR_{CO2} or regulation than the front brain.

Regulation of Cerebral Blood Flow

It is important to understand how normal CBF is maintained when challenged metabolically and by gravity. A failure in the autoregulation and reactivity systems causes decreases in blood flow to certain areas of the brain. The brain is a very complex and delicate organ. It is highly metabolic and as such, requires waste removal as well as adequate nutrition at all times from blood [40,41]. It has safeguards in place to ensure it always has sufficient blood flow, which can be altered by changes in P_aCO_2 and in blood pressure.

The brain is extremely sensitive to changes in P_aO2, P_aCO_2 and pH [42–44]. More specifically, it is the smaller arteries that are most sensitive and affected by changes in P_aCO_2 [45]. Tissues release oxygen and adenosine in order to change vascular resistance in response to hypercapnia [12]. Decreases in oxygen increases the smooth muscle membrane potential by opening the Ca^{2+} activated K^+ channels or ATP- sensitive K^+ channels in the cell membrane [12]. Local changes in the tissue causing hypoxia, hypercapnia and acidosis result in vasodilation of the arteries [42]. This causes the vessels to "wash out" CO_2 from the tissue [3,46]. If the tissue is hypocapnic, vasoconstriction occurs, decreasing CBF and resulting in the fall of P_aCO_2 [3,46,47].

Reactivity in response to hypocapnia has been shown to work very rapidly, responding by dropping CBF to half after 1.9 seconds [48]. Thus, the vascular response to changes in P_aCO_2 is local and extremely rapid to maintain pH and blood flow. When blood pressure rises or falls, or cerebral perfusion pressure changes, there are changes in cerebrovascular tone modulated by myogenic, metabolic, shear-dependent and neurovascular regulation [42,48–50]. This intricate process of maintaining stable cerebral perfusion and tissue oxygenation against changes in blood pressure is termed *Cerebral Autoregulation* [40,51–53]. When autoregulation is functioning properly, CBF is maintained at around 40–50 ml/ 100 g/min over the range of ABPs from 50 to 150 mmHg [52,54]. As with CR_{CO2}, the smooth

muscular cells vasodilate or vasoconstrict by changing the activation state of the K^+ and Ca^{2+} channels in the cell membrane [12]. When blood pressure is low, vessels dilate, and when pressure is high vessels constrict [12]. This process happens rapidly, taking merely seconds due to the myogenic response [55]

The final way cerebral circulation can be changed is through direct neural input. The cerebral vessels are innervated by both sympathetic and parasympathetic nerves which can also cause vasoconstriction and vasodilation [12]. This is an area is less studied, but still plays a role in vasodilation and vasoconstriction of blood vessels.

Orthostasis

The body is challenged every time a human stands up and the brain responds with rapid cerebral autoregulation. Assuming an upright posture causes blood to shift, resulting in the pooling of about 700mL of blood in the lower abdomen and legs [12,56,57]. Venous pooling is not complete for upwards to 5 minutes [58,59]. Pooling causes a decreased venous return to the heart, decreased ventricular filling, resulting in lower end diastolic volume and ejection, and lower cardiac output resulting in a lower mean arterial pressure [12,56]. There is a fall of systolic blood pressure of 5-10 mmHg and a slight diastolic blood pressure rise [60]. Due to the hydrostatic gradient of the brain now being above the heart, the perfusion pressure in the brain decreases and CBF is at risk of becoming inadequate, just from simply standing [3,12,44].

Peripherally, in order to adjust to these changes, the arterial mechanoreceptors, located in the aortic arch and carotid sinuses, cue for neural reflex adjustments [58]. The drop in total peripheral resistance causes the arterial baroreceptors to respond to the hypotension; this is a reflex which acts to maintain carotid blood pressure and therefore increases blood pressure at heart level [61]. The cardiopulmonary receptors respond to the decrease in preload, increasing total peripheral resistance [12,40,57]. The baroreflex also detects the blood pressure decrease and stimulates sympathetic and parasympathetic activity, increasing heart rate and peripheral vasoconstriction in order to restore blood pressure [12,57]. Stimulation of the autonomic nervous system results in release of epinephrine, which helps return blood to the heart by contributing to an increase in heart rate of 10-25 beats per minutes to counteract the reduction in cardiac stroke volume for the maintenance of cardiac output [57,60].

In some people, this blood pressure regulation system fails resulting in a prolonged, decreased blood pressure. A fall in systemic blood pressure of more than

20mmHg of systolic blood pressure or of more than 10mmHg of the diastolic blood pressure within three minutes of standing is termed *Orthostatic Hypotension* (OH)[60,62,63]. It occurs due to the autonomic nervous system failing to respond to the challenges moving to an upright posture presents, causing significant pooling of blood in the lower limbs, which results in a decreased end diastolic filling, a reduced circulation and insufficient blood to the brain [64]. Symptoms of OH include postural dizziness, fatigue and blurred vision [63]. Orthostatic hypotension can also be a symptom of *presyncope,* a time where a patient feels as though syncope is imminent [57,64]. Normal symptoms of presyncope can be seen in Table 1.

Syncope, a sudden, transient loss of consciousness and postural tone with spontaneous recovery, is a concern for those with OH [49]. OH is greatly due to peripheral blood pressure dysregulation, and it places the brain at risk. Syncope is caused, ultimately, by a lack of oxygen in the brain. The symptoms of OH, presyncope as well as BAO are all very similar suggesting they may be caused by a similar area of the brain. If these symptoms exist as a precursor to syncope, there must be a decrease in blood flow to a specific area of the brain.

Table 1: Common symptoms experienced during presyncope [22–25]

Presyncope Symptoms
Uncomfortable in an ill-defined way Sweating
Nausea
Desire to sit down or leave the room Light-headed
Weakness
Visual disturbances/blurring/dimming Neck pain
Headache Cognitive slowing
Seizure-like tonic movements Yawning/fatigue
Dizziness

Regional Differences

It is understood that different arteries in the brain have different blood velocities but it has long been believed that the frontal and posterior regions of the brain have similar CR_{CO2} and cerebral autoregulation responses [3]. Due to this understanding, several commonalities have emerged in the literature. It is assumed that anterior Cerebral blood velocity (CBV), measured in the MCA, is adequate for estimating whole brain CBV and that changes seen in the MCA are reflected by all other cerebral arteries [3,4]. The MCA is the artery of choice as it is easy to insonate through the transtemporal window, has the highest blood velocity, and when the two MCAs are combined, they perfuse 70-80% of the whole brain (reviewed by Willie et al., 2011). Additionally, it has an insonation angle close to zero reducing error. New data are starting to suggest that assuming everything reacts like the MCA is an inaccurate overestimation, and that there may be differences in CR_{CO2} between the frontal and posterior brain [7,8,66,67].

The posterior arteries are more difficult to insonate. The BA is best found through the foramen magnum at a depth of 80mm to 120mm, and no commercial head set exists to hold a probe in that position [5]. The PCA can be insonated through the temporal window at a depth of 60mm to 70mm [5]. The challenge of finding and maintaining a quality signal becomes apparent when looking at the literature. Skow et al. (2013) compared the CR_{CO2} of the BA to the PCA during a hyperoxic rebreathing test and found no significant difference between the two arteries. This, plus the challenge the BA has presented scientists in the past, has caused the BA to not often be used in literature, with methodologies opting for the PCA or the VA instead. Insonating the BA requires the chin to be tucked in order to widen the foramen magnum window, which places participants in an uncomfortable position that is hard to maintain for any length of time. Also, small movements of the head and neck

frequently cause a lack of signal, so the participant has to remain almost completely still. Both the PCA and VA are easy to insonate from a neutral head position and the signal is easier to maintain for a long period of time.

There is a growing body of evidence which states the frontal and posterior brain regulate their circulations differently. Differences have been found in CR_{CO2}[7–9]. Some work has shown the BA reacts in a similar way as the MCA [45,68]. In more recent literature, the MCA velocity was found to have ~70% more reactivity than the PCA velocity reactivity in a task of end-inspiratory breath holding [6]. This is similar to past rebreathing and steady state models which have found the MCA to be more reactive than the PCA [7,8]. In a breath holding task, research has suggested that both the VA and BA react similarly, and both arteries are less reactive than the ICA and MCA [9]. The most convincing work thus far has been produced by Sato et al. which showed that the internal carotid arteries were more reactive than the vertebral arteries during a rebreathing task[67]. Additionally, they collected data on both the MCA and the BA in 6 subjects, which was insufficient to run statistics, but did show that the MCA was more reactive than the BA. From this research, it seems as though the CR_{CO2} of the arteries is greater in the frontal aspect of the brain.

Another way of challenging cerebral hemodynamics is through a posture change, either actively through a sit-to-stand protocol, or passively through a head- up tilt (HUT) which stimulates cerebral autoregulation through changes in blood pressure. A passive HUT presents slightly different hemodynamic properties than an active stand [48]. While both stimulate cerebral autoregulation to occur, a stand causes an initial drop in blood pressure as well as an increase in heart rate whereas a HUT has no initial orthostatic hypotension and a slow and slight increase in heart rate [69]. Oddly enough, HUT does not increase in breathing rate, but the change in end tidal CO_2 and P_aCO_2 that is seen is thought to be due to the changes in alveolar ventilation that occurs during tilt [40,70,71]. HUT also causes a decrease in cerebral

blood flow velocity which is thought to be caused by increased CR_{CO2} [40,70].

The majority of sit-to-stand procedures have only looked at the MCA in the front brain, and have ignored the posterior brain. Assuming an upright posture is linked to an initial decrease in the CBF of the frontal and parietal areas of the brain which does return to baseline with time [72,73]. It has been seen that active standing causes a decrease in prefrontal cortex oxygenation that remains decreased throughout a steady state period even without any visible symptoms [74]. Significant frontal flow decreases have been observed during both steady state active and passive standing [72,73,75]. These affected areas of the brain are important, as they play a huge role in integration of visual sensory inputs, a crucial element to a successful posture change to standing [72]. The sole sit-to-stand study that looked at the frontal and posterior brain found both the MCA and the PCA had a slight increase in velocity before a distinct nadir and recovery occurred, but there were no differences observed in PCA and MCA blood flow velocity or cerebrovascular resistance index (CVRi) throughout the transition or standing period [10].

In a passive posture change, HUT testing has shown decreases in ICA blood flow while the VA remained constant [68]. Another group did both a HUT and a head down tilt combined with a modified rebreathing task [11]. Their findings suggested that in all positions, the MCA had an absolute greater CR_{CO2} and greater CVRi, but neither HUT or head down tilt had a differential effect on either the MCA or the PCA [11].

In terms of other measures used to assess the differences between the front brain and the hind brain, CVRi has been used in a lower body negative pressure study[113]. At syncope, MCA CVRi was found to be less than the CVRi in the PCA. Little else exists with regards to CVRi or pulsatility index (PI). To get a better, all-around understanding of the differences between the front brain and the posterior brain both of these measures will be used in the following study.

All these studies use the MCA to represent the frontal brain, but there is little consistency in the artery used for the posterior brain, with most opting for the PCA or the VA. Rarely has the BA been used, possibly because it has been reported it acts similarly to the PCA to changes in P_aCO_2 [7]. But, it is the largest and main posterior artery and the majority of the posterior circulation stems from a BA branch. As mentioned, it's these branches that supply many of the areas affected in orthostatic hypotension and presyncope. Information regarding the actions of the BA during challenges of cerebral autoregulation and CR_{CO2} remain either non-existent or incomplete. In fact, no HUT, sit-to-stand or hyperventilation and breath hold studies have been completed using the BA as a posterior circulation marker. Using these methods in combination can offer a well-rounded explanation of cerebral autoregulation and CR_{CO2} in the BA in comparison to the MCA. A HUT causes a passive change in blood pressure and P_aCO_2 [70]. Because it is passive, blood pools and presents more of a challenge to the cerebral autoregulation process. A sit-to-stand protocol causes a change in blood pressure but maintains the muscle pump, again causing a pressure regulation challenge, but does not have the same type of change in P_aCO_2 that a HUT causes; HUT is a slow decrease while a sit-to-stand causes a more abrupt change in response to the posture change [70,76]. A hyperventilation and breath hold task isolates CR_{CO2} by challenging the cerebral arteries ability to respond to changes in P_aCO_2.

Purpose

The purpose of this thesis was to examine regional cerebral blood flow differences between the MCA and the BA. This was accomplished by challenging cerebral autoregulation through an active and passive posture change, and by challenging cerebrovascular reactivity through hyperventilation and breath hold tasks.

Hypotheses

1. The MCA will be more reactive to changes in $P_{ET}CO_2$ than the BA[67].
2. Decreasing $P_{ET}CO_2$ and blood pressure will cause greater changes in MCA velocity than in BA velocity [72,73,75]
3. The relative change in BA velocity will be less than the MCA during a passive posture change[68].
4. The relative change in BA velocity will be less than the MCA during an active posture change[72,73,75]
5. Cerebrovascular resistance will be higher in the BA than in the MCA when blood pressure decreases[113].

METHODS

SUBJECTS

Two groups of 11 participants were recruited through posters on campus and in the community, and through word of mouth. There were two groups as testing was completed on separate days. Volunteers who had peripheral vascular disease, diabetes, cardiovascular disease, kidney disease, chronic inflammatory disease, neurological disorders, skin sensitivity, or were currently pregnant were excluded from participating in this study. There was some overlap in volunteers for the two groups but this is not expected to have an effect on the data as the model accounts for random effects.

Table 2:Participant characteristic for all tests.

	Sit to stand, HV, BH, Interaction	**HUT**
Sex	3M 7F	3M 7F
Age (years)	21.0 ± 2.67	24.0 ± 2.67
Height (cm)	173 ± 8.40	181.7 ± 28.0
Weight (kg)	70.7 ± 12.0	65.5 ± 13.2
MCA Depth (mm)	48.9 ± 6.47	53.2 ± 6.41
BA Depth (mm)	85.1 ± 4.18	88.0 ± 4.83

HV= hyperventilation trial. BH= breath hold trial. HUT= head up tilt trial. MCA= middle cerebral artery.

BA=basilar artery.

For the sit to stand, hyperventilation and breath hold as well as the interaction trial (Table 2), 3 males and 8 females volunteered but one female was not included in the final data analysis due to a machine malfunction. For the HUT (Table 2), 11 participants volunteered, including 3 males and 8 females. One female was not included due to an inability to maintain signal from the BA.

Participants were asked to refrain from consuming food and caffeine 2 hours

prior to testing, and not to consume alcohol in the 24 hours leading to testing time. No strenuous exercise was done 24 hours prior to testing, and only light walking was done on the day of testing.

PROTOCOL

Tests were divided between two days, and two different groups of participants participated in the different days. The first group only did a HUT, while the other group at a later date participated in the randomized hyperventilation/breath hold, followed by the sit to stand and then the randomized interaction trials including a hyperventilation/breath hold combined with a sit to stand

Participants were asked to read an information letter and were instructed to ask questions if they had any, complete a health status form and provide a written, informed consent form prior to any measure being taken. An overview of the protocols can be seen in Figure 2. Research ethics were obtained from the University of Waterloo Clinical Research Ethics Committee (ORE21797 and ORE21728). The protocol was also verbally discussed to ensure clarification. Participants were familiarized with the equipment as well as the lab before instrumentation and were encouraged to ask questions to ensure their comfort during testing. Then, height and weight were measured and an ID number was assigned. Room temperature, barometric pressure and relative humidity was also be noted to ensure the testing environment was similar between participants. At this point, instrumentation occurred in a seated position.

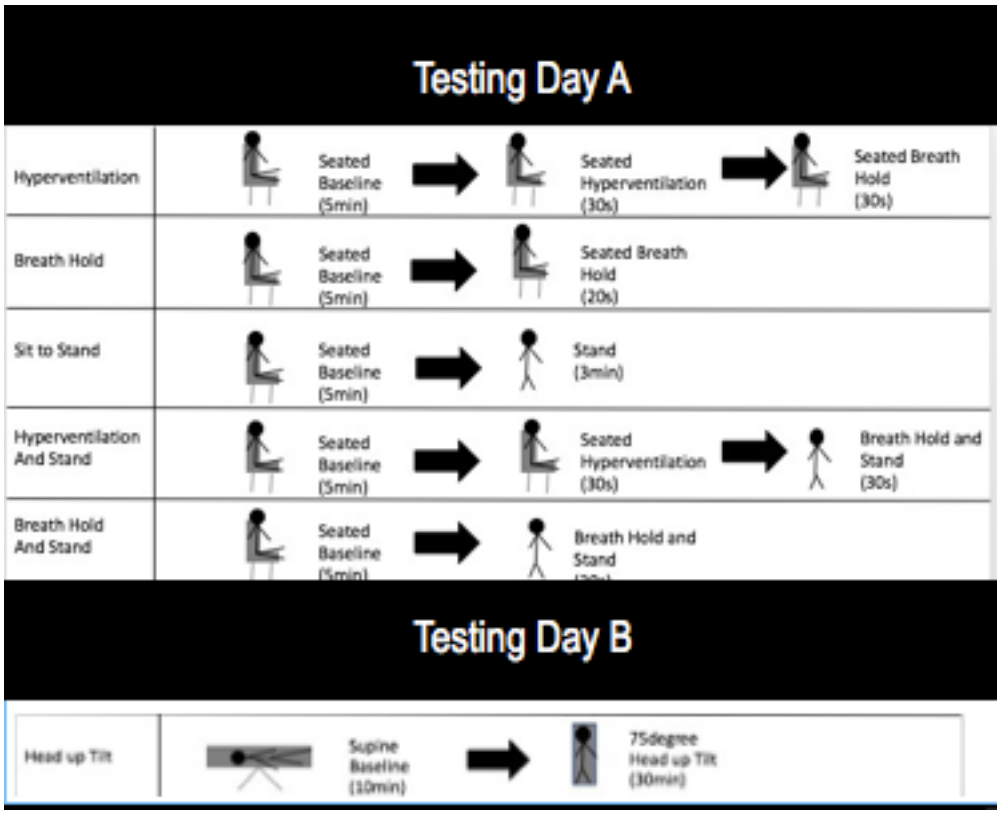

Figure 2: An overview of the 6 different protocols used in this thesis and the order of testing.

SIT-TO-STAND

Once set up with equipment, participants were asked to remain in a seated position with their feet on the floor for 5 minutes to collect baseline resting data.After this time, participants were asked to quietly plant their feet and stand for 3 minutes. After this period, they returned to a seated position with their feet on the floor for 5 minutes. This was repeated for a total of 3 trials. After this, participants were asked to complete the orthostatic hypotension questionnaire (OHQ).

HYPERVENTILATION AND BREATH HOLD

After instrumentation (see *Physiological Measures* below), participants underwent a voluntary hyperventilation and hypoventilation protocol. It began with a 5 minute seated rest period with their feet on the floor, and their back supported by a chair. Participants were asked to stare at a dot placed on the wall ahead of them to avoid any confounding effects visual stimuli might have on the BA blood flow velocity [77]. Then, in random order they were asked to either hold their breath for 30 seconds, or hyperventilate at a rate of 30 deep breaths per minute for 30 seconds, immediately followed by a 30 second breath hold. The hyperventilation was not to be shallow; breaths were of normal volume, just faster. Additionally, the breath hold was not strained, and participants were as relaxed as possible for the 30 seconds. Each condition was completed twice and was separated by a minimum of 2 minutes of normal breathing. Once all 4 trials were complete, participants were asked to complete two OHQ, one for breath holding and the other for hyperventilation.

The participants then sat and stared at the dot on the wall in preparation for the interaction trial. They rested in this position for a 5-minute baseline period. Then randomly participants were asked asked to either hold their breath for 30 seconds and simultaneously stand at the commencement of breath hold or hyperventilate at a rate of 30 deep breaths per minute for 30 seconds, immediately followed by a simultaneous 30 second breath hold and stand at the commencement of breath hold. Each condition was completed twice, and each trial was separated by a 5- minute baseline period. Once all 4 trials were completed, equipment was removed

and the participant was asked to complete the OHQ twice, once for breath holding with stand and once for hyperventilation with stand.

HEAD UP TILT

Before undergoing instrumentation, the tilt table was adjusted to each participant's size. This ensured the head was in the head cradle in such a way that exposed their occipitus to access the BA; shoulders were on the shoulder board and that their feet laid flat on the foot board. This position was made as comfortable as possible by adjusting the necessary pieces of the table. Participants moved from the table to a chair for instrumentation. This included NIRS, transcranial Doppler for the MCA, transcranial Doppler for the BA, ECG and beat by beat blood pressure measurements.

After instrumentation, participants were asked to carefully reposition themselves on the tilt table placing their feet flat on the foot board, and their head in the modified cradle with the help of researchers. At this time, adjustments were made to the Doppler probes as necessary to ensure a strong signal. Once complete, the participants were given a pillow to hold over their chest before being secured onto the table using a two ratchet straps placed in an "X" formation over their shoulders and chest. This helped to ensure their feelings of safety and comfort when the tilt table was moved. The participant was verbally instructed to alert the researchers if at any time they began to experience nausea, narrowing of vision, loss of vision, severe light-headedness or dizziness throughout the test.

Upon completion of set up, participants remained in the supine position quietly for 5 minutes of resting data collection. Then, two researchers verbally

coordinated removing the brake, and smoothly transitioning the table from 0^{o} to 75^{o} where they once again locked the table in place. The passive transition took 5-10 seconds to complete, during which time participants remained quiet. The test was prematurely terminated by the researchers if systolic blood pressure dropped below 90mmHg, or there is a sudden drop in heart rate (>15 bpm), or if the participant requested termination for any reason. Only one participant had a drop in systolic blood pressure to the termination point. Another participant's test was ended early due to a loss of signal. Otherwise, participants remained in this new position for 30 minutes. After 30 minutes, two researchers once again coordinated removing the brake from the table and smoothly transitioning from 75^{o} back down to 0^{o} before locking the table in place. Equipment was removed and the participant was asked to complete the OHQ.

PHYSIOLOGICAL MEASURES

Arterial blood pressure was continuously measured non-invasively by finger plethysmography (Portapres, Finapres Medical Systems, Hogehilweg, Amsterdam, The Netherlands). Hand was held at heart level so that finger blood pressure data was representative of the blood pressure at the heart level.

Heart rate was determined by an electrocardiogram (Philips iE33 Ultrasound, Philips, Koninklijke, N.V) using electrodes placed on the right and left shoulder below the collar bone and the left fifth intercostal space.

During the sit-to-stand, the hyperventilation and breath hold and interaction trials, a reusable mask attached to a sample line sampled exhaled CO_2. Gas was analysed by infrared spectroscopy (DATEX-OHMEDA 5200 CO_2 Monitor, Mundelein, IL, USA). The peak concentration of CO_2 at the end of an expired breath was considered the end-tidal CO_2 ($P_{ET}CO_2$).

A near infrared spectroscopy probe (Portalite, Artinis Medical Systems, Elst, Netherlands) was be placed above the right eyebrow to measure prefrontal cortex oxygenation.

