

Competing effects of arterial pressure and carbon dioxide on cerebrovascular regulation during
exercise and orthostatic stress

by

Eric Thomas Hedge

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Kinesiology

Waterloo, Ontario, Canada, 2024

© Eric Thomas Hedge 2024

Examining Committee Membership

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

External Examiner	Ronney B. Panerai Professor Emeritus, Department of Cardiovascular Sciences, University of Leicester
Supervisor	Richard L. Hughson Distinguished Professor Emeritus, Department of Kinesiology and Health Sciences, University of Waterloo Senior Director of Research, Schlegel-UW Research Institute for Aging
Internal Member	Jason S. Au Assistant Professor, Department of Kinesiology and Health Sciences, University of Waterloo
Internal-external Member	Alfred C. H. Yu Professor and Assistant Vice-President (Research and

International), Department of Electrical and Computer
Engineering, University of Waterloo

Other Member

Paolo B. Dominelli

Assistant Professor, Faculty of Kinesiology, University of Calgary

Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the 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.

Statement of Contributions

Chapter 1: Eric T. Hedge drafted the chapter; Eric T. Hedge and Richard L. Hughson edited, revised and approved the final version of the chapter.

Chapter 2: Eric T. Hedge and Richard L. Hughson conceived and designed the research; Eric T. Hedge performed the experiments and analyzed the data; Eric T. Hedge and Richard L. Hughson interpreted the data; Eric T. Hedge prepared the figures and drafted the chapter; Eric T. Hedge and Richard L. Hughson edited, revised and approved the final version of the chapter.

Chapter 3: Eric T. Hedge, Andrew D. Robertson and Richard L. Hughson conceived and designed the research; Eric T. Hedge performed the experiments and analyzed the data; Eric T. Hedge, Andrew D. Robertson and Richard L. Hughson interpreted the data; Eric T. Hedge prepared the figures and drafted the chapter; Eric T. Hedge, Andrew D. Robertson and Richard L. Hughson edited, revised and approved the final version of the chapter.

Chapter 4: Eric T. Hedge, Carmelo J. Mastrandrea and Richard L. Hughson conceived and designed the research; Eric T. Hedge and Carmelo J. Mastrandrea performed the experiments; Eric T. Hedge and Courtney A. Patterson analyzed the data; Eric T. Hedge, Carmelo J. Mastrandrea and Richard L. Hughson interpreted the data; Eric T. Hedge prepared the figures and drafted the chapter; Eric T. Hedge, Carmelo J. Mastrandrea, Courtney A. Patterson and Richard L. Hughson edited, revised and approved the final version of the chapter.

Chapter 5: Eric T. Hedge, and Richard L. Hughson conceived and designed the research; Eric T. Hedge, Andrew D. Robertson, Jeremy N. Cohen, Roxanne Fournier, Carol G. Bryans, Jeremy Rabineau, Danielle K. Greaves and Richard L. Hughson performed the experiments; Eric T. Hedge, Andrew D. Robertson, Jeremy N. Cohen, Jeremy Rabineau and Richard L. Hughson

analyzed the data; Eric T. Hedge, Andrew D. Robertson, Jeremy N. Cohen and Richard L. Hughson interpreted the data; Eric T. Hedge prepared the figures and drafted the chapter; Eric T. Hedge, Andrew D. Robertson, Jeremy N. Cohen and Richard L. Hughson edited, revised and approved the final version of the chapter.

Chapter 6: Eric T. Hedge drafted, edited, revised and approved the final version of the chapter.