Cerebral blood velocity (CBV) of the MCA was measured using transcranial Doppler ultrasound (DWL Doppler-Box, Compumedics, Germany GmbH, Singen, Germany) through a 2 MHz transducer placed on the right temporal cranial window. The probe was attached to a modified, adjustable headband (Marc 600, Spencer Technologies, Seattle, WA USA) which was fit snugly to the participant's head, and held the probe steady on the MCA for a continuous velocity signal. The MCA was insonated through the transtemporal window at a depth of ~50mm with an anticipated mean velocity of 55 ± 12 cm/sec [5].

CBV of the BA was measured using transcranial Doppler ultrasound (DWL Doppler-Box, Compumedics, Germany GmbH, Singen, Germany) through a 2 MHz transducer placed on the occipital region for the foramen magnum cranial window

(Figure 3). Based on a previous TCD headband model for the BA[78], the probe was attached the posterior of the same headband used to secure the MCA probe to hold this probe tightly in place. Following the previously outlined standardized protocol, the BA was insonated through the transforaminal window at a depth of ~80mm with an anticipated mean velocity of 41 ± 10 cm/sec [5]. This involved finding the notch at the bottom of the occipitus and then insonating the probe at an angle towards the eyes. Researchers always confirmed the vertebral artery was found more shallow to confirm the deeper artery was in fact the BA.

All of these inputs, with the exception of NIRS, were immediately integrated and time aligned by the program Lab Chart (ADInstruments, Dunedin, New Zealand). They were collected at 1000Hz. NIRS was collected at 50Hz.

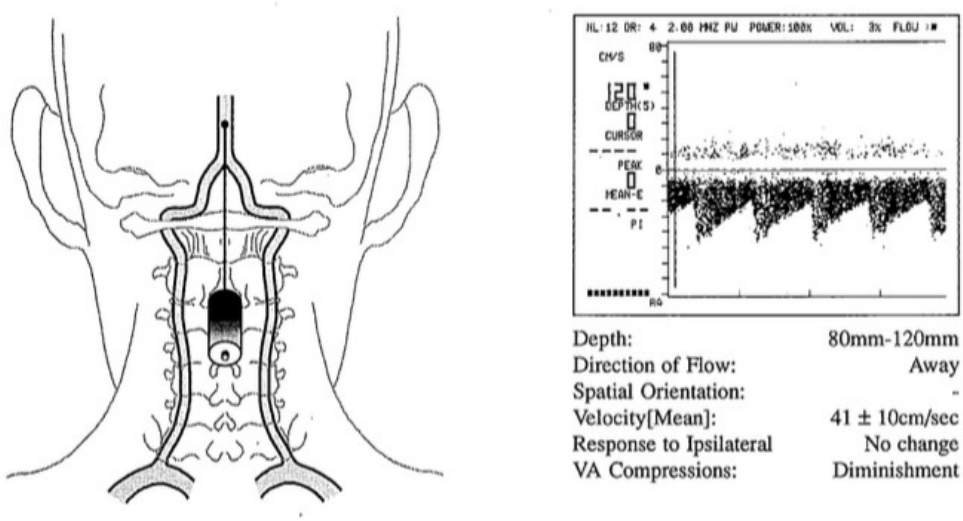

Figure 3: Position of the Doppler to obtain the signal of the BA and its subsequent waveform and properties. From Newell et al. (1992) Anatomy and Freehand Techniques.

DATA ANALYSIS

Using Lab Chart (ADInstruments, Dunedin, New Zealand), a macro was coded to extract beat-by-beat maximum, minimum and mean values of the TCD waveforms as well as CO_2. The outer envelope of the TCD signal was averaged over each cardiac cycle to determine CBV in both the BA and the MCA.

NIRS was collected at 50Hz; ECG was down sampled from 1000Hz to 50Hz to time align NIRS with the rest of the data. Once time aligned, a macro was used to pull data in a beat-to-beat format. Once all data sources were time aligned, beat-by-beat data were cleaned and linearly interpolated into a second-by-second format for further analysis using Sigmaplot (Systat Software, Krakow, Poland). Further analysis was carried out using Statistical Analysis Software (SAS Institute, Cary, NC, USA) using a proc mixed procedure with random effects.

Statistical significance was set at $p < 0.05$. The flow chart seen in Figure 4 illustrates the numerous statistical tests that were completed. During HUT, which consisted of 1 trial, a 2 way (Time X Artery) RM ANOVA with a post hoc Tukey test was used to analyse velocity, change in velocity, PI, RI, CVRi and CR_{CO2}. A 1 (Time) way repeated measures ANOVA with a post hoc Bonferroni test was used to analyse the change in TSI and the change in oxygenated haemoglobin. Both the hyperventilation and breath hold trials, the interaction trials and the sit to stand trials consisted of multiple trials. Every trial was used, thereby including a random effect of participant into the model. Due to this, a 2 factor (Time X Artery) mixed linear model with random effects was used to analyse velocity, change in velocity, PI, RI, CVRi and CR_{CO2}. For the change in TSI and the change in oxygenated haemoglobin, a 1 factor (Time) mixed linear model with random effects was used for analysis. A Tukey post hoc test was always used with the mixed linear model.

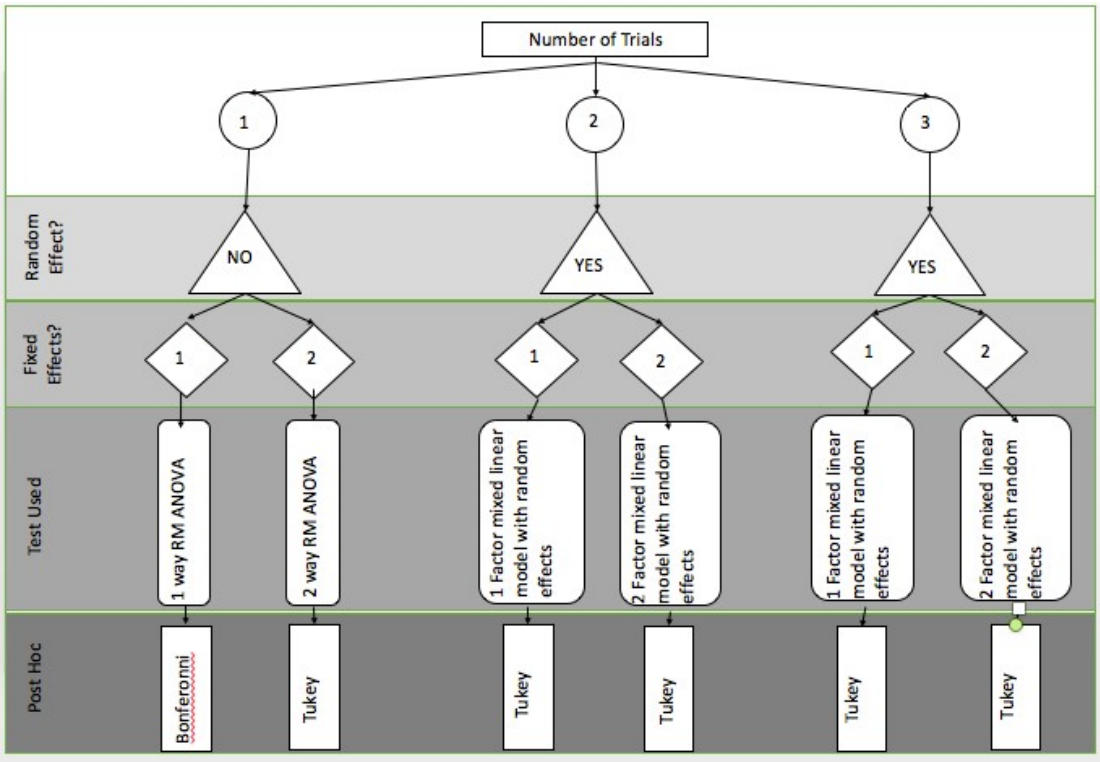

Figure 4: Flow chart of the statistical models used for analysis (adapted from Moye, l, Statistical Methods for Cardiovascular Researchers, 2016)).

HYPERVENTILATION AND BREATH HOLD

Each trial was treated separately and trials were not averaged across subjects. Mean CBV was averaged in the final 30s of baseline, the final 5s hyperventilation and the final 5 s of breath hold conditions. $P_{ET}CO_2$ was averaged in the 30s of baseline, the final 5s of hyperventilation and in the first breath after breath hold. These values were used to calculate cerebrovascular reactivity (CR_{CO2}) (Equation 1) of the BA and the MCA between baseline and hyperventilation, and between hyperventilation and breath hold or between baseline and breath hold.

Mean, minimum and maximum velocity were averaged in the final 30s of baseline, and in the final 5s of every ten second increment of the trial. These values were used to calculate PI (Equation 2) and RI (Equation 3). Mean arterial pressure was averaged during these same time points and used with velocity to calculate CVRi (Equation 4).

Thus, using the final 30s of baseline, the final 5s of every 10 second increment, the MCA and BA CBV, change in velocity, PI, RI, CVRi and CR_{CO2} were calculated. Hyperventilation and breath hold were separated in the same trial, and analysed separately; baseline, 10s, 20s and 30s were compared, then 30s, 40s, 50s and 60s were compared. As seen in Figure 4, a 2 factor mixed linear model with random effects was used. The change in TSI and the change in O_2Hb from baseline were calculated at the same time points but a one factor mixed linear model with random effects was run on the full 60s trial for hyperventilation trials (30s of hyperventilation and 30s of breath hold), and only 30s for breath hold trials. This was done to separate and monitor the effect of each maneuver.

$$CR_{CO2} = \frac{\Delta CBV}{\Delta P_{ET}CO_2} \qquad \text{Equation 1}$$

$$PI = \frac{Systolic\ Vessel\ Velocity - Diatsolic\ Vessel\ Velocity}{Mean\ Vessel\ Velocity}$$ Equation 2

$$RI = \frac{Systolic\ Vessel\ Velocity - Diastolic\ Vessel\ Velocity}{Systolic\ Vessel\ Velocity}$$ Equation 3

$$CVRi = \frac{BP\ Vessel}{CBV\ Vessel}$$ Equation 4

For hyperventilation, the scores of the OHQ were totaled and the change in oxygenated haemoglobin at the end of hyperventilation was averaged for each participant. The Pearson correlation coefficient was calculated using Sigmaplot. For breath hold, the OHQ scores were correlated with the change in oxygenated haemoglobin at 20s of breath hold.

SIT-TO-STAND

For both the MCA and the BA, velocity, change in velocity, PI, RI, CVRi, $CR_{CO2,}$ change in TSI and change in oxygenated haemoglobin were calculated for a 30s baseline average, and for 5s averages at the end of every 30s increment of the 3min stand. In addition to looking at the changes over time, a 30s baseline average was also compared the value of each variable at the mean arterial pressure nadir caused by the stand. The nadir was considered the point at which mean arterial pressure was the lowest after the stand. Corresponding statistical tests were run as per Figure 4.

The scores of the OHQ were totaled and the change in oxygenated haemoglobin at 30s into the stand was averaged for each participant. The Pearson correlation coefficient was calculated using Sigmaplot.

INTERACTION TRIALS

For both the MCA and the BA, velocity, change in velocity, PI, RI, CVRi, $CR_{CO2,}$ change in TSI and change in oxygenated haemoglobin were calculated for a 30s baseline average, and for 5s averages at the end of every 10s increment of the first minute of the 3min stand. Hyperventilation and breath hold were separated in the same trial, and analysed separately; baseline, 10s, 20s and 30s were compared, then 30s, 40s, 50s and 60s were compared. In addition to looking at the changes over time, a 30s baseline average was also compared the value of each variable at the mean arterial pressure nadir caused by the stand. Corresponding statistical tests were run as per Figure 4.

For hyperventilation + stand trials, the scores of the OHQ were totalled and the change in oxygenated haemoglobin at the end of hyperventilation was averaged for each participant. The Pearson correlation coefficient was calculated using Sigmaplot. For breath hold + stand trials, the OHQ scores were correlated with the change in oxygenated haemoglobin at 20s of breath hold.

HEAD UP TILT

For both the MCA and the BA, velocity, change in velocity, PI, RI, CVRi, change in TSI and change in oxygenated haemoglobin were calculated for a 30s baseline average, and for 5s averages at the end of every 1min increment of the 10min HUT. Corresponding statistical tests were run as per Figure 4.

The scores of the OHQ were totalled and the change in oxygenated haemoglobin at the end of tilt was averaged for each participant. The Pearson correlation coefficient was calculated using Sigmaplot.

RESULTS

HYPERVENTILATION

For the hyperventilation trials (Figure 5), all variables were analyzed for the first 30s of hyperventilation at 60 breaths/min, then separately for the subsequent 30s breath hold. As per Figure 4, we expected to see a decrease in velocity during hyperventilation, and an increase during breath hold. Using absolute values, there was a main effect of artery ($p<0.0001$) and a main effect of time ($p<0.0001$) but there was no statistical significance between the two arteries with the interaction effect (HV: $p=0.160$ BH: $p=0.57$). The non-significant difference between arteries reflected lower absolute values for the BA relative to the MCA. When CBV data were expressed as %change in velocity (Figure 6B) from baseline, for the 30s of hyperventilation, there was a main effect of artery ($p<0.0001$) and of time ($p<0.0001$) but the interaction effect was not significant ($p=0.079$). The not significant difference between arteries showed the BA experienced a greater change in the change of velocity than the MCA change in velocity, but again this was not significant. For the following 30s breath hold, there was again a main effect of both artery ($p=0.0003$) and time ($p<0.0001$) but the interaction effect was not significant ($p=0.30$).

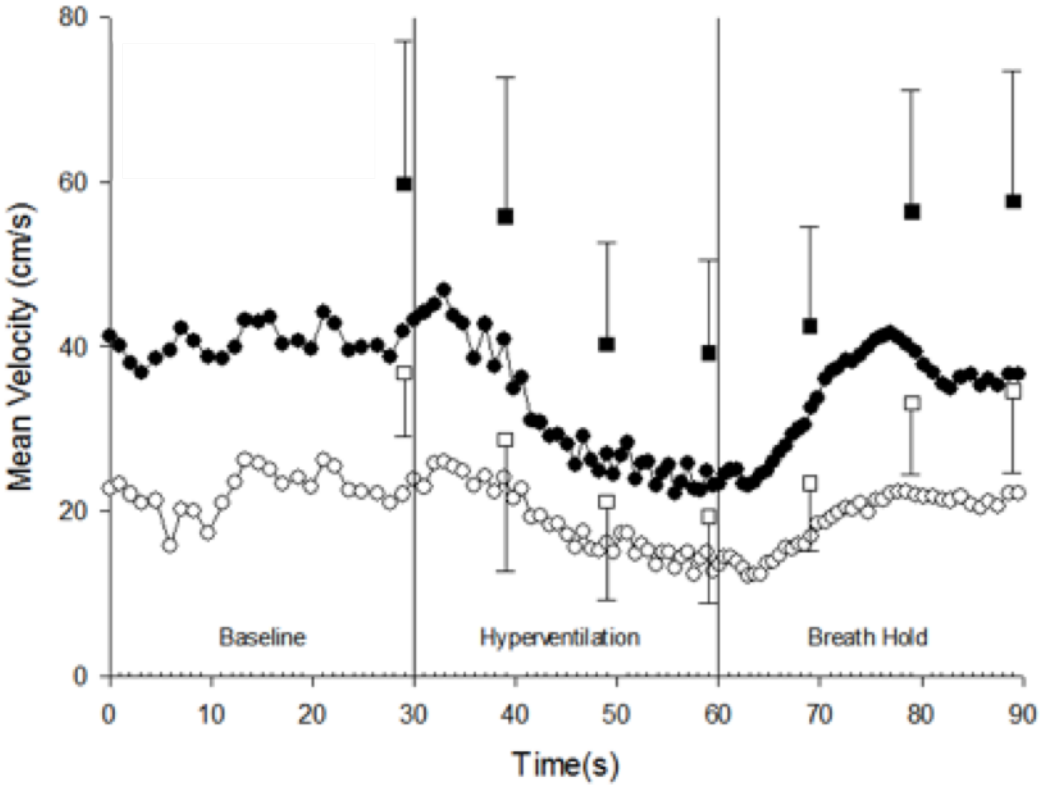

Figure 5: The raw mean velocity of both the MCA and the BA of Participant 1 MCA data is represented by filled circles, and BA data is represented by open circles. Group averages for MCA are illustrated as filled squares and group averaged for the BA are represented by open squares

.

CVRi (Figure 6C) was compared between the MCA and the BA and during the first 30s of hyperventilation. There was a significant main effect of artery ($p<0.0001$), time ($p=0.00006$) and an interaction effect ($p=0.0054$). A Tukey post hoc test revealed significance between arteries at 10s ($p<0.0001$), 20s ($p=0.036$) and 30s ($p<0.0001$) during hyperventilation, with the BA having a higher CVRi than the MCA. The following 30s breath hold had a significant main effect of artery ($p<0.0001$), time ($p<0.0001$) and interaction effect ($p=0.0073$). A post hoc Tukey test a difference at 30s ($p<0.0001$), at 40s ($p=0.0047$), and 50s ($p=0.016$) which persisted as a change caused by hyperventilation, and the BA having a higher CVRi than the MCA.

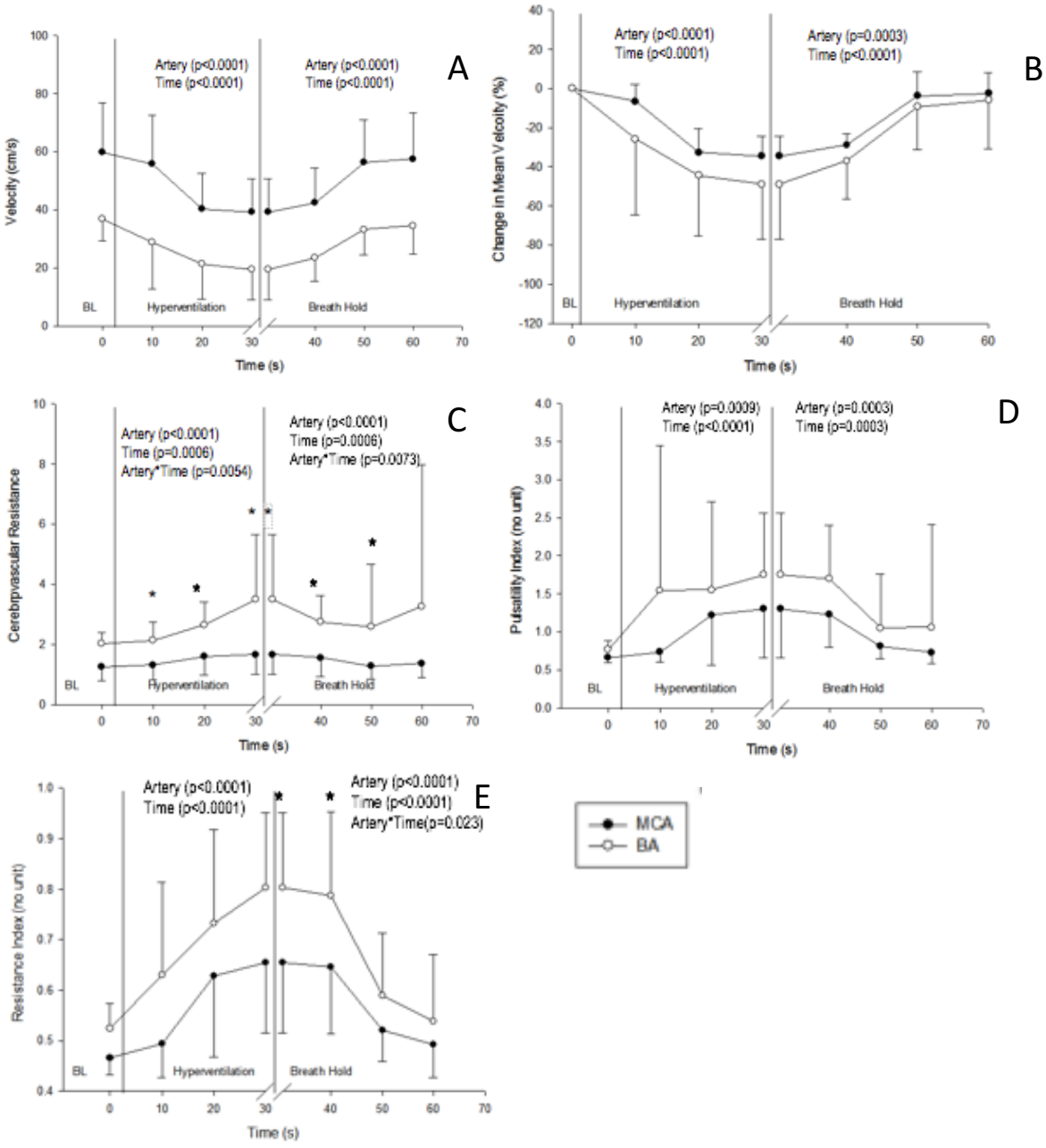

*Figure 6: Results of the Hyperventilation trials (mean±SD). Participants hyperventilated for 30s, and then held their breath for 30s. Figure A illustrates the raw mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. MCA represented by filled circles. BA represented by open circles. Significant interaction effect in the first 30s is denoted by *.Significant interaction effect is the final 30s denoted by *.*

PI (Figure 6D) was calculated and compared between the MCA and the BA for the first 30s of hyperventilation. The main effect of artery ($p=0.0009$) and the main effect of time ($p<0.0001$) were significant, but their interaction was not significant ($p=0.254$). During the breath hold portion, the main effect of artery ($p=0.0003$) and main effect of time ($p<0.0001$) were significant, but their interaction effect was not significant ($p=0.84$).

RI (Figure 6E) was calculated and compared between the two arteries during hyperventilation and breath hold. During hyperventilation, the main effects of artery ($p<0.0001$) and time ($p<0.0001$) were significant, but their interaction effect was not significant ($p=0.19$). During the breath hold that followed hyperventilation, both the effect of artery ($p<0.0001$) and of time ($p<0.0001$) were significant, as was their interaction effect ($p=0.023$). The BA was significantly higher in RI than the MCA at 30s (MCA: 0.65 ± 0.14 BA: 0.803 ± 0.15, $p<0.0001$) as well as 40s (MCA: 0.645 ± 0.13 BA:0.79 ± 0.17, $p<0.0001$).

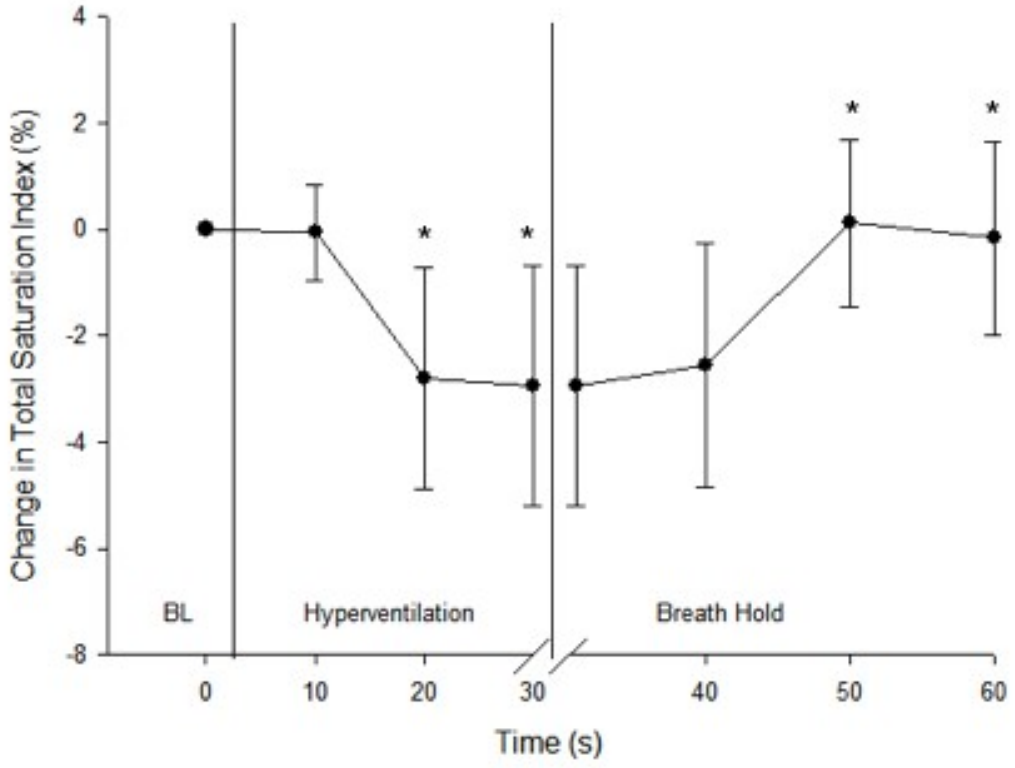

*Figure 7: Total saturation Index during a 30s hyperventilation and 30s breath hold (mean±SD).. Significance from baseline in the first 30s is denoted by *. Significance from the 30s time point during the last 30s is denoted by *.*

TSI (Figure 7) was compared during the hyperventilation portion of the trial, and there was a significant difference ($p<0.0001$) across time at 20s ($p<0.0001$, -2.89±2.08%) and 30s (2.95±2.27%, $p<0.0001$). When TSI was compared during the final 30s to the TSI value at the end of hyperventilation, a significant effect of time ($p<0.0001$) was found at 50s (0.12±1.58%, $p<0.0001$) and 60s (-0.17±1.81%, $p<0.0001$). Oxygenated haemoglobin was compared across time for the first 30s of hyperventilation but the main effect of time was not significant ($p=0.56$). In the 30s of breath hold, the main effect of time was also not significant ($p=0.70$). The OHQ was not correlated with the oxygenated haemoglobin difference at 30s ($r= -0.0735$, $p=0.840$).

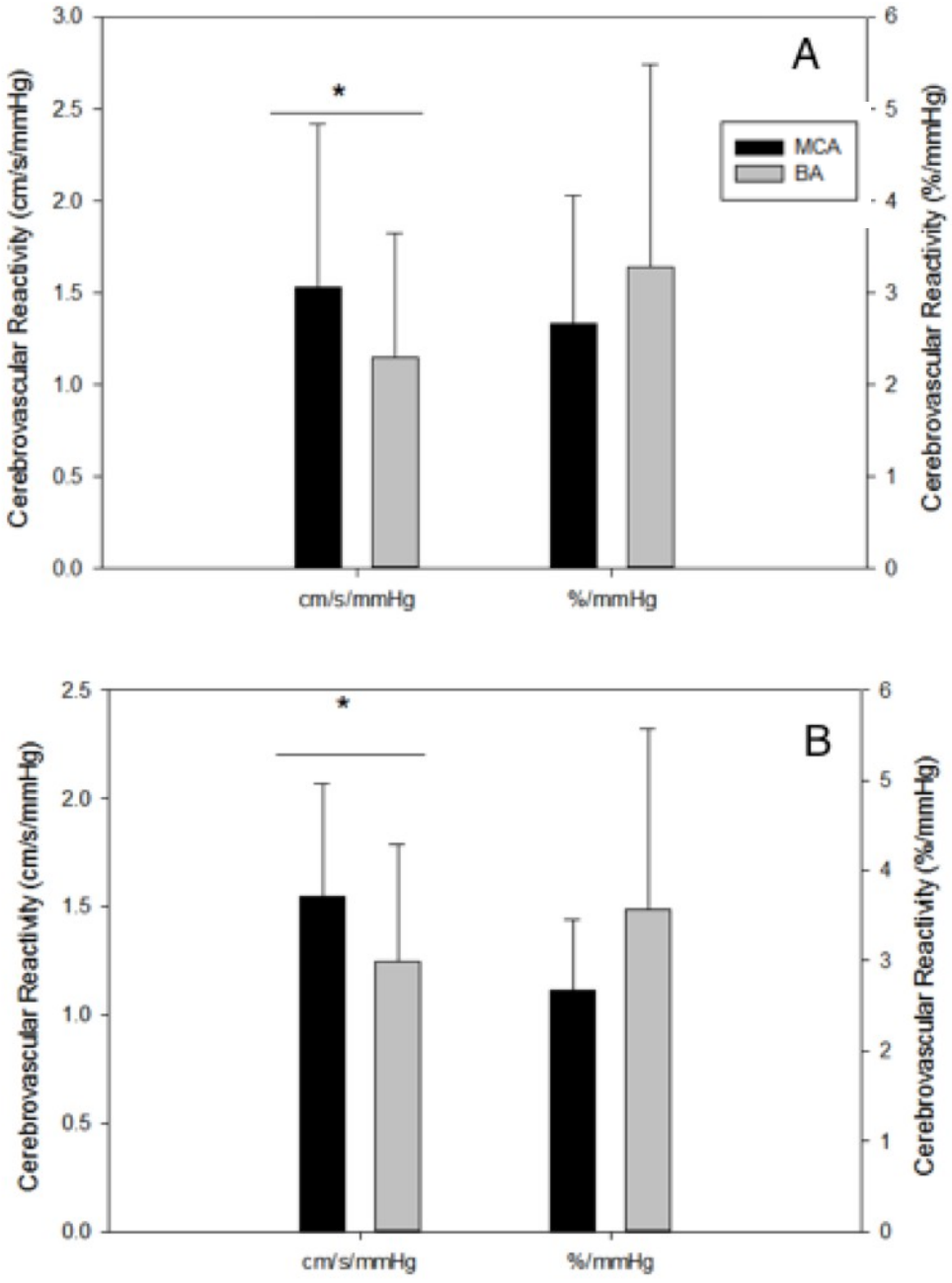

*Figure 8:Cerebrovascular Reactivity of the MCA and the BA calculated in two ways (mean±SD).. Figure A represents reactivity during hyperventilation. Figure B represents reactivity during breath hold. MCA is represented by black bars. BA represented by grey bars. Significance between arteries ($p<0.05$) is denoted by *.*

Mean CBV and $P_{ET}CO_2$ was averaged in the final 30s of baseline, the final 5s hyperventilation and the final 5 s of breath hold conditions. $P_{ET}CO_2$ changed by an average of -13.66 ± 4.48mmHg over these first 30s and these values were used to calculate cerebrovascular reactivity (CR_{CO2}) (Equation 1) of the BA and the MCA

between baseline and hyperventilation, and between hyperventilation and breath hold. The CR_{CO2} (Figure 8A) during hyperventilation was found to be significantly different between the MCA and the BA, with the MCA being more reactive than the BA (MCA: 1.58± 0.516cm/s/mmHg BA: 1.23 ± 0.551cm/s/mmHg, p = 0.028). During breath hold, the change in $P_{ET}CO_2$ was 14.50 ± 6.07 mmHg. The CR_{CO2} (Figure 8B) was calculated for breath hold period and was found to be significantly different between arteries, again with the MCA being more reactive than the BA (MCA: 1.61 ± 0.941mmHg/cm/s BA: 1.20 ± 0.720mmHg/cm/s, p = 0.030). When calculated with the relative change in velocity, the difference in reactivity during hyperventilation (Figure 8A) was not significant, this time with the BA being more reactive than the MCA (MCA: 2.67± 0.778 %/mmHg BA: 3.56± 2.01%/mmHg p=0.12). During the breath hold that followed (Figure 8B), the difference between arteries was not significant (p=0.80).

BREATH HOLD

The strictly breath hold trials were only analyzed up to 20s into breath hold as that was an achievable time for all participants to hold their breath. The average change in $P_{ET}CO_2$ was 7.39 ± 4.05 over the 20s. Using the final 30s of baseline and the final 5s of each ten second increment of the following breath hold, both the MCA and BA mean CBV (Figure 9A) was compared and found to have a significant main effect in both artery ($p<0.0001$) and time ($p<0.0001$) but had a not significant interaction effect ($p=0.85$). The %change from baseline (Figure 9B) had a significant time main effect ($p<0.0001$) but had a not significant artery main effect ($p=0.66$) and a not significant interaction effect ($p= 0.90$). CVRi (Figure 9C) had statistically significant main effects of artery ($p<0.0001$) and of time ($p=0.0058$) but had a not significant interaction effect ($p= 0.38$). Similarly, PI (Figure 9D) had significant main effects of artery ($p<0.0001$) and time ($p=0.0001$) but not in the interaction effect ($p=0.43$). RI (Figure 9E) continued the trend with significant main effect of artery ($p<0.0001$) and time ($p<0.0001$) but not in their interaction effect ($p= 0.56$).

In order to calculate CR_{CO2}, the change $P_{ET}CO_2$ had to be calculated; $P_{ET}CO_2$ increased an average of 7.39 ± 4.05 mmHg during the 20s breath hold. CR_{CO2} (Figure 10) was calculated and compared between arteries, but showed no significance ($p=0.71$). CR_{CO2} was also calculated using the %change in velocity per mmHg of CO_2 but was also not significant ($p=0.99$).

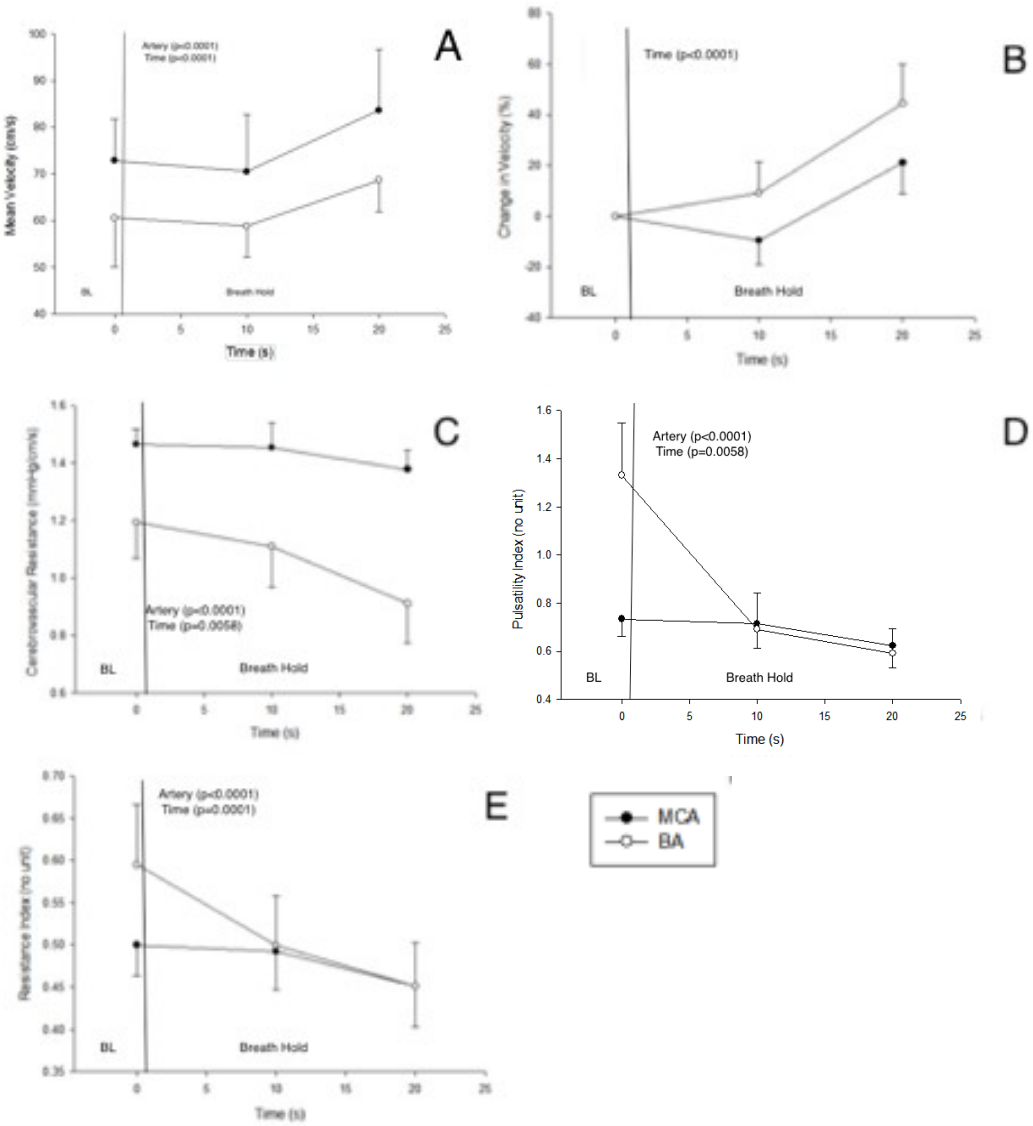

*Figure 9:Results of the breath hold trials.Participants held their breath for 20s (mean±SD).. Figure A illustrates the raw mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA.MCA is represented as filled circles. BA represented by open circles. Significant interaction effect in the first 20s is denoted by *.*

TSI was found to be significantly different across time (p = 0.024) but only between 10s (-0.43±1.36%,)and 20s (0.53±0.98%, p=0.018). Oxygenated haemoglobin was not significant across the breath hold duration (p=0.37). The OHQ was not correlated with the change in oxygenated haemoglobin at 20s (r=-0.266, p=0.489).

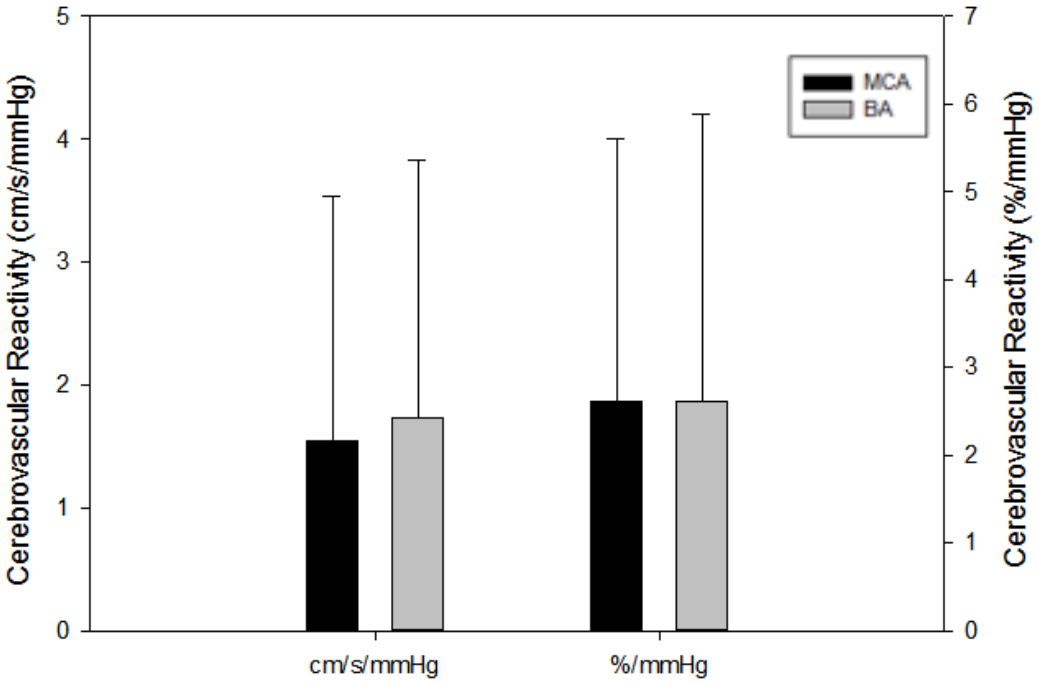

*Figure 10: Cerebrovascular reactivity calculated in two ways of the MCA and the BA during a 20s breath hold (mean±SD).. MCA represented by black bars. BA represented by grey bars. Significance between arteries ($p<0.05$) is denoted by *.*

SIT-TO-STAND: ACROSS TIME

The raw data of a sit to stand trial for participant 1 can be seen in Figure 11. Participants underwent a 5min seated baseline, followed by a stand that lasted for 3min. This section focusses on the changes that occurred over the 3min stand. The values at nadir will be discussed later. Absolute values of mean velocity (Figure 12A) of the MCA and the BA compared across time points and found to have a significant main effect of artery ($p<0.0001$) and of time ($p=0.0001$), but the interaction effect between the two was found to be not significant ($p= 0.95$). The %change from baseline (Figure 12B) values were found for each time point and compared but only the main effect of time was significant ($p<0.0001$). Both the main effect for artery ($p=0.59$) and the interaction effect ($p=0.50$) were not significant.

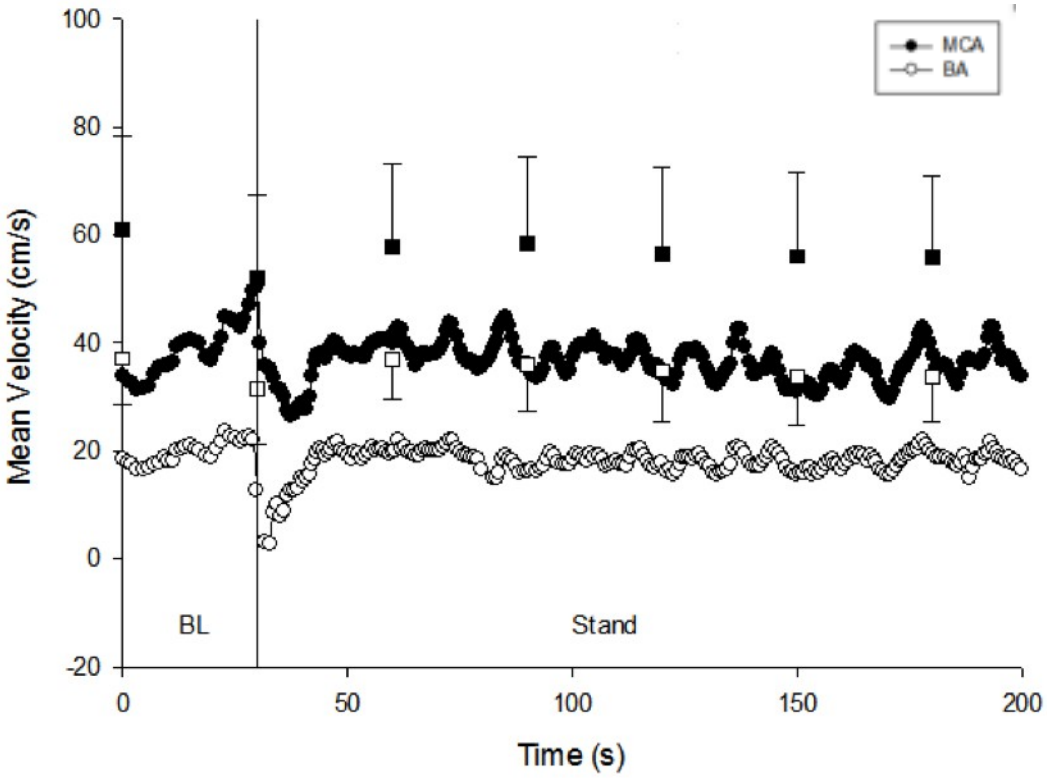

Figure 11: Raw mean velocity of participant 1 along during a 3min sit to stand protocol (mean±SD).. MCA of the participant is represented by filled cirlces. BA of the participant is represented by open circles. Group averages of the MCA mean velocity represented by squares. Group averages for BA mean velocity represented by open squares.

CVRi (Figure 12C) was compared between arteries across the 3min stand and the main effects of artery ($p<0.0001$) and time ($p<0.0001$) were significant but their interaction was not ($p=0.49$). PI (Figure 12D) was compared between arteries and was found to have statistically significant main effects of artery ($p<0.0001$) and time ($p<0.0001$) but not significant interaction effect ($p= 0.18$). RI (Figure 12E) was calculated and compared between arteries and while there was a significant main effect of artery ($p<0.0001$) and time ($p<0.0001$), the interaction effect was not significant (0.66).

The change in TSI was significant ($p< 0.0001$) from baseline at 30s (-2.79 ± 1.74%, $p<0.0001$). Oxygenated haemoglobin was not significant ($p=0.63$) across time.

The OHQ was not correlated to the change in oxygenated haemoblogin at 30s (r=-0.572, p=0.084).