Abstract

The human brain is highly sensitive to changes in cerebral blood flow. There are multiple integrated and redundant regulatory mechanisms acting simultaneously to ensure adequate cerebral perfusion and removal of waste products. However, the contribution of different cerebrovascular control mechanisms to the increase in cerebral blood flow during exercise or the reduction in flow during orthostatic stress are controversial, especially for the competing roles of arterial pressure and CO₂. Therefore, the purpose of this thesis was to identify which regulatory factors play prominent roles in modulating cerebral blood flow during and following transitions in exercise intensity or posture change. This was accomplished through a series of experiments that evaluated cerebrovascular responses to moderate- and high-intensity interval exercise, bed rest, and orthostatic stress tests to pre-syncope. Through causal time-series modeling, it was identified that cerebral autoregulation effectively minimized the effects of exercise-induced increases in mean arterial pressure (MAP) on middle cerebral artery blood velocity (MCAv), and that changes in estimated arterial partial pressure of CO₂ (P_aCO₂) largely dictated MCAv dynamics in response to step changes in work rate. These findings sharply contrast with recent attempts to characterize the increase in MCAv at the onset of exercise as a mono-exponential response. Sex-specific effects of MAP and end-tidal PCO₂ on MCAv were identified while standing following two weeks of bed rest in post-menopausal women and similar-aged men, with reduced end-tidal PCO₂ contributing to reductions in men and lower MAP contributing to reductions in women. Vertebral artery blood flow was also identified as an important factor potentially mediating cerebrovascular-respiratory interactions during orthostatic stress in the progression to syncope. Overall, the results of these experiments demonstrate important connections between the cerebral vasculature and respiratory control during exercise and

orthostatic stress, enhancing our fundamental understanding of cerebrovascular control and the integrative cerebrovascular cascade leading to syncope.

Acknowledgements

First, thank you to all the participants who volunteered to participate in the many studies that I have conducted over the last 4 years, which made all of this work possible.

To my mentor, Richard Hughson, thank-you for giving me the opportunity to succeed and grow from a student into a young scientist. The training opportunities, connections, knowledge and mentorship that you have shared with me are irreplaceable.

To my thesis committee members, Jason Au, Paolo Dominelli, Alfred Yu, and Ronney Panerai, thank-you for contributing your expertise to this thesis. Paolo and Jason, thank-you for also including me in many facets of your lab activities both on- and off-campus.

To my lab-mates, thank you for always being willing to help and support me during my PhD program. The experiences living abroad with many of you for bed rest studies were some of the most unique in my life.

To my fellow grad students, the roller coaster ride of a PhD program would not be the same without the bike rides, squash matches, and of course, beverages.

Lastly, to my family, thank-you for your steadfast support throughout my academic journey.

Table of Contents

Examining Committee Membership	ii
Author's Declaration.....	iv
Statement of Contributions	v
Abstract	vii
Acknowledgements.....	ix
List of Figures	xiv
List of Tables	xvi
List of Equations	xvii
List of Abbreviations	xviii
Chapter 1. Introduction	1
Overview of Cerebrovascular Control	2
Metabolic Control of Cerebral Blood Flow.....	3
Cerebral Autoregulation	4
Chemical Control of Cerebral Blood Flow.....	4
Neurogenic Control of Cerebral Blood Flow	5
Effect of Cardiac Output on Cerebral Blood Flow	6
Effect of Exercise on Cerebral Blood Flow	7
Effects of Orthostatic Stress on Cerebral Blood Flow	10
Measurement Techniques for Cerebral Blood Flow	13

Measurement Techniques for Systemic Hemodynamics	16
Measurement Techniques for Arterial PCO ₂	17
Thesis Rationale	18
Research Questions and Hypotheses.....	19
Chapter 2. Competing influences of arterial pressure and carbon dioxide on the dynamic cerebrovascular response to step transitions in exercise intensity	20
Abstract	20
Introduction	21
Methods	22
Participants	22
Data Collection	23
Data Analysis.....	25
Statistical Analysis	26
Results	27
Discussion	34
Chapter 3: Effects of dynamic changes in arterial pressure and carbon dioxide on middle cerebral artery blood velocity during moderate- and high-intensity interval exercise	42
Abstract	42
Introduction	44
Methods	46