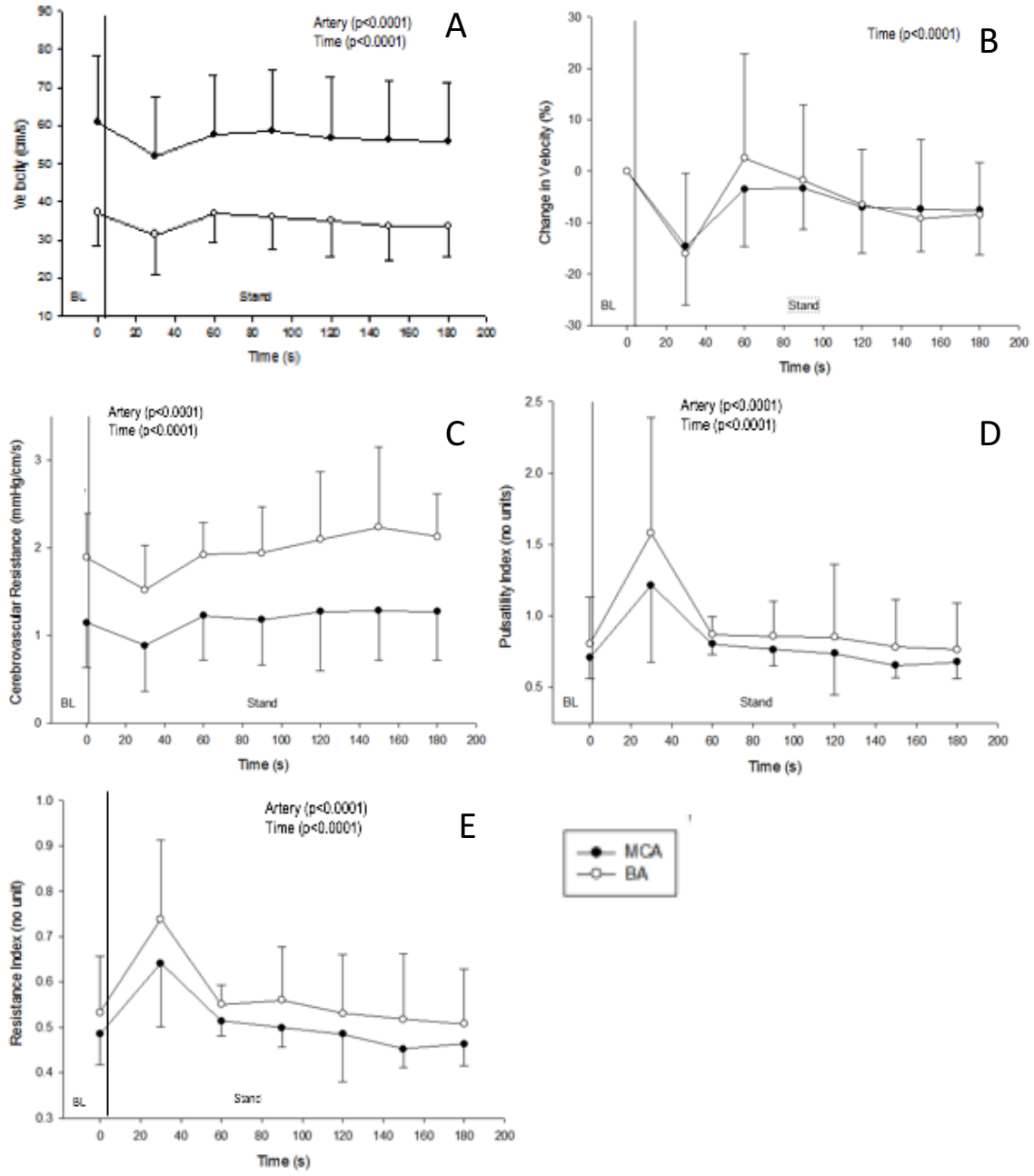

Figure 12: Results of the sit to stand trials during the entire 3min (mean±SD).. Figure A illustrates the raw mean velocity of the MCA and BA. Participants went from a seated baseline to standing for 3min, Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. MCA is represented by filled circles. BA represented by open circles. Significant interaction effect ***is denoted by*** **.*

SIT TO STAND: BASELINE VS NADIR

In addition to comparing time points during the 3min for analysis, baseline values for all measures were also compared to the value at the mean arterial pressure nadir, which was considered the lowest blood pressure after stand. This is because it is the main stimulus in changing blood pressure and for the protocol. This point was determined for each trial and the corresponding values at this time were used for analysing baseline to nadir. The average mean arterial pressure decrease to nadir was 27.59 ± 6.66 mmHg. The absolute mean velocity (Figure 13A) values were compared from baseline to nadir but no difference was found in the interaction effect ($p= 0.18$, but there was significance in the main effect of artery ($p<0.0001$) and in the main effect of time ($p<0.0001$), meaning there was a significant difference between baseline and nadir. The %change in mean velocity (Figure 13B) values were also analyzed and found to be not significant in the interaction effect ($p= 0.36$) and in the main effect of artery (0.36). The main effect of time was significantly different ($p<0.0001$). The change in CVRi (Figure 13C) from baseline to nadir was not significant ($p= 0.45$), but the main effect of artery ($p<0.0001$) and time ($p<0.0001$) were significant across the 3min stand.

When PI (Figure 13D) was compared between arteries, there were significant differences in the main effects of artery ($p<0.0001$) and time ($p<0.0001$) as well as the interaction effect ($p=0.021$) with the BA having a higher PI than the MCA. The change for the BA from baseline to nadir was significant ($p<0.0001$), as was the change from baseline to nadir in the MCA ($p<0.0001$). The two arteries were also

significantly different at nadir (p=0.0001) with the BA having a higher PI than the MCA.

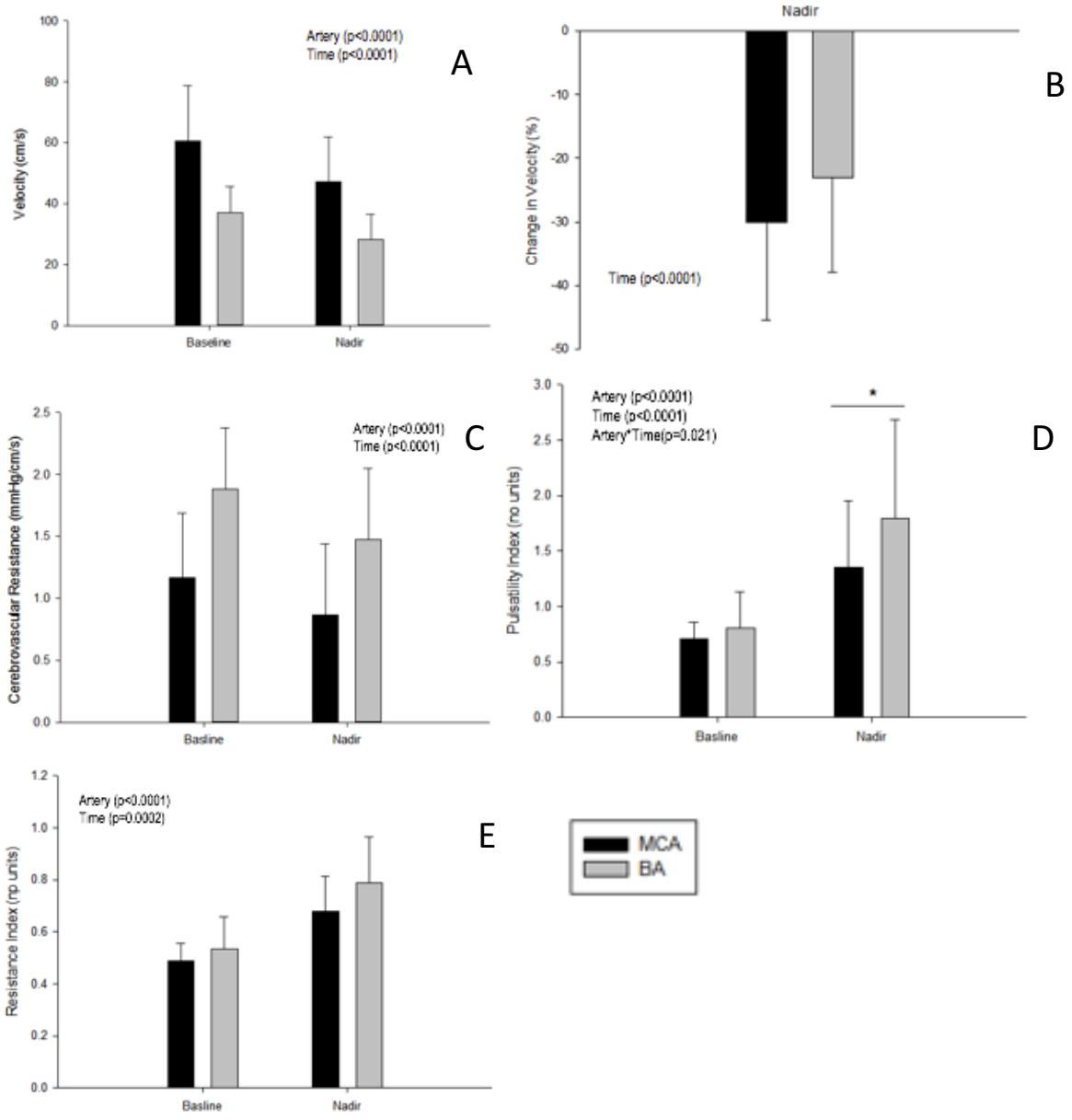

Figure 13: Results of the sit to stand trials in comparing baseline values to nadir(mean±SD).. Particpants went from a seated baseline to standing for 3min. Figure A illustrates the raw mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. MCA represented by black bars. BA represented by grey bars. Significance between arteries ***is denoted by*** **.*

RI (Figure 13E) was calculated and compared from baseline to nadir and there was significance in the main effects of time ($p=0.0002$) and artery ($p<0.0001$) but not in the interaction effect ($p=0.19$)

With NIRS variables, TSI was significantly different ($p<0.0001$) from baseline to nadir (-3.03 ± 1.94%). Oxygenated haemoglobin was not significantly different from baseline to nadir ($p=0.11$)

INTERACTION TRIALS: HYPERVENTILATION AND STAND: ACROSS TIME

The interaction trials combined the sit to stand with hyper- and hypoventilation. For the Hyperventilation with sit to stand trials, the hyperventilation portion and the breath hold portion were analyzed separately. Participants underwent a 5min seated baseline, followed by a hyperventilation at a rate of 60 breaths/min, then a stand and 30s breath hold during the standing position. Absolute values of mean velocity (Figure 14A) were compared across time points and although the main effects of artery ($p<0.0001$) and time ($p<0.0001$) were significant during hyperventilation, their interaction effect was not ($p=0.14$). Breath hold was similar and had significant main effects of time ($p<0.0001$) and artery ($p<0.0001$), but a not significant interaction effect ($p=0.29$). Using these same data, the %change in mean velocity (Figure 14B) from baseline was calculated for both the MCA and BA at the same time points. During hyperventilation, although both main effects of artery ($p<0.0001$) and time ($p<0.0001$) were significant, the interaction effect where the BA changed more than the MCA was not significant ($p=0.079$).

During breath hold, there was significance in the main effect time ($p<0.0001$) and the main effect of artery ($p=0.00003$) but the interaction effect was not significant ($p=0.30$).

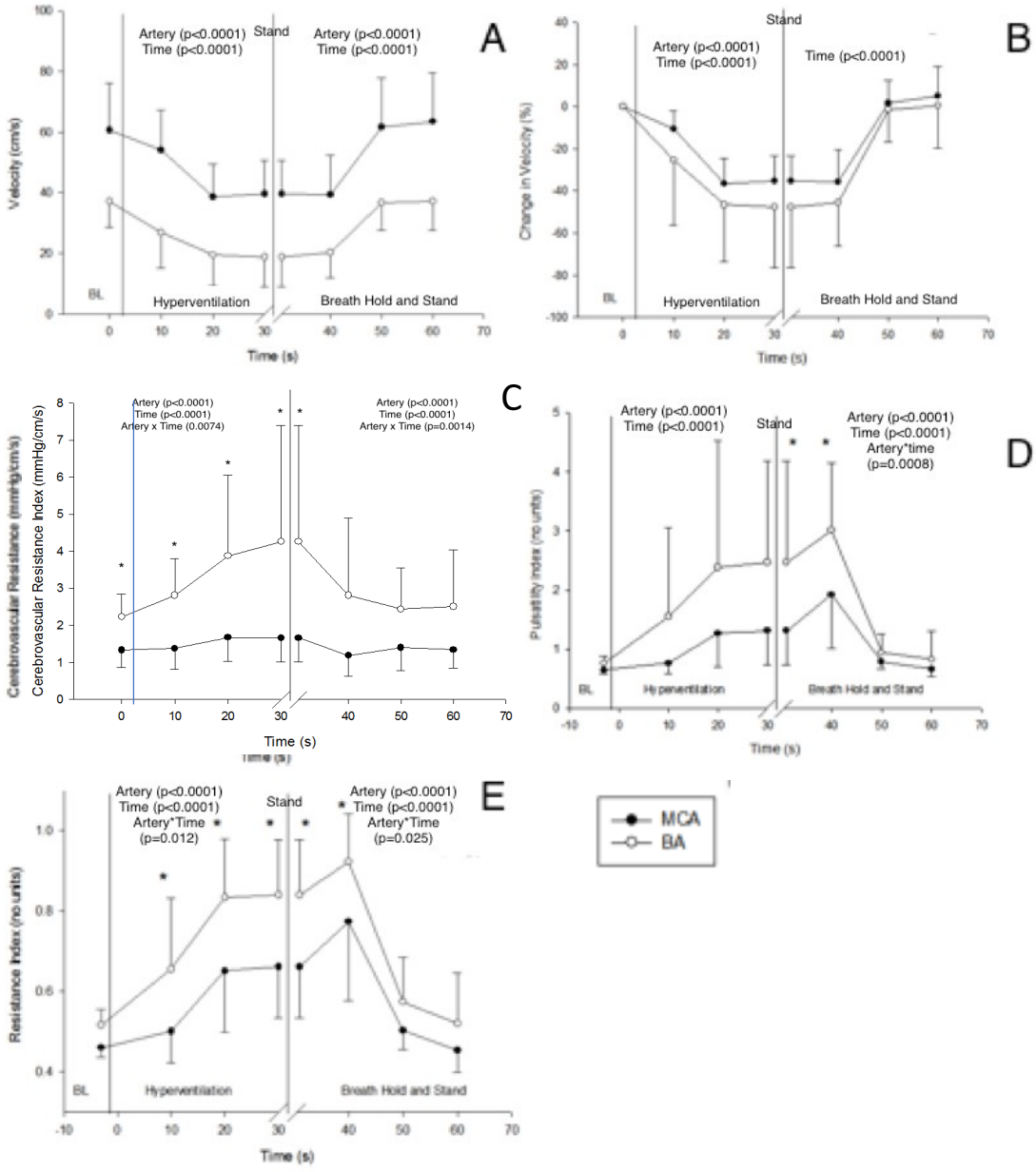

Figure 14: Results if the Hyperventilation and Stand trials (mean±SD).. Participants went from a seated baseline, to hyperventilating for 30s, to standing and holding their breath for 30s. Figure A illustrates the raw mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. MCA is represented by filled circles. BA represented by open circles. Stand occurred at 30s time point. Significant interaction effect in the first 30s denoted by. Significant interaction effect in the final 30s denoted by*

CVRi (Figure 14C) was calculated during the first 30s of hyperventilation, and the main effect of artery ($p<0.0001$) and time ($p=0.0001$) were significant as was the interaction effect ($p=0.0074$). A Tukey post hoc test revealed the BA was significantly

higher than the MCA at 0s ($p=0.029$), 10s ($p<0.0001$), at 20s ($p<0.0001$) and at 30s ($p<0.0001$) with the BA having higher CVRi values than the MCA. During the breath hold portion of the trial, the main effect of artery ($p<0.0001$) and time ($p<0.0001$) as well as their interaction effect ($p=0.0014$) were significant. A Tukey post hoc test revealed significance between the MCA and the BA at 30s ($p<0.0001$), with the BA having a higher CVRi than the MCA.

In analyzing PI (Figure 14D) during the 30s hyperventilation, the main effects of time ($p<0.0001$) and artery ($p<0.0001$) were significant, but were not significant in their interaction effect ($p=0.051$). During the breath hold that followed, PI has significant time ($p<0.0001$) and artery ($p<0.0001$) main effects, as well as a significant interaction effect ($p=0.0007$). A Tukey post hoc test revealed the BA was significantly higher in PI than the MCA at 30 (MCA: 1.31 ± 0.57 BA: 2.47 ± 1.71, $p<0.0001$) and 40s (MCA: 1.92 ± 0.90 BA: 3.01 ± 1.15, $p<0.0001$).

RI (Figure 14E) was calculated and compared during hyperventilation, there was significance in both main effects of time ($p<0.0001$) and artery ($p<0.0001$), as well as in their interaction effect ($p=0.0095$). A Tukey post hoc test revealed the BA was significantly higher than the MCA at 10s (MCA: 0.50 ± 0.078 BA: 0.65 ± 0.18, $p<0.0001$), 20s (MCA: 0.65 ± 0.15 BA: 0.83 ± 0.15, $p<0.0001$) and 30s (MCA 0.66 ± 0.13 BA: 0.84 ± 0.14, $p<0.0001$). RI during breath hold followed the same pattern having significance in both main effects of artery ($p<0.0001$) and time ($p<0.0001$) as well as in the interaction effect ($p=0.028$). A Tukey post hoc test revealed the BA to be higher than the MCA at 30s (MCA 0.66 ± 0.13 BA: 0.84 ± 0.14, $p<0.0001$) and 40s (MCA: 0.77 ± 0.20 BA: 0.92 ± 0.12, $p=0.0002$).

The change in TSI (Figure 15) data was compared during just the hyperventilation portion of the trial, and was found to be significant ($p < 0.001$), and a Tukey post hoc test revealed it was significant from baseline at 20s (-3.71 ± 2.74%, $p < 0.001$), and 30s (-3.83 ± 3.43%, $p<0.0001$). During breath hold, TSI was significantly different ($p<0.0001$) from baseline at 40s (-4.69 ± 3.51%, $p<0.0001$) and 50s (0.98 ± 2.80%, $p<0.0001$). TSI is a continuous variable and as such, it was always compared to baseline for differences. Oxygenated haemoglobin during hyperventilation was not different across time ($p=0.90$). This was also true for the breath hold duration ($p=0.51$). The OHQ was not correlated with the change in oxygenated haemoglobin at 30s ($r= 0.272$, $p=0.447$).

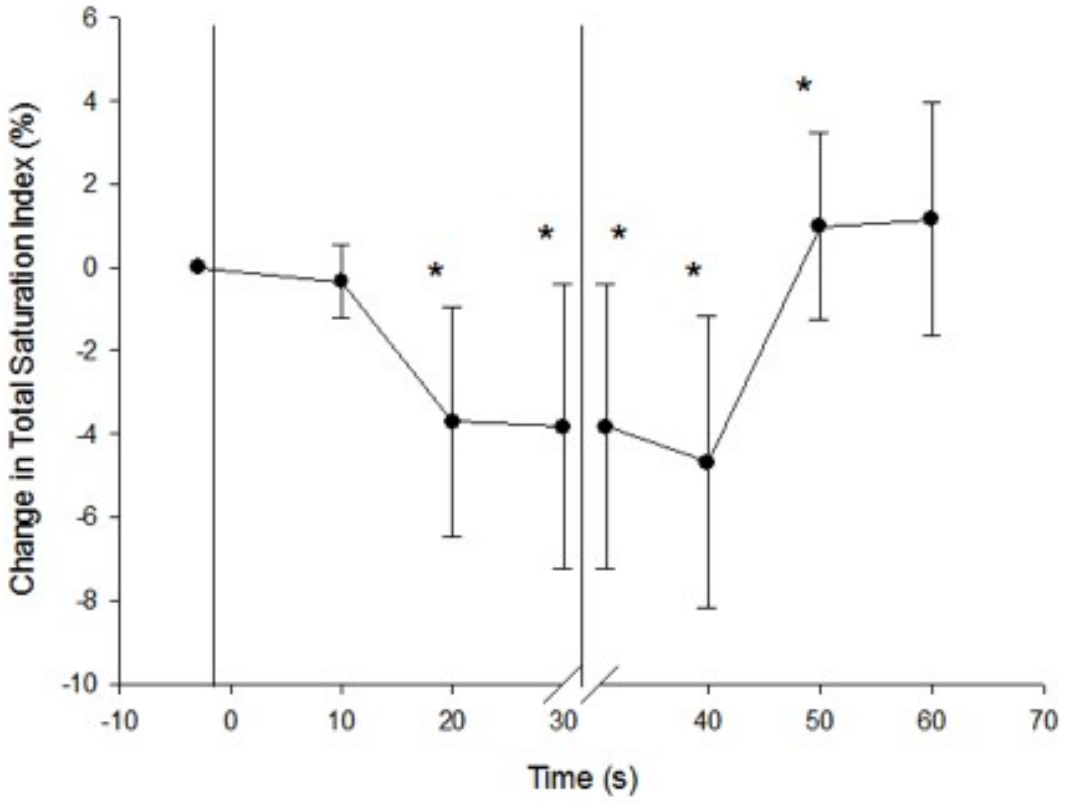

*Figure 15: Change in total saturation index over time(mean±SD).. The first 30s was compared to baseline, an dthe final 30s compared to the value at 30s. Significance from baseline is denoted by *.*

From baseline to the end of hyperventilation, $P_{ET}CO_2$ decreased -12.7 ± 6.91 mmHg. CR_{CO2} (Figure 16A) was calculated for both the MCA and the BA from baseline to the end of hyperventilation. The difference between arteries was significant during the hyperventilation (Figure 16A) portion of the task, and data showed the MCA was significantly more reactive than the BA (MCA: 2.24 ± 2.12mmHg/cm/s BA: 1.75 ± 1.46mmHg/cm/s, p= 0.049). From the end of hyperventilation to the end of breath hold, $P_{ET}CO_2$ increased 13.0 ± 5.86 mmHg. CR_{CO2} was also calculated from the end of hyperventilation to the end of breath hold (Figure 16B) and this difference was significant, again with the MCA being more reactive (MCA: 2.28 ± 1.19mmHg/cm/s BA: 1.81 ± 1.35mmHg/cm/s, p= 0.036). When calculated with the %change in velocity, cerebrovascular reactivity was not significant between arteries (p=0.082). During breath hold, the difference in reactivity was also not significant (p=0.066).

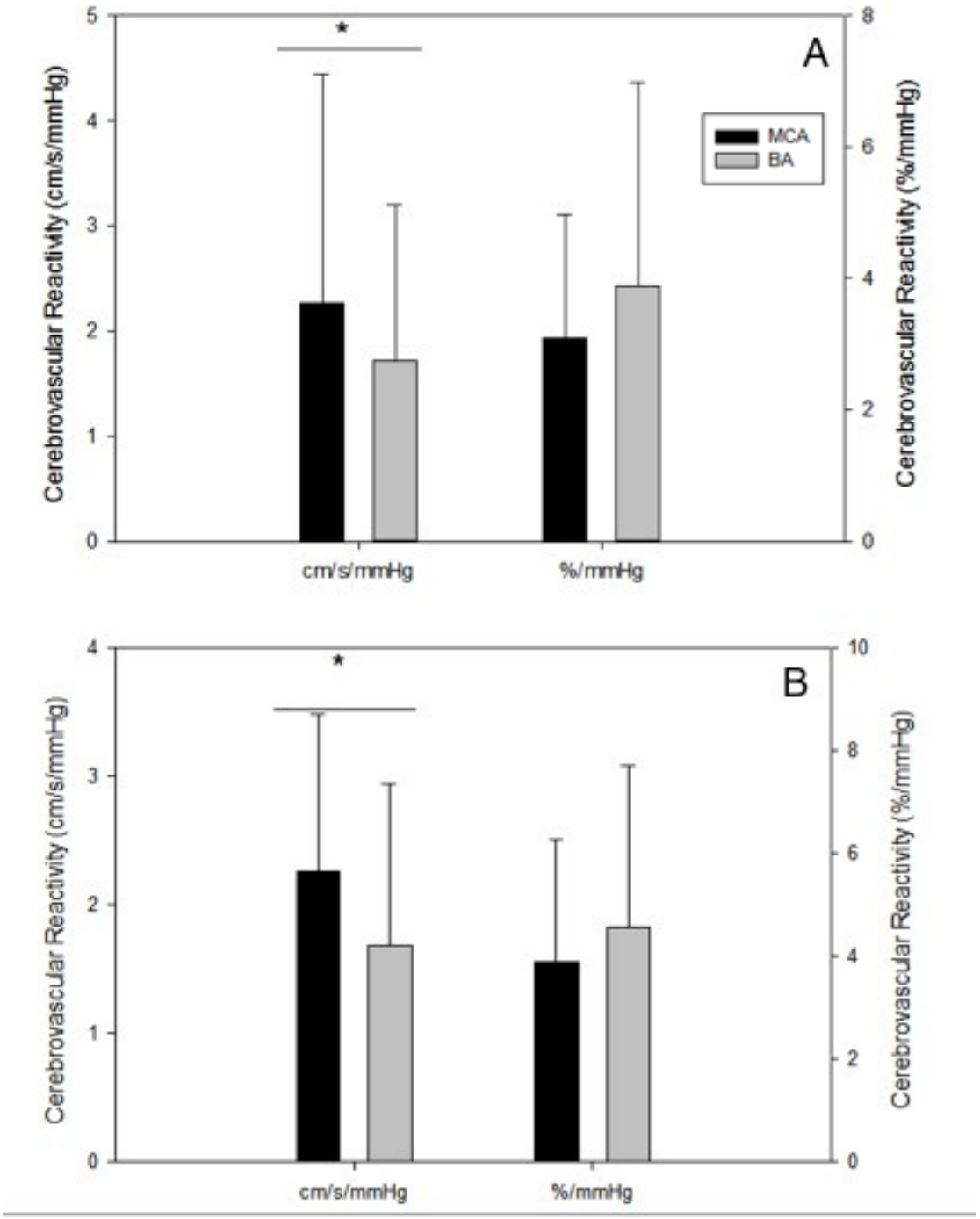

*Figure 16: Figure A: Cerebrovascular reactivity for the MCA and the BA (mean±SD). Figure A. CVR during hyperventilation. Figure B. Cerebrovascular reactivity during breath hold. MCA represented by black bars. BA represented by grey bars. Significance between arteries ($p<0.05$) is denoted by *.*

INTERACTION TRIALS: HYPERVENTILATION AND STAND: BASELINE VS NADIR

Using the same trial including a 30s hyperventilation, stand and 30s breath hold, values were compared at baseline and at the value of the mean arterial pressure nadir that occurred with the stand. Mean arterial pressure decreased an average of 39.02 ± 11.92 mmHg from baseline to nadir. For the absolute mean velocity (Figure 17A) of both the MCA and the BA there was significance in the main effects of artery ($p<0.0001$) and time ($p<0.0001$) but there was no significance in the interaction effect ($p= 0.18$). The %change in mean velocity (Figure 17B) values were also calculated there was a significant difference in the main effects of time ($p<0.0001$) and artery ($p<0.0001$). There was a significant difference in the interaction effect ($p=0.030$) and the Tukey post hoc test revealed these differences between arteries at nadir (MCA: -41.37 ± 12.08% BA: -51.21 ± 18.35%, $p=0.015$), with the BA having a greater change in velocity.

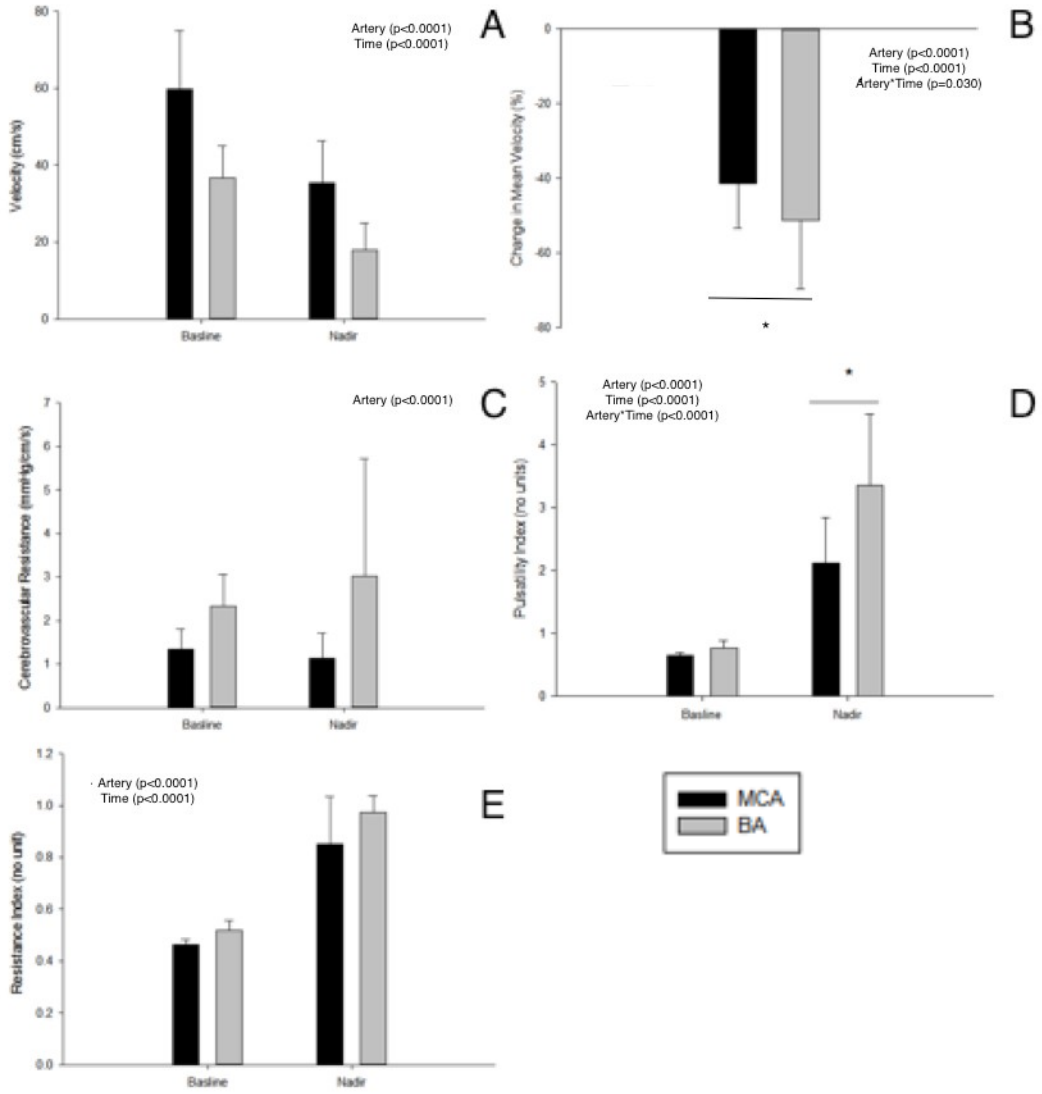

Figure 17: Results of the hyperventilation and stand trials (mean±SD)., looking at differences between baseline and the value at nadir. Participants went from a seated bseline, to hyperventilating for 30s, to standing and holding their breath for 30s. Figure A illustrates the raw mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. ***Significance interaction effect is denoted by*** **.*

The differences in CVRi (Figure 17C) between arteries from baseline to nadir were compared but no significance was found in the interaction effect (p= 0.14) or the main effect of time (p=0.435). The main effect of artery was significant (p<0.0001). The difference in PI (Figure 17D) was compared and both main effects of time (p<0.0001) and artery (p<0.0001) were significant, as was the interaction effect

($p<0.0001$). A Tukey post hoc test revealed the difference to be between arteries at nadir, with the BA having a higher pulsatility than the MCA (MCA: 2.12 ± 0.724 BA:3.35 ± 1.13, $p< 0.0001$). BA was significantly different from baseline to nadir ($p<0.0001$) as was the MCA ($p<0.0001$). The difference in RI (Figure 17E) between arteries was not significant ($p= 0.10$). But the main effects of time ($p<0.0001$) and artery ($p<0.0001$) were significant. The %change in TSI was significant ($p<0.0001$) from baseline to nadir ($-5.43 \pm 3.78\%$). The change in oxygenated haemoglobin was not significant ($p=0.78$).

INTERACTION TRIALS: BREATH HOLD + STAND: ACROSS TIME

For the breath hold interaction trial, a 5min seated baseline preceded a stand and breath hold for 20s. The absolute mean velocity for both arteries was compared during baseline, at 10s and at 20s of breath hold, as 20s was as long as all participants could hold their breath. The absolute mean velocity (Figure 18A) values had time ($p<0.0001$) and artery ($p<0.0001$) main effects, but their interaction was not significant ($p=0.57$. The %change in velocity (Figure 18B) was also calculated, and while the main effect of time was significant ($p<0.0001$), the main effect of artery ($p=0.27$) and the interaction effect ($p=0.57$) were not significant. The change in CVRi (Figure 18C) had significant main effects of time ($p<0.0001$) and artery ($p=0.0026$), but the interaction effect was not significant ($p=0.60$). This trend continued with PI (Figure 18D), which has significant main effects of artery ($p=0.0005$) and time ($p<0.0001$) but not significant interaction effects ($p=0.091$). Finally, RI (Figure 18E) was calculated and compared. The interaction effect was not significant ($p=0.31$) but the main effects of time ($p<0.0001$) and artery ($p<0.0001$) were significant. CR_{CO2} (Figure 19) was calculated using the change in $P_{ET}CO_2$ (6.19 ± 1.40 mmHg) from baseline to the end of breath hold, and the change was significant (MCA: 2.37 ± 2.82mmHg/cm/s BA: 1.74 ± 1.93mmHg/cm/s, $p=0.019$). When calculated with relative velocity values, the difference was not significant ($p=0.22$).

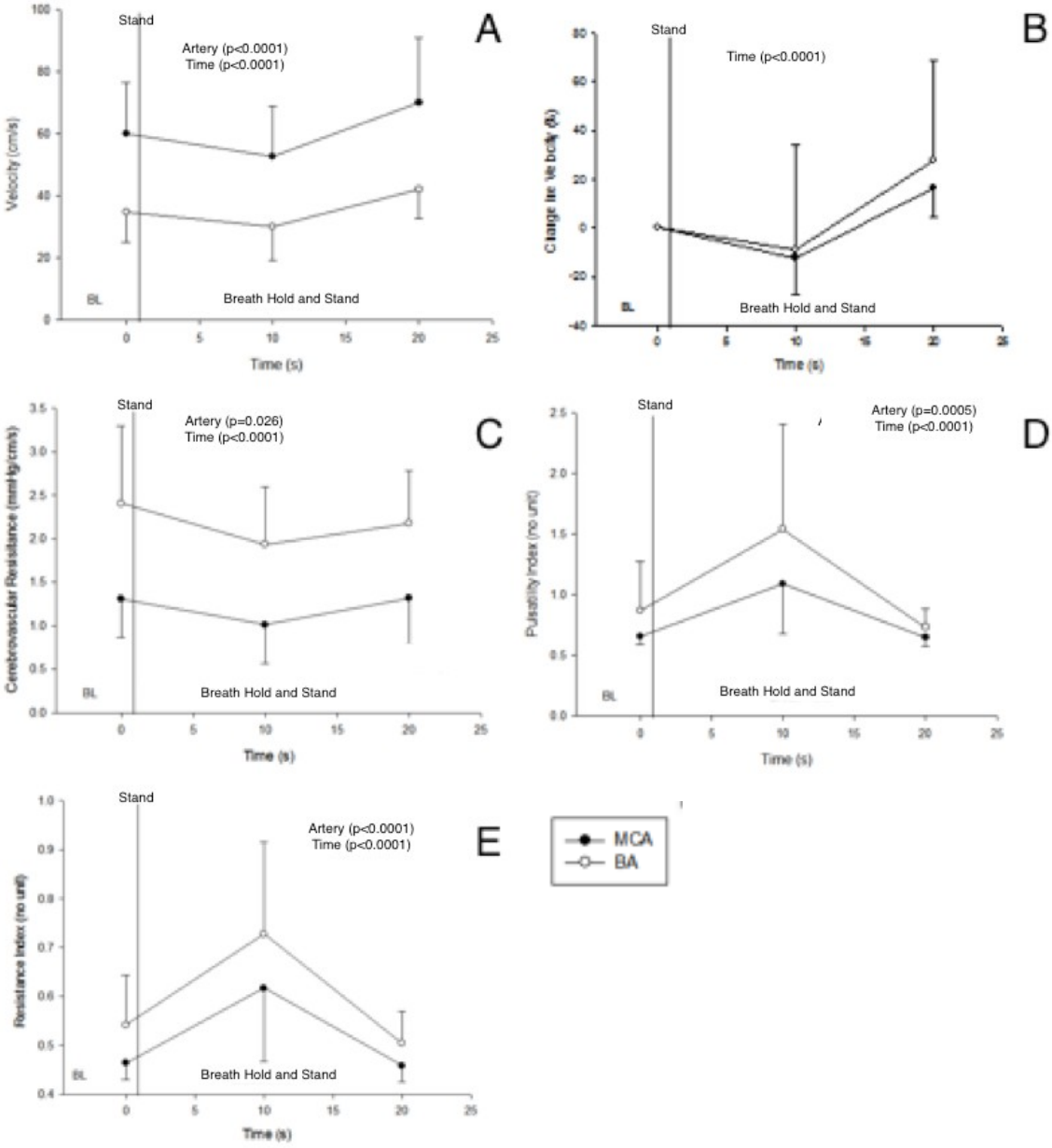

Figure 18: Results of the breath hold and stand trials (mean±SD).. Participants went from a seated baseline, to standing and holding their breath for 20s. Figure A illustrates the raw mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. MCA represented by closed circes. BA represented by open circles. Stand occurred at the end of he 30s baseline (BL). ***Significant interaction effect is denoted by*** **.*

TSI was significantly different ($p<0.0001$) across time, and a Tukey post hoc test revealed differences from 0 to 10s (-2.42 ± 2.03%, $p=0.0004$) and from 10 to 20s (1.42 ± 2.03%, $p=0.049$). But the change in oxygenated haemoglobin was not significant ($p=0.367$). The OHQ was not correlated with the change in oxygenated haemoglobin at 20s of breath hold ($r=-0.012$, $p=0.973$).

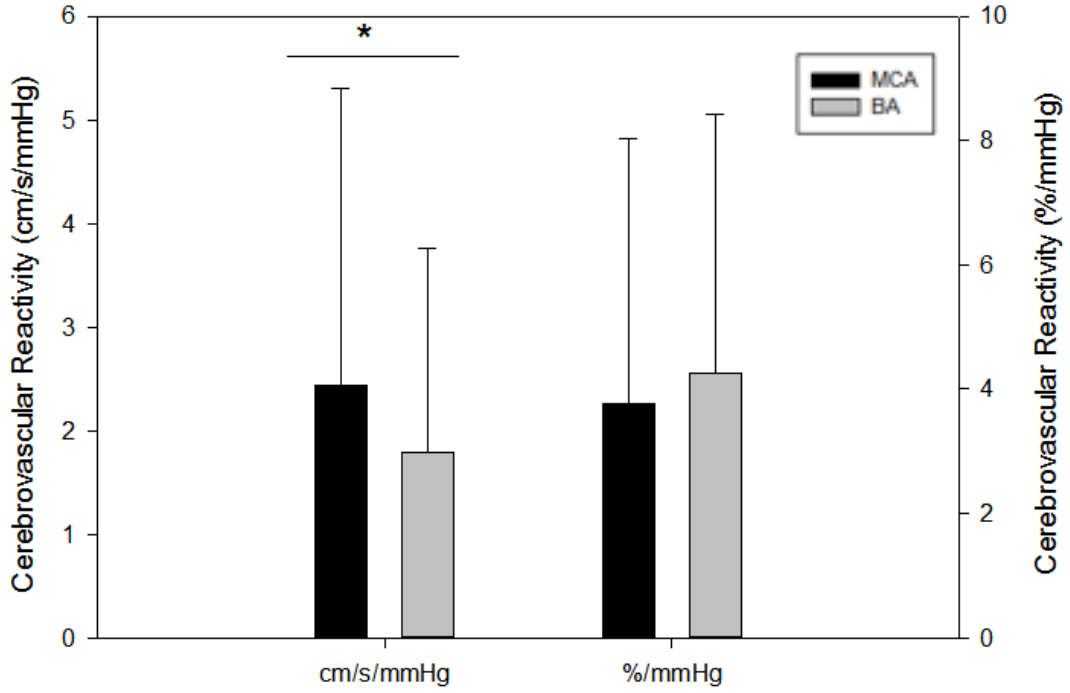

*Figure 19: Cerebrovascular reactivity during the breath hold and stand (mean±SD).. Reactivity calculated over th2 20s breath hold. MCA is represented by black bars. BA is represented by grey bars. Significance between arteries is denoted by *.*

INTERACTION TRIALS: BREATH HOLD + STAND: BASELINE VS NADIR

Baseline values were also compared to the values at the mean arterial pressure nadir.

The absolute velocity (Figure 20A) values were found to have significant main effects of time ($p<0.0001$) and artery ($p<0.0001$) but not for their interaction ($p=0.34$). The %change in velocity (Figure 20B) was also calculated but only the main effect of time was significant ($p<0.0001$). The interaction effect ($p=0.91$) and the main effect of artery ($p=0.91$) were not significant. CVRi (Figure 18C) had a not significant interaction effect ($p=0.920$) but had significant main effects of artery ($p<0.0001$) and time ($p=0.026$). The interaction effect for PI (Figure 18D) was almost significant ($p=0.062$) but the main effect of time ($p<0.0001$) and artery ($p=0.0002$) were significant. Finally, RI (Figure 18E) was calculated and compared, but the interaction effect was not significant ($p=0.084$). Though, the main effect of time ($p<0.0001$) and the main effect of artery ($p<0.0001$) were significant. From baseline to nadir ($-2.98 \pm 2.32\%$), TSI was significantly different ($p<0.0001$), but the change in oxygenated haemoglobin was not significant ($p=0.245$).

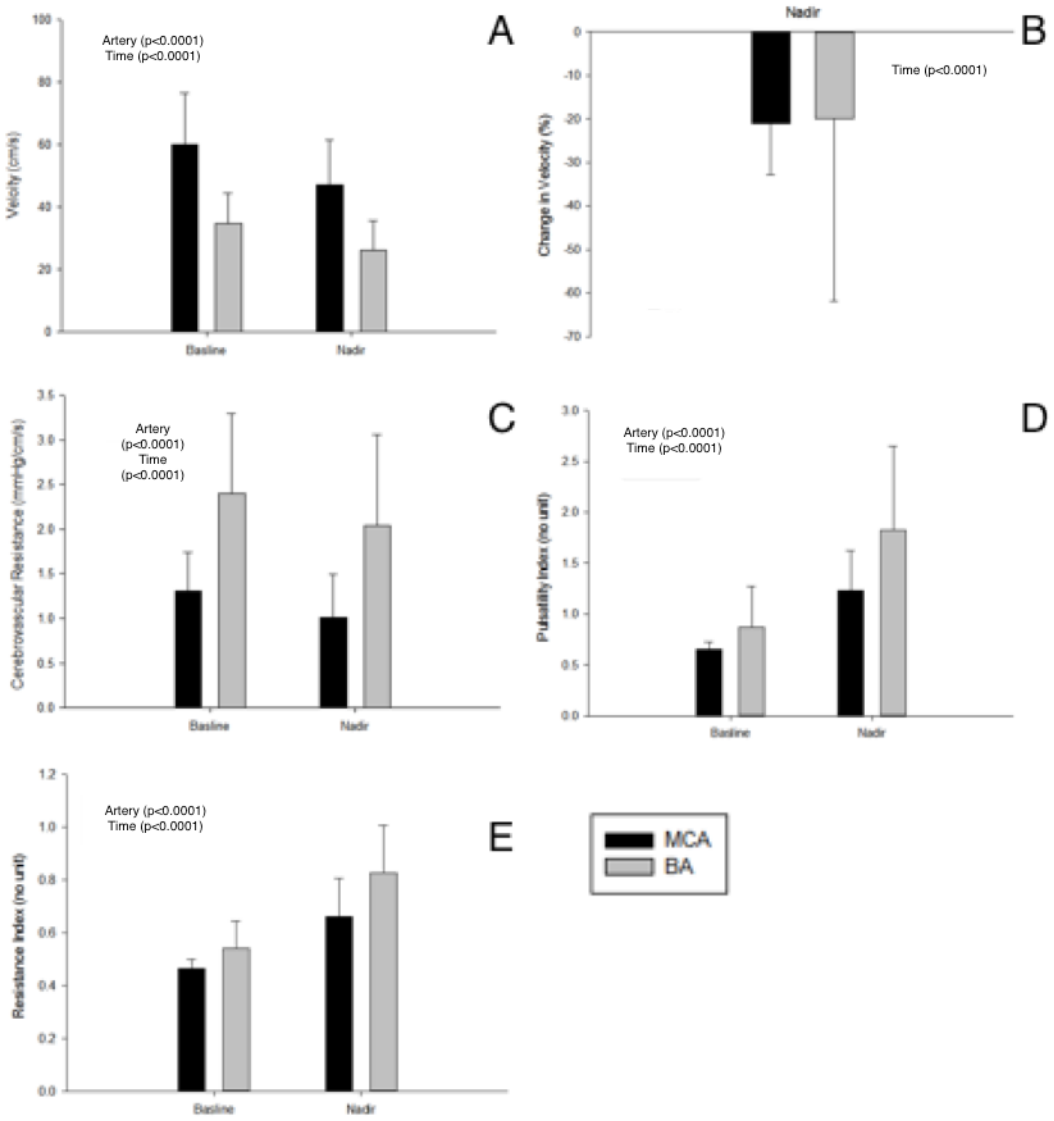

Figure 20: Results of the breath hold and stand trials in comparing baseline to nadir Figure A illustrates the raw mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. MCA is represented by black bars. BA is represented by grey bars. ***Significance between arteries is denoted by*** **.*

HEAD UP TILT

For the HUT, a 5min supine baseline was followed by a passive HUT to 75degrees which was maintained for 10min in all participants. The 30s supine baseline and 10min HUT raw mean velocity data for participant 11 can be found in Figure 21. Absolute mean velocity (Figure 22A) was compared between the MCA and the BA at baseline, 60s, 120s, 180s, 240s, 300s, 360s, 420s, 480s, 540s, and 600s and the difference between arteries at these time points was significant ($p= 0.007$) at all time points. The %change in mean velocity (Figure 22B) from baseline was also not significant ($p= 0.659$). There was no difference in CVRi (Figure 22C) ($p= 0.512$) or RI (Figure 22E) ($p= 0.101$) but there was a significant difference in PI (Figure 22D) ($p=0.042$) that existed between arteries at the tenth minute (MCA: 0.664 ± 0.082, BA: 0.834 ± 0.299, $p< 0.001$). For NIRS variables, the change in TSI was not significant ($p= 0.294$) across time points as was oxygenated haemoglobin ($p=0.623$). The OHQ was not correlated with the change in oxygenated haemoglobin in the tenth minute of tilt ($r=-0.084$, $p=0.843$).