Participants	46
Exercise Testing	47
Data Collection	48
Data Analysis.....	49
Statistical Analysis	51
Results	52
Discussion	63
Chapter 4: Sex differences between post-menopausal women and similar age men in response to orthostatic stress following two weeks of bed rest	71
Abstract	71
Introduction	72
Methods.....	74
Participants	74
Bed Rest and Exercise Countermeasures	76
Supine-to-Stand Test	77
Statistical Analysis	80
Results	80
Discussion	94
Chapter 5: Vertebral artery blood flow and respiratory interactions during graded lower-body negative pressure to pre-syncope	104

Abstract	104
Introduction	105
Methods	107
Participants	107
Experimental Protocol	107
Data Collection	109
Data Analysis	110
Statistical Analysis	112
Results	112
Discussion	124
Chapter 6: General Discussion.....	131
Summary of Findings	131
Significance.....	133
Limitations	136
Future Directions.....	137
Thesis Conclusion	138
References.....	140

List of Figures

Figure 1. Group mean moderate-intensity interval exercise responses	30
Figure 2. Comparison of group mean measured and predicted changes in mean middle cerebral artery blood velocity during exercise.....	31
Figure 3. Group mean solutions from autoregressive moving average analysis of moderate intensity exercise.....	32
Figure 4. Group mean on- and off-transient cerebrovascular responses to exercise	33
Figure 5. Group mean heart rate responses and schematic for the changes in work rate during the moderate- and high-intensity pseudorandom interval exercise protocols.....	56
Figure 6. Comparison of group mean measured and predicted changes in mean middle cerebral artery blood velocity using autoregressive moving-average analysis during moderate- and high-intensity interval exercise	57
Figure 7. Group mean moderate- and high-intensity interval exercise responses	58
Figure 8. Comparison of group mean measured and predicted changes in mean middle cerebral artery blood velocity for the first and second halves of high-intensity interval exercise	59
Figure 9. Group mean autoregressive moving average analysis solutions obtained from the first and second halves of the high-intensity interval exercise protocol	60
Figure 10. Group mean autoregressive moving average analysis solutions obtained from the first and second halves of the high-intensity interval exercise protocol, and the whole moderate-intensity interval exercise test.....	61
Figure 11. Group mean moderate- and high-intensity interval exercise responses highlighting the rapid increase in mean middle cerebral artery blood velocity at the onset of high-intensity intervals.....	62

Figure 12. Comparison of stand test tolerance times between men and women, with tests performed before and after bed rest	91
Figure 13. Mean cardiovascular and cerebrovascular time-series responses of men and women to the first 3 min of the supine-to-stand test.....	93
Figure 14. Individual time-series responses to progressive lower-body negative pressure.....	117
Figure 15. Individual vertebral artery diameter and mean velocity responses to progressive lower-body negative pressure	118
Figure 16. Repeated measures correlations between vertebral artery blood flow or middle cerebral artery blood velocity and the end-tidal partial pressure of carbon dioxide throughout the lower-body negative pressure protocol	119
Figure 17. Repeated measures correlations between vertebral artery blood flow and middle cerebral artery blood velocity	120
Figure 18. Individual time-series responses to lower-body negative pressure for resistance-area product and critical closing pressure.....	121
Figure 19. Individual plots of the minute ventilation–vertebral artery blood flow relationship during lower-body negative pressure.....	122
Figure 20. Relationship between the change in vertebral artery blood flow and the change in minute ventilation from before lower-body negative pressure to pre-syncope	123

List of Tables

Table 1. Summary of responses to cycling at 25 W and 90 % of the ventilatory threshold	29
Table 2. Comparison of the magnitude of rapid changes in mean arterial pressure and middle cerebral blood velocity at the onset of step increases in work rate following different recovery interval durations	55
Table 3. Pre-bed rest participant characteristics	75
Table 4. Summary of cardiovascular and respiratory variables while supine before and after 14 days of head-down bed rest.....	85
Table 5. Summary of initial orthostatic response to standing before and after 14 days of head-down bed rest	86
Table 6. Summary of magnitude of change in cardiovascular variables from supine to nadir during the initial orthostatic response to standing before and after 14 days of head-down bed rest	88
Table 7. Summary of cardiovascular and respiratory variables while standing before and after 14 days of head-down bed rest.....	89
Table 8. Summary of cardiovascular and respiratory responses at rest and pre-syncope.....	115
Table 9. Modified rebreathing test results	116