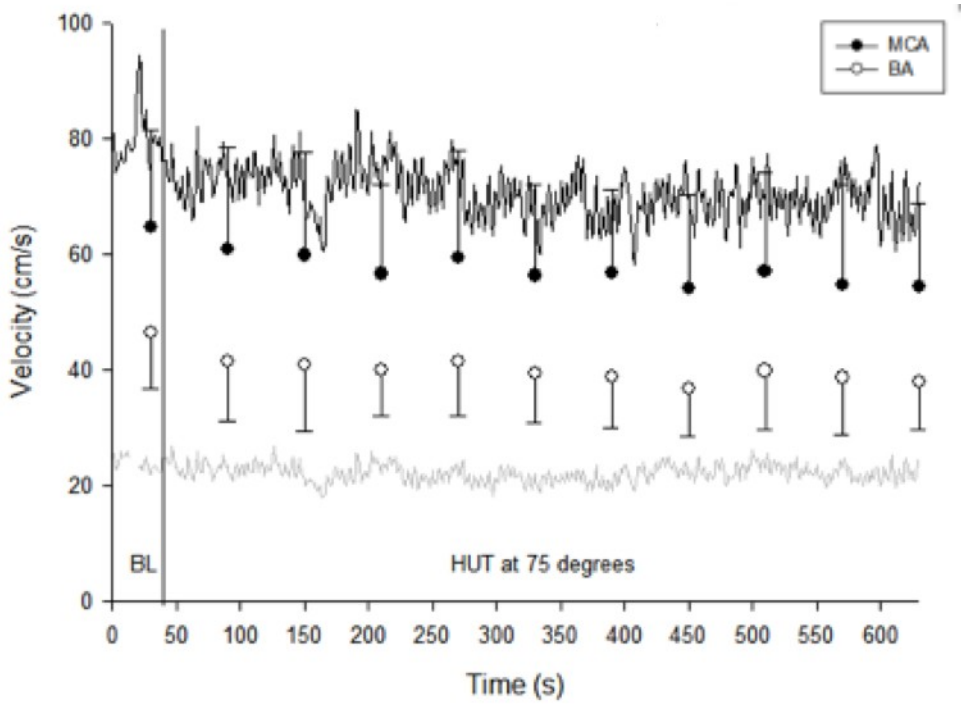

Figure 21: Raw mean velocity of the MCA and the BA during a 10min tilt for a single participant (lines), and group means (circles) with standard deviation.

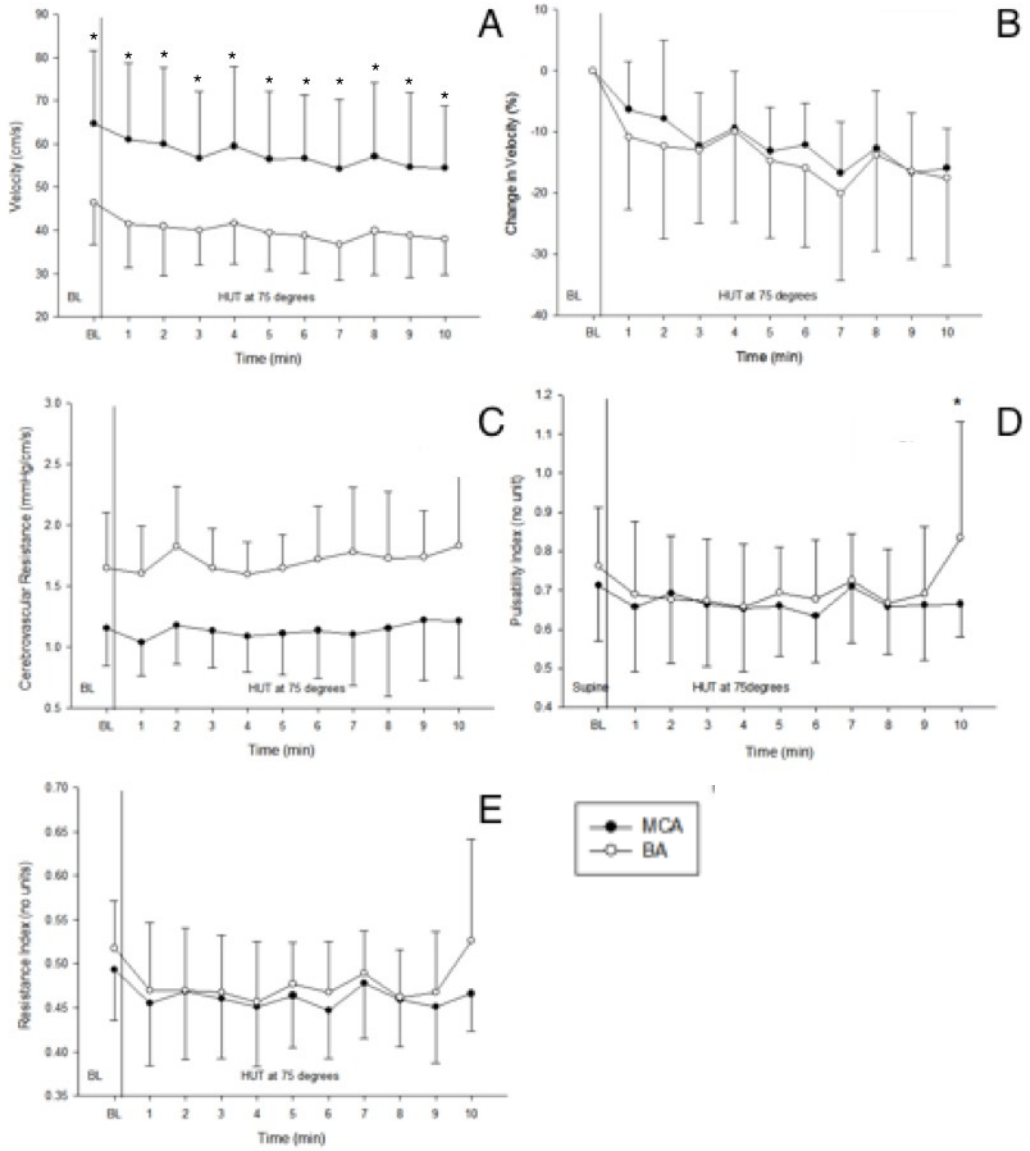

Figure 22: Results of a 10min heat up tilt (mean±SD).. Figure A illustrates the mean velocity of the MCA and BA. Figure B represents the change in velocity of the MCA and the BA. Figure C represents the cerebrovascular resistance of the MCA and the BA. Figure D represents the pulsatility index of the MCA and the BA. Figure E represents the resistance index of the MCA and BA. ***Significance is denoted by*** **.*

DISCUSSION

This thesis set out to explore if regional cerebral blood flow differences exist between the MCA and the BA through a combination of hyperventilation, breath hold and standing along with HUT protocol. The hyperventilation protocol showed increased CVRi in the BA at 10s, 20s, 30s and 40s as well as increased RI in the BA at 30s and 40s. The breath hold tests showed no differences between arteries. The sit to stand tests showed increased PI in BA at nadir. The hyperventilation followed by a stand and breath hold showed increased CVRi in the BA at 10s, 20s, 30s, and 40s as well as higher PI at 30s and 40s in the BA. It also showed increased RI in the BA at 10s, 20s, 30s and 40s. The BA decreased velocity more than the MCA and nadir and also had a higher PI. During the breath hold and stand trials there were no significant differences. During the HUT trials, data showed significant differences in absolute velocity between arteries at all time points and also the BA had an increased PI at the tenth minute. Overall, these data do not support the hypothesis that the MCA is more reactive than the BA, and they do not support the hypothesis that the BA would change velocity less with changes in blood pressure. But, CVRi was shown to be increased in the BA during hyperventilation, which was caused by both a change in CO_2 and in blood pressure.

In the past, regional cerebral blood flow differences have been demonstrated in response to changes in P_aCO_2, where the MCA was more reactive than the posterior artery, and in lower body negative pressure where the hind brain is better at preserving blood flow during hypervolemia than the front brain[14,79,80] and during changes in stroke volume with tilt [11]. These data have produced inconclusive results

with research suggesting that the frontal brain circulation is more responsive than posterior circulation during tests with a reduction in stroke volume and cerebral blood flow, that the posterior brain is more responsive than the anterior brain [80] and that both circulation systems react the same way [81,82]. Depending which data are used in calculations significantly alters the outcome. When the raw velocity changes are used, data suggest that the MCA is more reactive than the BA. When normalized, the trend is the BA is more reactive than the MCA but the difference in this study was never significant Due to the little research that includes the BA, many of these results are novel and do tend to support the theory that regional cerebral blood flow differences exist in a young, healthy population; the MCA is a better autoregulatory vessel than the BA but their relative reactivity is the same.

CARBON DIOXIDE REACTIVITY

Blood pressure regulation and cerebrovascular reactivity are closely related, and increases in CO_2 result in increases in blood pressure, decreases in CO_2 result in decreases in blood pressure. The hyperventilation and breath hold trials were designed to specifically alter the partial pressure of CO_2 in the cerebral vasculature with minimal changes in blood pressure. Several studies have recently shown differences in CR_{CO2} between anterior and posterior circulation using steady state and rebreathing methods[8,83–85] but to date, limited information exists regarding breath hold maneuvers, and the regional cerebrovascular response remains greatly unclear[86], which is why we opted for a voluntary protocol. The interaction trials also include the element of changing the partial pressure of CO_2, but the inclusion of the stand was meant to challenge cerebrovascular autoregulation during a period of altered arterial PCO2. Thus, these carbon dioxide related interaction trial results must be interpreted with the notion of a purposeful change in blood pressure.

In comparing ICA and VA during hypo- and hypercapnia in the past, diameters were not seen to change[85]. Although we do not have diameter data on the intracranial arteries, we assume the changes we saw are due strictly to changes in velocity and not due to diameter. The change during hyperventilation was -13.66±4.45mmHg and -12.0±6.91mmHg, and during breath hold it was7.39 ±4.05mmHg and 6.19 ±1.40mmHg. Given that the hyperventilation lasted just 30s and the breath holds just 20s, it is very unlikely there were any changes in these major vessel diameters as in the past, cross sectional area changes in the MCA after 5min of changing $P_{ET}CO_2$[87] . Additionally, autoregulation of the PCA territory has been shown to be altered metabolically by the activation of eye opening in healthy adults; with eyes open, the PCA territory blood flow is increased [77]. Due to this confounding

factor, participants were asked to stare at a dot placed on the wall to reduce this effect to the greatest extent possible.

Hypercapnia

A voluntary breath hold produces arterial hypercapnia. When this occurs, CBF increases to rid the brain tissues of the increased CO_2 which affects pH[3]. The average change in $P_{ET}CO_2$ was 7.39 ± 4.05 mmHg during the breath hold trial and 6.19 ± 4.10mmHg during the breath hold during stand trial. The breath hold that occurred after hyperventilation changed $P_{ET}CO_2$ by 13.78 ± 6.74mmHg and the breath hold that occurred after the hyperventilation and stand trials changed $P_{ET}CO_2$ by 13.02 ± 5.86mmHg. The change in $P_{ET}CO_2$ was comparable in the just breath hold trial and in the breath hold and stand trial. Yet, there were no significant differences in velocity, change in velocity, CVRi, PI, RI or in CR_{CO2} in the either trial. It is important to note that this was a voluntary breath hold, and due to this we were limited by the psychological and physical break points of our participants. In a recent voluntary breath hold study, participants were able to hold their breath for an average of 50.2± 3.2s, which greatly exceeds the performance of our subjects, who often struggled to reach 20s[6]. Several factors may have played a role in the length of breath hold, including initial lung volume, psychological factors, blood gas concentrations (prior hyperventilation), metabolic rate, and chemoreceptor stimulation[88]

In the past, hypercapnia with a $P_{ET}CO_2$ level of 6% CO_2 gas has caused the velocity of the MCA to increase by 28 ± 9cm/s and the velocity of the BA to increase by 14 ± 4cm/s[85]. With the exception of the breath hold trial, CR_{CO2} was always higher in the MCA than in the BA, similar to changes previously reported. Thus, it would seem the MCA is capable of experiencing greater fluctuations in velocity than the BA. Yet, when looking at the normalized CR_{CO2} values, the trend was for the BA to have a higher reactivity, and this difference was a trend during the hyperventilation portion of the hyperventilation trial. It is hard to make comparisons in this area of research because there is little consistency in how reactivity is calculated, and as mentioned

above, the method in which this value is calculated has an impact in how it is interpreted. Sato et al in the past have done a rebreathing protocol which calculated reactivity in the same normalized manner used in this study. What they found was that the MCA was more reactive than the BA, although no statistics were run. This is opposite to the trend seen in this study[67]. It is possible the difference in methods may have been responsible for the different results, but this is unlikely. These results were unexpected and the reason behind it is unclear.

At the turn of the century, research showed no different regional responses to CO_2 had been observed [4,45,89]. More recently, this has been challenged with some authors citing the reactivity of the vertebro-basilar circulation is lower compared to the internal carotid circulation[67]. Additionally, the MCA has been compared to the PCA and authors concluded that the reactivity of the MCA is not reflected by the PCA[7]. Although we did not see a significant difference in the CR_{CO2} of the breath hold trial, we did have a significant difference between the reactivity of arteries during the breath hold interaction trial, with the MCA having a greater reactivity than the BA using absolute values. This was observed during the breath hold portion of the hyperventilation trial, and the interaction hyperventilation trial; the MCA has a greater CR_{CO2} than the BA during hypercapnia. Using relative values (%/mmHg) the result is reversed; the BA is more reactive than the MCA but the difference is not significant. This is contrary to Sato et al. who observed a greater reactivity in the MCA (%/mmHg), but was unable to statistically determine if it was significant as they had too few BA observations to include in analysis. They did though, find the ICA was more reactive than the vertebral artery, suggesting the front vasculature is more reactive than the hind brain.

In the past, lower reactivity has been observed in the arteries of the posterior brain and researchers hypothesized that this was due to less autonomic tone at baseline, leaving less reserve for dilation with CO_2 stimulation[85,90]. This notion has

been supported by a positron emission computed tomography which showed lower dilatory reactivity in the cerebellum relative to the cerebrum[91]. Contrary to this, we showed that both PI and RI are elevated in the BA relative to the MCA at baseline, as well as during hyperventilation and breath hold. These variables do not account for blood pressure as CVRi does, as they simply use velocities to be calculated. PI and RI are reflective of the downstream arteries whereas CVRi is reflective of the MCA or the BA in this study. This suggests elevated tone in the downstream arteries of the BA relative to the MCA, contrary to past hypotheses.

Although this study assumed the diameter of the artery did not change, a past study which looked at the posterior inferior cerebellar artery (PICA) and the MCA assumed there was a change in diameter that could be responsible for the changes they saw[92]. They saw higher pulsatility values in their hind brain artery, PICA, and they suggested that during hypercapnia, the PICA dilates more due to the smooth muscle relaxing in the vessel wall than in the MCA[92]. This in turn causes local arterial compliance to increase, leading to an increase in local compliance flow which increases PI[93]. PI was observed to be significantly different between arteries at 30s and at 40s during hyperventilation and stand trial suggesting the change in CO_2 caused greater restriction of the downstream arteries of the BA than in the MCA.

Hypocapnia

During hypocapnia, CBF decreases as the vasculature constricts to attenuate the fall of CO_2[3]. Thus it was expected to see decreases in the velocities of both arteries during hyperventilation. In the past, hypocapnia achieved by hyperventilation, driving $P_{ET}CO_2$ down by 15mmHg, has been shown to decrease MCA velocity by -28 ± 10cm/s and reduce BA velocity by -9 ±4cm/s[85]. In the hyperventilation trial, $P_{ET}CO_2$ was decreased -13.66 ± 4.48mmHg, the MCA velocity decreased -20.5 cm/s and the BA decreased -17.4cm/s. In the hyperventilation interaction trial, $P_{ET}CO_2$ was driven down -12.72 ± 6.91mmHg, MCA velocity decreased by -21.2cm/s and the BA reduced velocity by -18.3cm/s, again following the same trend as previously reported[85]. Although these differences were not significant, the reactivity between vessels was, with the MCA being more reactive than the BA in both trials that included hyperventilation. Our results are similar to those in the past as in response to step hypoxia in normal individuals, the reactivity of the BA has been shown to be reduced relative to the MCA[94].

During the hyperventilation with stand and breath hold trial, RI was higher in the BA at 10s, 20s and 30s of hyperventilation. When CO_2 is decreased, it is expected that there is restriction of the downstream arteries in response to the change. What was found though, was also increased PI and RI after the hyperventilation at 40s, 10s into the breath hold as the arteries slowly returned towards baseline, with the BA having higher values than the MCA.

We observed a significant difference in the CR_{CO2} between arteries in both the hyperventilation trial and the interaction trial with hyperventilation but again, only when absolute velocity values were used. The relative velocity values produced not significant differences but the BA was higher than the MCA and these are the results which bear weight. The reason for not significant differences in CR_{CO2} with both breath hold and hyperventilation may be due to the fact that regional sympathetic

innervation of the posterior brain vasculature is less extensive than in the anterior cerebral circulation[95]. It is contrary to Reinhard et al. who found greater reactivity in the cerebrum relative to the cerebellum[90]. The reason we see these differences in CR_{CO2} between the MCA and the BA may be due to different characteristics in the vasculature itself, like basal vascular tone[96], regional microdensity[97], autonomic innervation[98] and regional heterogeneity in ion channels or production of NO[99].

BLOOD PRESSURE REGULATION

As previously mentioned blood pressure regulation and cerebrovascular reactivity are closely related. In order to try to look at the response to just manipulating blood pressure participants completed a passive tilt (75^{o} HUT) and an active stand (sit to stand protocol). Although slight changes in CO_2 were expected, the main effect of these trials was the change in blood pressure. The interaction trials also included the element of an active stand. Hyperventilation was meant to drive decreases in velocity through reduction in PETCO2, and the stand was meant to further decrease this by a change in blood pressure, creating a self-induced hypocapnic, low blood pressure state. The breath hold and stand trials were meant to initially drive down velocity by lowering blood pressure, and then increase velocity again as $P_{ET}CO_2$ increased over the 20s breath hold. Again, subjects were asked to stare at a dot on the wall to reduce the variability that can occur in the posterior brain with the activation of the eyes and the visual cortex[77]

ACTIVE STAND

In the past, no one has done a sit to stand protocol which looks at both the MCA and the BA. The values at nadir will be discussed later, but an active posture change has been shown in the past to decrease blood velocity of the MCA with values dropping to 57 ± 14cm/s [10] and 42.1 ± 1.6cm/s[100] over time. At the end of the third minute of standing, we observed a minor, not significant decrease in both MCA and BA blood velocity, with the MCA dropping from 60.93 ± 17.23 cm/s to 55.75 ± 15.28 cm/s and the BA dropping from 37.08 ± 8.56 cm/s to 33.78 ± 8.40 cm/s.

In terms of CVRi, the MCA and the BA were not significantly during the sit to stand trials, with the BA being significantly higher than the MCA; for the same change in blood pressure there is less change in velocity of the BA relative to the MCA. Both arteries had their lowest CVRi values at 30s and this was probably due to the change of blood pressure at the stand which would have caused arterioles to dilate. This dilation decreased resistance in order to increase blood flow and correct for the reduction in blood pressure. Although the two arteries saw similar changes in velocity, the BA was significantly higher in CVRi than the MCA at all time points, as it had lower flow at the same blood pressure. In terms of which artery is superior at autoregulation, the data are inconclusive. In looking at the %change in velocity of each artery at nadir, sometimes the BA changes more than the MCA, and vice versa, often being not significant. The sole significant difference in the change in velocity occurred in the stand that followed hyperventilation, and the MCA decreased velocity -41.37 ± 12.08% and the BA decreased -51.21 ± 18.35% which would suggest that the MCA is superior at autoregulation than the BA. This difference could be explained by regional heterogeneity in ion channels and reactive oxygen species or nitric oxide[101] .

Standing has also been shown to decrease CVRi from a seated (1.9 ± 0.1

mmHg/cm/s) to a standing (1.6 ± 0.6) position[100]. Contrary to this observation, we saw an increase in CVRi from baseline to the third minute of stand with the MCA increasing from 1.14 ± 0.51mmHg/cm/s to 1.27 v 0.55mmHg/cm/s and the BA increasing from 1.89 ± 0.50mmHg/cm/s to 2.13 ± 0.49mmHg/cm/s. This increase in resistance could be due to the slight (2.31mmHg) increase in blood pressure observed, that happens over time from seated to standing but this is highly unlikely. The increase in resistance was most likely not caused by reactivity as the change in $P_{ET}CO_2$ was miniscule, just -0.058mmHg.

A 5min stand in the past caused a drop in MCA PI to 0.67 ± 0.12[102] and in the current study the MCA also decreased in the 3min stand from 0.70 ± 0.15 to 0.67 ± 0.11, and the BA decreased similarly starting at a baseline value of 0.80 ± 0.32 and decreasing to 0.76 ± 0.32 in the final minute of stand. This study observed no significant differences in PI across time, but did see a significant difference between arteries at nadir. This may be reflective increased pulsatiliy of the smaller arteries during the decrease in blood pressure which would normally cause cerebral arteries to dilate.

In addition to looking at how an active stand affects both arteries over time, we looked at values at the time of the mean arterial pressure nadir. In the past, an active stand has been shown to reduce MCA velocity by 14% ± 13%[76], 18.8 ± 1.5% [100] and by 20 ± 7%[10]. Although the BA has not yet been observed in a sit to stand, the PCA has been shown to reduce velocity by 18 ± 13%[10]. Our MCA decreased by 30.06 ± 15.32% and our BA reduced velocity by 23.07 ± 14.80%, which are slightly higher values, but the difference between the two arteries was not different (p= 0.343 During the interaction trial with prior hyperventilation, we observed a significantly different velocity change between the MCA and the BA. After 30s of hyperventilation, the nadir at stand caused the BA to decrease velocity by -51.21 ± 18.34% and the MCA to decrease velocity by -41.37 ± 12.08%. (p=0.031). This

suggests that blood pressure changes can cause greater changes in the CBV of the posterior brain than the front brain. Thus, these data may reflect that the BA and the hind brain is not as efficient as the MCA with regard to autoregulation as changes in blood pressure result in greater velocity changes of the BA. But again, depending in the method of calculation, this fact can change

CVRi at nadir in the MCA has been observed to drop -0.18 ±0.5 mmHg/cm/s [76] and by -0.23 ± 0.04 mmHg/cm/s[100]. Our MCA CVRi decreased 0.3mmHg/cm/s at nadir and the BA decreased 0.41mmHg/cm/s at nadir but the difference was not significant. The increase in PI at nadir was significantly different between arteries, with the BA increasing more than the MCA in both variables. In the interaction trial with hyperventilation, the BA PI also increased significantly more than the MCA at nadir. This shows higher resistance in the downstream, smaller arteries of the BA which makes sense as at baseline, CVRi was higher in the BA and at nadir BA velocity was lower due to this increased resistance. Why this is the case is not clear. All data seem to suggest that the BA is a more resistive artery than the MCA Passive Posture Change

Passive Posture Change

A passive posture change is different from an active posture change in that there is no initial orthostatic hypotension caused by the muscle contraction and relaxation in the transition to upright posture. Instead, blood pressure decreases over time as blood pools in the lower legs, and blood pressure in the head also decreases as the head moves above the heart. The BA has not yet been observed in a tilt setting, but it has been observed in the supine position using a handheld probe[89]. Baseline velocity of the BA was recorded at 46.43 ± 9.63 cm/s and the MCA at a velocity of 64.76 ±16.79 cm/s, both very comparable values to those in the past literature[89]. In the past, velocity in the MCA has been observed to drop from 67 ± 7cm/s at baseline to 54 ± 10cm/s average for the final three minutes of a HUT[103].

Another author reported a baseline value of 58.2 ± 2.6 cm/s and during tilt it dropped to 50.2 ± 2.4cm/s[104]. The MCA velocity in this study decreased 10.27 cm/s and our BA decreased 8.45cm/s. The MCA values are similar, but the BA has not been observed in the past, and thus we cannot make comparisons to past works. The relative decrease was -15.98 ± 6.55% for the MCA and -17.56 ± 14.36% for the BA during the tilt which is a small difference between the two arteries. What is important is the BA seems to have a hindered ability for autoregulation as evidenced by the greater change in BA velocity during the hyperventilation with stand and breath hold trial; a greater change in blood pressure produces these results.

Baseline supine CVRi values have been observed in the MCA with a value of 1.23 ± 0.16mmHg/cm/s[104] and 1.3 ± 0.5 mmHg/cm/s[10]. The baseline MCA value in our HUT study was 1.15 ± 0.31mmHg/cm/s which is again very comparable, but not identical as different participants were used. The PCA has been observed with a supine value 2.5 ± 0.9 mmHg/cm/s [10], as it also has a slower velocity than the MCA, which suggests the BA should have a higher value as well. This was observed in our

baseline value which was 1.65 ± 0.45mmHg/cm/s for the BA. CVRi in the MCA has been shown to increase from a supine baseline of 1.23 ± 0.16 mmHg/cm/s to 1.60 ± 0.37mmHg/cm/s in the final three minutes of tilt[102]. Our MCA increase was modest, rising from 1.16 mmHg/cm/s to 1.22 mmHg/cm/s, and from 1.65 mmHg/cm/s to 1.83 mmHg/cm/s in the BA. The hind brain seems to be a more resistive vessel than the MCA, and in the increase seen with tilt seems contrary to the decrease in blood pressure (-19.02mmHg) from supine to tenth minute of tilt which should have resulted in decreased CVRi. This suggests that CO_2 may have been a factor but this study does not have the data to support or refute this notion.

The only significant difference we observed with HUT was a difference in PI in the tenth minute, where the BA had a significantly higher pulsatility index than the MCA. This study saw an increase in PI with the BA (0.834 ± 0.299) in the tenth minute of the HUT, where the BA PI was significantly higher than the MCA. This change did not alter velocity significantly. Past data have suggested an increase in resistance and PI at presyncope in the MCA[102]. In the past it has been observed that the decrease in blood flow seen during presyncope is related to increases in the resistance area product, denoting increases in resistance, caused by a myogenic response to a decrease in blood pressure[105]. Data from the current study shows an increase in pulsatility index that occurs in the BA before the MCA. Perhaps this suggests resistance begins increasing in the downstream arterioles of the BA, reducing blood flow in areas that produce symptoms like seeing spots or dizziness due to the increased resistance, before experiencing the change in resistance in the MCA which ultimately results in syncope.

NIRS

NIRS was included in this study to see if it could be a viable measure for predicting orthostatic hypotension. It has been shown in the past that an upright posture causes a decrease in prefrontal cortex oxygenation[58,106–108]. We only observed significant differences in the change in TSI when moving from the seated to standing position. We also saw no changes during tilt. It is possible this is because we only had a young, healthy population. Increased age has been shown to increase the severity of the change in oxygenation[109]. During the hyperventilation and breath hold trials, TSI decreased with hyperventilation, and increased with breath hold. These results suggest that vasoconstriction was occurring with hyperventilation decreasing TSI, and with breath hold, there was dilation increasing TSI.

In each protocol, participants were asked to complete an OHQ, a questionnaire which has been validated in the past [110]. In each protocol, the change in oxygenated haemoglobin was found, and we selected a point where subjects claimed to feel the greatest sensations. These protocols were unsuccessful in provoking strong orthostatic symptoms in many participants and many reported low scores. But, when symptoms were experienced, the questionnaire captured what was felt. Unfortunately, we had no significant correlations with the change in oxygenated haemoglobin. This suggests that using NIRS on the prefrontal cortex to monitor oxygenated haemoglobin is possibly not the best indicator of orthostatic symptoms.

LIMITATIONS

This was an exploratory study that used several tests to help identify if and in which ways the MCA and BA differ. The sample size affected the statistical power of the HUT tests, often producing low alpha values suggesting Type II error. In the tests with repeated trials, this was not an issue as all trials were used in analysis, increasing the statistical power.

Additionally, all participants were young and healthy, without a history of random fainting or orthostatic hypotension. This is the population where the regulation of brain blood flow is presumably functioning normally. It is possible we will see greater regional cerebral blood flow differences in populations with known dysregulation, like orthostatic hypotension.

Another limitation was mechanical; the CO_2 analyser used during the HUT test was faulty and resulted in the loss of all CO_2 data from the HUT. This resulted in the loss of CR_{CO2} calculations which would have been novel for the BA in this setting.

Something that is extremely difficult to control is the length of a voluntary breath hold. Several factors influence the length of a voluntary breath hold including initial lung volume, psychological factors, chemoreceptor stimulation, blood gas concentrations and metabolic rate[88]. It is human condition to want to breathe and it is possible 20s was insufficient amount of time to see any significant amount of change in the arteries. This may be a reason why we saw very few significant differences during our strictly breath hold trials.

An assumption made with this research was that the diameter of the arteries did not change throughout testing. This has been validated in the past with chabges in PETCO2 and in lower body negative pressure protocols (changes in blood pressure)[111]. All changes seen were assumed to be strictly due to changes in velocity. This was something we were unable to quantify as TCD can only produce values regarding blood

velocity, not any other characteristics. This is a common limitation of regional blood flow studies.

PRACTICAL IMPLICATIONS AND FUTURE DIRECTIONS

These findings help us better understand the basic functioning of the brain by producing novel results regarding the BA and how it responds to changes in blood pressure and $P_{ET}CO_2$. They challenge the fundamental understanding of how our cerebral vasculature functions. Indices like CVRi and CR_{CO2} using TCD are increasingly being used to help manage patients with cerebrovascular diseases [89]. By continuing to explore what is normal in each area of the brain we are better able to determine "normal" values which can be used to help diagnose those in diseased states [89]. Our work used a healthy population, which to date is needed to better our understanding of the regional differences that exist in a healthy brain. In the future, more work should be done to create a better understanding of the basic differences in a healthy brain.

Additionally, this new information regarding regional differences during changes in blood pressure and in CR_{CO2} may be able to help us better predict falls in our aging population. Data showed that there was no correlation between oxygenation of the prefrontal cortex and orthostatic symptoms. It's possible the protocol was not sufficient to elicit these symptoms. Thus, it is difficult to determine if the wrong area is being used to predict these symptoms. For so long, scientists have only looked at the MCA to explain what is happening in the brain. Perhaps through studying regional differences, we will better be able to find areas that relate to symptoms and in turn help us predict falls.

CONCLUSIONS

This study set out to explore if regional blood flow differences exist between the MCA and the BA. It was determined that the MCA has a higher reactivity to CO_2 than the BA but only when absolute velocity values are used which was predicted by hypothesis 1. When reactivity is calculated with the %change in velocity, the trend is that BA is more reactive than the MCA but the difference is not significant. In terms of autoregulation, the MCA is superior to the BA as shown by greater changes in BA velocity during the hyperventilation and stand trial. Additionally, the BA is higher in CVRi than the MCA. These results, like several recent studies, suggest that regional blood flow differences between the front brain and the hind brain do exist, but unlike past works reactivity is not different[7]. This study suggests autoregulation is superior in the front brain than in the hind brain and that the vasculature of the hind brain is more resistive than the front brain. These data contribute to the theory that regional blood flow differences exist in the brain.

References

1. Nakamura, T. *et al*. Effective detection of cardiac sympathetic denervation with dobutamine infusion in Parkinson's disease: Correlation with cardiac 123I-metaiodobenzylguanidine (MIBG) uptake. *Auton. Neurosci*. **130,** 64 (2006).
2. Marieb, E. N. & Hoehn, K. *Human Anatomy & Physiology*. *Physiology* **7,** (2010).
3. Ainslie, P. N. & Duffin, J. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. **296,** R1473-95 (2009).
4. Hida, W. *et al*. CO2 response for the brain stem artery blood flow velocity in man. *Respir. Physiol*. **104,** 71–75 (1996).
5. Newell, D. W. & Aaslid, R. in *Transcranial Doppler* 9–35 (Raven Press, 1992).
6. Bruce, C. D. *et al*. Quantifying cerebrovascular reactivity in anterior and posterior cerebral circulations during voluntary breath holding. *Exp. Physiol*. **12,** 1517–1527 (2016).
7. Skow, R. J. *et al*. Differential cerebrovascular CO2 reactivity in anterior and posterior cerebral circulations. *Respir. Physiol. Neurobiol*. **189,** 76–86 (2013).
8. Willie, C. K. *et al*. Regional brain blood flow in man during acute changes in arterial blood gases. **14,** 3261–3275 (2012).
9. Sato, K. *et al*. Differential blood flow responses to CO2 in human internal and external carotid and vertebral arteries. *J. Physiol*. **590,** 3277–90 (2012).
10. Sorond, F. A., Khavari, R., Serrador, J. M. & Lipsitz, L. A. Regional cerebral autoregulation during orthostatic stress: age-related differences. *Journals Gerontol. Ser. A Biol. Sci. Med. Sci*. **60,** 1484–1487 (2005).
11. Tymko, M. M., Skow, R. J., MacKay, C. M. & Day, T. a. Steady-state tilt has no effect on cerebrovascular CO2 reactivity in anterior and posterior cerebral

circulations. *Exp. Physiol.* **100,** 839–51 (2015).

12. Folino, A. F. Cerebral Autoregulation and Syncope. *Prog. Cardiovasc. Dis.* **50,** 49–80 (2007).

13. Baird, T. A., Muir, K. W. & Bone, I. Basilar artery occlusion. *Neurocrit. Care* **1,** 319–329 (2004).

14. Ogoh, S. *et al.* Blood flow in internal carotid and vertebral arteries during graded lower body negative pressure in humans. *Exp. Physiol.* **100,** 259–266 (2015).

15. Folino, A. F., Migliore, F., Marinelli, A., Iliceto, S. & Buja, G. Age-related hemodynamic changes during vasovagal syncope. *Auton. Neurosci. Basic Clin.* **156,** 131–137 (2010).

16. Hendrikse, J., van Raamt, a F., van der Graaf, Y., Mali, W. P. T. M. & van der Grond, J. Distribution of cerebral blood flow in the circle of Willis. *Radiology* **235,** 184–189 (2005).

17. Barret, K., Barman, S., Boitano, S. & Brooks, H. *Ganong's Review of Medical Physiology*. (McGraw-Hill, 2016).

18. Vrselja, Z., Brkic, H., Mrdenovic, S., Radic, R. & Curic, G. Function of circle of Willis. **34,** 578–584 (2014).

19. Hoksbergen, A. W. J., Majoie, C. B. L., Hulsmans, F. J. H. & Legemate, D. A. Assessment of the collateral function of the circle of Willis: Three-dimensional time-of-flight MR angiography compared with transcranial color-coded duplex sonography. *Am. J. Neuroradiol.* **24,** 456–462 (2003).

20. Zhu, G., Yuan, Q., Yang, J. & Yeo, J. H. The role of the circle of Willis in internal carotid artery stenosis and anatomical variations: a computational study based on a patient-specific three-dimensional model. *Biomed. Eng. Online* **14,** 107 (2015).

21. Zhang, C. *et al.* Modeling the circle of Willis to assess the effect of anatomical variations on the development of unilateral internal carotid artery stenosis. *Biomed.*

Mater. Eng. **24,** 491–499 (2014).

22. Devault, K. *et al*. Blood Flow in the CIrcle of Wiliis: Modeling and Calibration. **4006,** 1–18

23. Zhu, G., Yuan, Q., Yang, J. & Yeo, J. Experimental study of hemodynamics in the circle of Willis. *Biomed. Eng. Online* **14,** S10 (2015).

24. Alastruey, J., Parker, K., Piero, J., Byrd, S. & SHerwin, S. Modelling the Circle of WIllis to Asses the Effects of ANatomical Variations and Occlusions on Cerebral Flows. *J. Biomech.* **40,** 1794–1805 (2007).

25. Bradac, G. B. in *Cerebral Angiography : normal anatomy and vascular pathology* 79–86 (2011). doi:10.1007/978-3-642-15678-6

26. Noback, C., Strominger, N., Demarest, R. & Ruggiero, D. *The Human Nervous System: Structure and Function*. (Humana Press, 2005).

27. Rutgers, D. R., Klijn, C. J. M., Kappelle, L. J., van Huffelen, a. C. & van der Grond, J. A Longitudinal Study of Collateral Flow Patterns in the Circle of Willis and the Ophthalmic Artery in Patients With a Symptomatic Internal Carotid Artery Occlusion. *Stroke* **31,** 1913–1920 (2000).

28. Grond, J. Van Der. Collateral Ability of the Circle of Willis in Patients With. *Stroke* 2768–2773 (2001). doi:10.1161/hs1201.099892

29. Kvistad, C. E., Oygarden, H., Thomassen, L., Waje-Andreassen, U. & Naess, H. Persistent middle cerebral artery occlusion associated with lower body temperature on admission. *Vasc. Health Risk Manag.* **9,** 297–302 (2013).

30. Imparato, a M., Riles, T. S. & Gorstein, F. The carotid bifurcation plaque: pathologic findings associated with cerebral ischemia. *Stroke.* **10,** 238–245 (2015).

31. Lee, J., Albers, G. W., Marks, M. P. & Lansberg, M. G. Capsular warning syndrome caused by middle cerebral artery stenosis. *J. Neurol. Sci.* **296,** 115– 120 (2010).

32. Kubik, B. Y. C. S. Whereas most of the vascular syndromes of the brain-stem have been the subject of a voluminous literature, basilar artery occlusion has. **69,** (1946).

33. Caplan, L. R. Occlusion of the vertebral or basilar artery. Follow up analysis of some patients with benign outcome. *Stroke*. **10,** 277–282 (1979).

34. Benson, F. D., Marsden, D. C. & Meadows, J. C. The Amnesic Syndrome of Posterior Cerebral Artery Occlusion. 133–145 (1974).

35. Yamasoba, T., Kikuchi, S. & Higo, R. Deafness associated with vertebrobasilar insufficiency. *J. Neurol. Sci*. **187,** 69–75 (2001).

36. Ferbert, A., Brückmann, H. & Drummen, R. Clinical features of proven basilar artery occlusion. *Stroke* **21,** 1135–1142 (1990).

37. Toyoda, K. *et al*. Bilateral deafness as a prodromal symptom of basilar artery occlusion. *J. Neurol. Sci*. **193,** 147–150 (2002).

38. Archer, C. R. & Horenstein, S. Basilar artery occlusion: clinical and radiological correlation. *Stroke* **8,** 383–390 (1977).

39. Hacke, W., Zeumer, H., Ferbert, a, Bruckmann, H. & del Zoppo, G. J. Intra-arterial thrombolytic therapy improves outcome in patients with acute vertebrobasilar occlusive disease 84. *Stroke* **19,** 1216–1222 (1988).

40. Van Beek, A. H. E. A., Claassen, J. A. H. R., Olde Rikkert, M. G. & Jansen, R. W. Cerebral autoregulation : an overview of current concepts and methodology with special focus on the elderly. 1071–1085 (2008). doi:10.1038/jcbfm.2008.13

41. Ainslie, P. N. Regional brain blood flow regulation during orthostatic stress: new insights from volumetric brain blood flow measurements. *Exp. Physiol*. **97,** 1247–8 (2012).

42. Abboud, F. M., Heistad, D. D., Mark, A. L. & Schmid, P. G. Reflex control of the peripheral circulation. *Prog Cardiovasc Dis* **18,** 371–403 (1976).

43. Grubb, B. P. & Kosinski, D. Tilt table testing: Concepts and limitations. *PACE - Pacing Clin. Electrophysiol*. **20,** 781–787 (1997).

44. Hainsworth, R. Pathophysiology of syncope. *Clin. Auton. Res*. **14,** 18–24 (2004).

45. Ogawa, S. *et al*. Carbondioxide reactivity of the blood flow in human basilar artery estimated by the transcranial doppler method in normal men: A comparison with that of the middle cerebral artery. *Ultrasound Med. Biol*. **14,** 479–483 (1988).

46. Colier, W. N., Binkhorst, R. a, Hopman, M. T. & Oeseburg, B. Cerebral and circulatory haemodynamics before vasovagal syncope induced by orthostatic stress. *Clin. Physiol*. **17,** 83–94 (1997).

Lagi, a, Cencetti, S., Corsoni, V., Georgiadis, D. & Bacalli, S. Cerebral vasoconstriction in vasovagal syncope: any link with symptoms? A transcranial Doppler study. *Circulation* **104,** 2694–2698 (2001).

47. Aaslid, R., Lindegaard, K. F., Sorteberg, W. & Nornes, H. Cerebral autoregulation dynamics in humans. *Stroke*. **20,** 45–52 (1989).

48. Henderson, M. C. & Prabhu, S. D. *Current Problems in Cardiology*. **22,** (1997).

49. Mader, G., Olufsen, M. & Mahdi, A. Modeling Cerebral Blood Flow Velocity During Orthostatic Stress. *Ann. Biomed. Eng*. **43,** 1748–58 (2015).

50. Ainslie, P. N. & Tzeng, Y. Blood pressure regulation IX : cerebral autoregulation under blood pressure challenges. **114,** 545–559 (2014).

51. Lagopoulos, J. Cerebral autoregulation. *Acta Neuropsychiatr*. **20,** 271–272 (2008).

52. Mbbs, A. T. Y. *et al*. Cerebral autoregulation and ageing. **12,** 643–646 (2005).

53. Guo, H. *et al*. Cerebral autoregulation is preserved during orthostatic stress superimposed with systemic hypotension. *J. Appl. Physiol*. **100,** 1785–1792 (2006).

54. Strandgaard, S. & Paulson, O. B. Cerebral Autoregulation. *Neurology* **15,** 413– 416 (1984).

55. Bayliss, J. effect may be exerted at lower doses than those required Since there is evidence that both benzo (a) pyrenel and carcinogenic activity of cigarette smoke , it is of more enzymes in human placental microsomes 33 and that nicotine and one of its major met. *J. Am. Coll. Cardiol*. 29–31 (1985).

56. Diedrich, a. & Biaggioni, I. Segmental orthostatic fluid shifts. *Clin. Auton. Res.* **14,** 146–147 (2004).

Lieshout, J. J. V. A. N., Wieling, W., Karemaker, J. M., Secher, N. H. & Syncope, N. H. S. invited review. 833–848 (2003).

57. Kong, K.-H. & Chuo, A. M. Incidence and outcome of orthostatic hypotension in stroke patients undergoing rehabilitation. *Arch. Phys. Med. Rehabil.* **84,** 559–562 (2003).

58. Medow, M. S. *et al*. Pathophysiology, diagnosis, and treatment of orthostatic hypotension and vasovagal syncope. *Cardiol. Rev.* **16,** 4–20 (2008).

59. Heitterachi, E., Lord, S. R., Meyerkort, P., McCloskey, I. & Fitzpatrick, R. Blood pressure changes on upright tilting predict falls in older people. *Age Ageing* **31,** 181–186 (2002).

60. Freeman, R. *et al*. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin. Auton. Res.* **21,** 69–72 (2011).

61. Deegan, B. M. T., O'Connor, M., Lyons, D. & Gearóid ÓLaighin. A new blood pressure and heart rate signal analysis technique to assess Orthostatic Hypotension and its subtypes. *Ieee Embs* 935–938 (2007).

62. Brignole, M. *et al*. Guidelines on Management (Diagnosis and Treatment) of Syncope - Update 2004 *. **6,** 467–537 (2004).

63. Willie, C. K. *et al*. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J. Neurosci. Methods* **196,** 221–237 (2011).

64. Sato, K., Ogoh, S., Hirasawa, A., Oue, A. & Sadamoto, T. The distribution of blood flow in the carotid and vertebral arteries during dynamic exercise in humans. *J Physiol* **589,** 2847–2856 (2011).

65. Sato, K. *et al*. Blood flow in internal carotid and vertebral arteries during

orthostatic stress. *Exp. Physiol.* **97,** 1272–80 (2012).

66. Barrett, K. M., Ackerman, R. H., Gahn, G., Romero, J. M. & Candia, M. Basilar and middle cerebral artery reserve: a comparative study using transcranial Doppler and breath-holding techniques. *Stroke* **32,** 2793–2796 (2001).

67. Imholz, B. P., Dambrink, J. H., Karemaker, J. M. & Wieling, W. Orthostatic circulatory control in the elderly evaluated by non-invasive continuous blood pressure measurement. *Clin Sci* **79,** 73–79 (1990).

68. Serrador, J. M., Hughson, R. L., Kowalchuk, J. M., Bondar, R. L. & Gelb, A. W. Cerebral blood flow during orthostasis: role of arterial CO2. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290,** R1087-93 (2006).

69. Wieling, W., Krediet, C. T. P. & Tschakovsky, M. E. Initial orthostatic hypotension : review of a forgotten condition. **165,** 157–165 (2007).

70. Ouchi, Y., Okada, H., Yoshikawa, E., Futatsubashi, M. & Nobezawa, S. Absolute changes in regional cerebral blood flow in association with upright posture in humans: an orthostatic PET study. *J. Nucl. Med.* **42,** 707–712 (2001).

71. Passant, U., Karlson, S., Edvinsson, L., Gustafson, L. & Risberg, J. Redistribution of blood f l o w in the cerebral cortex of normal subjects during head-up postural change. **2,** 119–124 (1992).

72. Mehagnoul-Schipper, D. J., Colier, W. N. & Jansen, R. W. Reproducibility of orthostatic changes in cerebral oxygenation in healthy subjects aged 70 years or older. *Clin. Physiol.* **21,** 77–84 (2001).

73. Passant, U. Cortical blood f l o w during head-up postural change in subjects w i t h orthostatic hypotension. **3,** 311–318 (1993).

74. Sorond, F. A., Serrador, J. M., Jones, R. N., Shaffer, M. L. & Lipsitz, L. A. The Sit-

to-Stand Technique for the Measurement of Dynamic Cerebral Autoregulation. **35,** 21–29 (2010).

75. Nakagawa, K. *et al*. Autoregulation in the Posterior Circulation Is Altered by the Metabolic State of the Visual Cortex. *Stroke* **40,** 2062–2067 (2009).

76. Hong, J. M., Joo, I. S., Huh, K. & Sheen, S. S. Simultaneous vasomotor reactivity testing in the middle cerebral and basilar artery with suboccipital probe fixation device: Technology update. *J. Neuroimaging* **20,** 83–86 (2010).

77. Kay, V. L. & Rickards, C. A. The role of cerebral oxygenation and regional cerebral blood flow on tolerance to central hypovolemia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **310,** R375-83 (2016).

78. Lewis, N. C. S. *et al*. Impact of transient hypotension on regional cerebral blood flow in humans. 169–178 (2015). doi:10.1042/CS20140751

79. Deegan, B. M. *et al*. Pressure in Healthy Humans. 2505–2508 (2010).

80. Lewis, N. C. S. *et al*. Impact of hypocapnia and cerebral perfusion on orthostatic tolerance. *J. Physiol.* **592,** 5203–19 (2014).

81. Harrell, J. W. & Yanke, P. A. Regional disparity of cerebral vessel reactivity John W. Harrell and Peter A. Yanke. **20,** 4981–4982 (2012).

82. Boulet, L. M. *et al*. Influence of prior hyperventilation duration on respiratory chemosensitivity and cerebrovascular reactivity during modified hyperoxic rebreathing. *Exp. Physiol.* **7,** 821–835 (2016).

83. Sato, K. *et al*. Differential blood flow responses to CO2 in human internal and external carotid and vertebral arteries. *J. Physiol.* **590,** 3277–90 (2012).

84. Barrett, K. M., Ackerman, R. H., Gahn, G., Romero, J. M. & Candia, M. Basilar and middle cerebral artery reserve: a comparative study using transcranial

Doppler and breath-holding techniques. *Stroke* **32,** 2793–2796 (2001).

85. Coverdale, N. S., Lalande, S., Perrotta, A. & Shoemaker, J. K. Heterogeneous patterns of vasoreactivity in the middle cerebral and internal carotid arteries. *Am. J. Physiol. Heart Circ. Physiol.* **308,** H1030-1038 (2015).

86. Parkes, M. J. Breath-holding and its breakpoint. 1–15 (2006). doi:10.1113/expphysiol.2005.031625

87. Park, C. W., Sturzenegger, M., Douville, C. M., Aaslid, R. & Newell, D. W. Autoregulatory response and CO2 reactivity of the basilar artery. *Stroke* **34,** 34–39 (2003).

88. Reinhard, M., Waldkircher, Z., Timmer, J., Weiller, C. & Hetzel, A. Cerebellar autoregulation dynamics in humans. *J. Cereb. blood flow Metab.* **28,** 1605– 1612 (2008).

89. Suzuki, Y., Oishi, M., Mizutani, T. & Sato, Y. Regional Cerebral Blood Flow Measured by the Resting and Vascular Reserve (RVR) Method in Chronic Alcoholics. **26,** 95–99 (2002).

90. Reinhard, M., Waldkircher, Z., Timmer, J., Weiller, C. & Hetzel, A. Cerebellar autoregulation dynamics in humans. **2,** 1605–1612 (2008).

91. Bude, R. O. & Rubin, J. M. Experimental Studies Relationship between the Resistive Index and Vascular Compliance and Resistance 1. (1999).

92. Giovanni, S. & Giovanni, S. S. Vascular reactivity in middle cerebral artery and basilar artery by transcranial Doppler in normals subjects during hypoxia. 135– 137 (1997).

93. Sjoberg, N. 377 autonomic nerves, mast cells, and amine receptors in human brainvessels. a histochemical and pharmacological study. **115,** 377–393

(1976).

94. Haubrich, C., Wendt, A., Diehl, R. R. & Klötzsch, C. Dynamic Autoregulation Testing in the Posterior Cerebral Artery. *Stroke* **35,** 848–852 (2004).

95. Cord, S. Comparative Studies of Regional CNS Blood Flow Autoregulation and Responses to CO 2 in the Cat.

96. Hamel, E., Edvinsson, L. & Mackenzie, E. T. Heterogeneous vasomotor responses of anatomically distinct feline cerebral arteries. 423–436 (1988).

97. Gotoh, J. U. N. *et al*. Regional differences in mechanisms of cerebral circulatory response to neuronal activation. **4030,** 821–829 (2001).

98. Lipsitz, L. A., Mukai, S., Hamner, J., Gagnon, M. & Babikian, V. Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension [In Process Citation]. *Stroke* **31,** 1897–1903 (2000).

99. Andresen, J. *et al*. Regulation of the Cerebral Circulation Endothelial influences on cerebrovascular tone. 318–327 (2006). doi:10.1152/japplphysiol.00937.2005.

100. Sung, R. Y. T., Du, Z. D., Yu, C. W., Yam, M. C. & Fok, T. F. Cerebral blood flow during vasovagal syncope induced by active standing or head up tilt. 154–158 (2000).

101. Murrell, C. J. *et al*. Cardiorespiratory and cerebrovascular responses to head- up tilt I: Influence of age and training status. *Exp. Gerontol*. **46,** 9–17 (2011).

102. Novak, V. *et al*. Autoregulation of Cerebral Blood Flow in Orthostatic Hypotension. *Blood* 104–111 (1998).

103. Zuj, K. A., Arbeille, P., Shoemaker, J. K. & Hughson, R. L. Cerebral critical closing pressure and CO 2 responses during the progression toward syncope.

801–807 (2013). doi:10.1152/japplphysiol.01181.2012

104. Mehagnoul-Schipper, D. J. *et al*. Cerebral oxygenation responses to standing in elderly patients with predominantly diastolic dysfunction. *Clin. Physiol. Funct. Imaging* **23,** 92–7 (2003).

105. Edlow, B. L. *et al*. The effects of healthy aging on cerebral hemodynamic responses to posture change. *Physiol. Meas.* **31,** 477–495 (2010).

106. Madsen, P. *et al*. Near-infrared spectrophotometry determined brain oxygenation during fainting. *Acta Physiol. Scand.* **162,** 501–507 (1998).

107. Mehagnoul-schipper, D. J., Vloet, L. C. M., Colier, W. N. J. M., Hoefnagels, W. H. L. & Jansen, W. M. M. Cerebral Oxygenation Declines in Healthy Elderly Subjects in Response to Assuming the Upright Position. (2000).

108. Kaufmann, H., Malamut, R., Norcliffe-Kaufmann, L., Rosa, K. & Freeman, R. The Orthostatic Hypotension Questionnaire (OHQ): Validation of a novel symptom assessment scale. *Clin. Auton. Res.* **22,** 79–90 (2012).

109. Serrador, J. M., Picot, P. A., Rutt, B. K., Shoemaker, J. K. & Bondar, R. L. MRI Measures of Middle Cerebral Artery Diameter in Conscious Humans During Simulated Orthostasis. 1672–1679 (2000).

110. Carey, B. J., Panerai, R. B. & Potter, J. F. Effect of aging on dynamic cerebral autoregulation during head-up tilt. *Stroke*. **34,** 1871–1875 (2003).

111. Tymko, M. M., Rickards, C. A., Skow, R. J., Ingram-Cotton, N. C., Howatt, M. K., & Day, T. A. The effect of superimposed tilt and lower body negative pressure on anterior and posterior cerebral circulations. *Physiol. Rep*. **4,** 1–14 (2016).

APPENDIX A – ORTHOSTATIC HYPOTENSION QUESTIONNAIRE (OHQ)

Orthostatic Hypotension Symptom Assessment (OHSA)[110]

Please check the number on the scale that best rates how severe your symptoms from low blood pressure have been. Please respond to every symptom. If you do not experience the symptom, circle zero (0). PLEASE RATE THE SYMPTOMS THAT ARE DUE ONLY TO YOUR LOW BLOOD PRESSURE PROBLEM

1. Dizziness, lightheadedness, feeling faint, or feeling like you might blackout.

NONE 0 - -	1	2	3	4	5	6	7	8	9	10 WORST POSSIBLE - -

2. Problems with vision (blurring, seeing spots, tunnel vision, etc.)

NONE 0 - -	1	2	3	4	5	6	7	8	9	10 WORST POSSIBLE - -

Weakness

NONE 0 - -	1	2	3	4	5	6	7	8	9	10 WORST POSSIBLE - -

3. Fatigue

NONE	1	2	3	4	5	6	7	8	9	10

0 - -										WORST POSSIBLE - -

1. Trouble concentrating.

NONE 0 - -	1	2	3	4	5	6	7	8	9	10 WORST POSSIBLE - -

2. Head/neck discomfort

NONE 0 - -	1	2	3	4	5	6	7	8	9	10 WORST POSSIBLE - -

APPENDIX B: Case study: Participant 11

Participant number 11 was a 22 year old female who met all inclusion criteria for the HUT trial. Her MCA was found at a depth 48mm and her BA at a depth of 88mm. All signals throughout the test were strong and without noise. What made this case unique was the fact that after 10min of tilt, she began to experience changes in vision and by 13min and 40s she had to assume the supine position as precaution to avoid syncope. According to her OHQ, she rated her sensation of dizziness, lightheadedness, faintness and the feeling of impending blackout to be an 8 out of 10, she rated her vision problems to be an 8 out of 10, and her weakness as a 4 out of 10.

Just before the participant mentioned seeing spots, researchers noticed a change in the velocity waveform of the BA. During the first 10min, the waveform had a typical systole and diastole wave separated with a dicrotic notch (Figure 23 A).

When we noticed a change starting to occur around 11min, the dicrotic notch was much deeper (Figure 23 B). When the participant started to complain of vision issues, the dicrotic notch deepened further and reached the irremovable wall filter at 8cm/s, masking the true velocity between systole and diastole (Figure 23 C and D).

At 12min, when the waveform looked like Figure 23 C, velocity began to decrease drastically which can be seen in Figure 24. The BA had a greater mean velocity %change than the MCA starting just after 650s, and this trend was maintained until supine was assumed. The MCA mean velocity reduced by -76.13% at its lowest, and the BA mean velocity was reduced by -83.69%. Although the BA has not been observed in syncope research, the MCA has been. In one study, syncope patients were observed in a 70^{o} HUT, and in the 29th minute the MCA had a mean velocity reduction of -48.21% whereas their non-

patient counterparts only had a -8.58% reduction [112]. CBV of the MCA during another tilt protocol was reduced to 21 ± 6 cm/s at syncope. The mean velocity of MCA of our participant was reduced to 5.9 cm/s, suggesting that syncope was imminent without the return to supine.

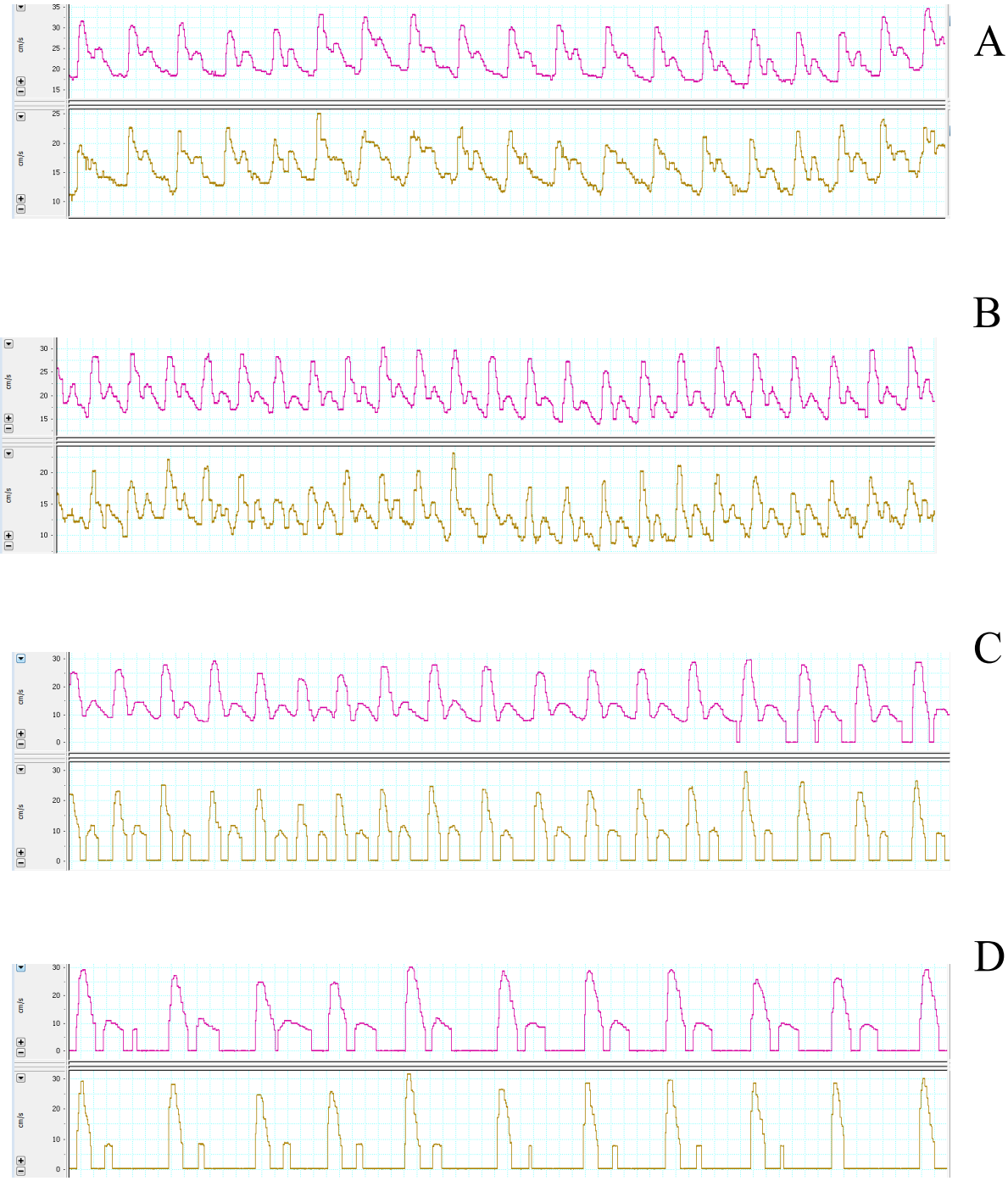

Figure 23:Velocity tracing of the MCA (pink) and the BA (gold) at different stages of tilt. A represents the flow pattern during the first 10min of tilt. B represents the change in waveform seen after 10min. C represents the first time the MCA reached the wall filter, 47s after the BA. D represents the waveform just before supine was assumed.

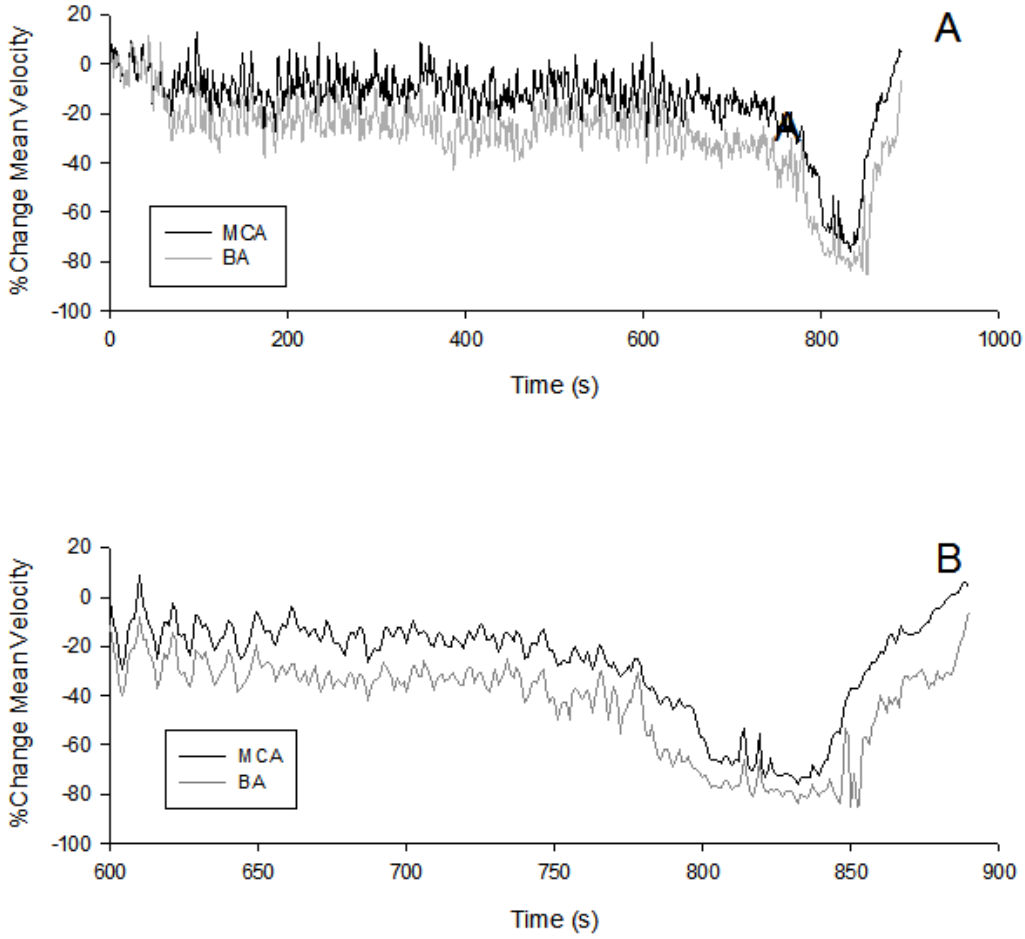

Figure 24: The change in mean velocity of the MCA and the BA of participant 11. A represents 30s of supine, roughly 820s of tilt and 40s of supine recovery. B represents a closer look at the changes that occurred during the time symptoms appeared.

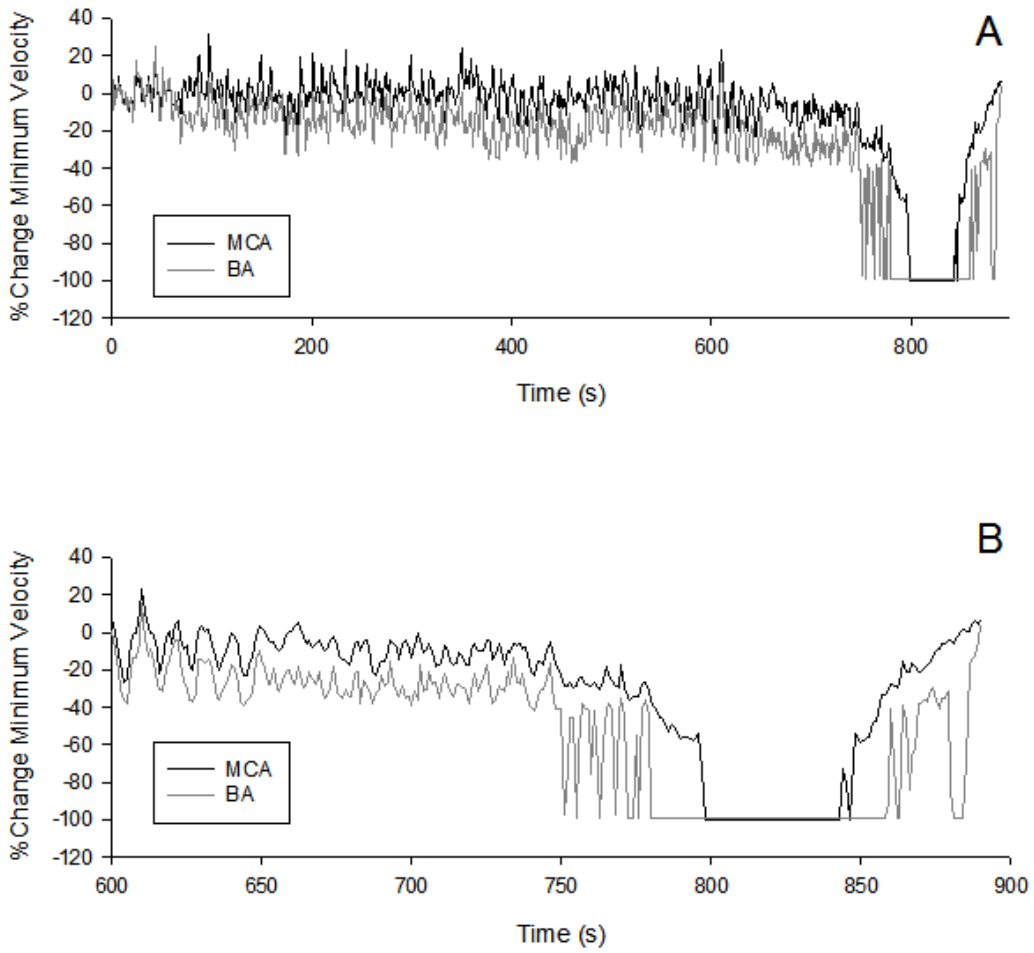

Figure 25: The change in minimum velocity of the MCA and the BA of participant 11. A represents 30s of supine, roughly 820s of tilt and 40s of supine recovery. B represents a closer look at the changes that occurred during the time symptoms appeared.

To look at this change further, the change in minimum velocity between arteries is plotted in Figure 28. At around 750s, the BA begins reaching the wall filter, denoted by a change of -100.00%. At this time, the MCA minimum velocity change is ~-27.01%. The MCA doesn't reach the wall filter until 797s, a full 47s after the BA hit the same minimum velocity. Due to the fact the BA has a slower mean velocity than the MCA[5], we'd expect the BA to reach the wall filter sooner, but 47s is a substantial time difference.

Participant 11 was an anomaly amongst all the participants. While other participants seemed to maintain their autoregulatory abilities, this participant showed what can happen in the front and hind brain when there is dysregulation. More specifically, it seems when syncope is imminent, there is greater dysregulation of the vasculature of the hind brain relative to the front brain. The symptoms like dizziness and "seeing spots" make sense in this given situation, as the ocular and vestibulocochlear systems are supplied by the BA which clearly had decreased blood flow. Further regional cerebral blood flow research should be done with syncope patients in order to determine if a factor of imminent syncope is dysregulation of the hind brain vasculature. Symptoms experienced by this participant were that of orthostatic intolerance, which often leads to falls. Perhaps furthering research in this area will help us clarify those at risk for falls and enhance our ability to prevent them.