List of Equations

Eq. 1 Cerebral blood flow equation	2
Eq. 2 Poiseuille's Law	3
Eq. 3 Rational function	111

List of Abbreviations

$\Delta 50\%$	The work rate that corresponds with 50% of the difference between the ventilatory threshold and peak oxygen uptake
η	Viscosity
ARMA	Autoregressive moving-average
BMI	Body mass index
BSA	Body surface area
CO ₂	Carbon dioxide
CrCP	Critical closing pressure
CVRi	Cerebrovascular resistance index
DBP	Diastolic blood pressure
eP _a CO ₂	Estimated arterial partial pressure of carbon dioxide
f _b	Breathing frequency
HDBR	Head-down bed rest
L	Vessel length
MAP	Mean arterial pressure
MCA	Middle cerebral artery blood
MCAv	Middle cerebral artery blood velocity
P _a CO ₂	Arterial partial pressure of carbon dioxide
P _a O ₂	Arterial partial pressure of oxygen
PCA	Posterior cerebral artery
PCAv	Posterior cerebral artery velocity
P _{ET} CO ₂	End-tidal partial pressure of carbon dioxide
PP	Pulse pressure
$\dot{Q}$	Cardiac output
$\dot{Q}$	Cardiac index
$\dot{Q}_{VA}$	Vertebral artery blood flow
RAP	Resistance-area product
RER	Respiratory exchange ratio
r	Radius
SBP	Systolic blood pressure
SNA	Sympathetic nerve activity
SVi	Stroke volume index
TPR	Total peripheral resistance
TS	Test terminated due to symptoms by participant
VA	Vertebral artery
$\dot{V}_A$	Alveolar ventilation
$\dot{V}_{CO_2}$	Carbon dioxide output
$\dot{V}_E$	Minute ventilation
$\dot{V}_{O_2}$	Oxygen uptake
VRT	Ventilatory recruitment threshold
VT	Ventilatory threshold

Chapter 1. Introduction

The health and function of the human brain is extremely sensitive to changes in cerebral blood flow. Higher resting cerebral blood flow is associated with better cognition and memory, whereas lower flow contributes to cognitive decline and the development of vascular dementia (Wolters *et al.*, 2017; van Dinther *et al.*, 2024). Humans encounter many stimuli during daily life that alter cerebral blood flow, with two of the most common stimuli being physical activity/exercise, and orthostatic stress. Habitual aerobic exercise increases cerebral blood flow and is promoted as beneficial for cerebrovascular and brain function (Barnes, 2015; Calverley *et al.*, 2020; Bliss *et al.*, 2021), with increased blood flow during a single bout of aerobic exercise still providing an acute benefit to cognitive function (Tari *et al.*, 2020). Conversely, orthostatic stress induces reductions in cerebral blood flow, with large reductions while standing being linked to cognitive impairment (Fitzgibbon-Collins *et al.*, 2024), and potentially provoking syncope (Van Lieshout *et al.*, 2003). Importantly, the contribution of different cerebrovascular control mechanisms to the increase in cerebral blood flow with exercise (Ogoh & Ainslie, 2009; Smith & Ainslie, 2017) and the reduction in flow during orthostatic stress (Serrador *et al.*, 2006; Zuj *et al.*, 2013; Immink *et al.*, 2014) are debated. Therefore, the aim of this thesis is to identify which regulatory factors play prominent roles in modulating cerebral blood flow during and following transitions in exercise intensity or posture change.

The following review provides an overview of mechanisms of cerebrovascular regulation and their roles during exercise and orthostatic stress, while highlighting important knowledge gaps. Methods to measure cerebral blood flow, systemic hemodynamics, and arterial PCO₂ are also discussed, with emphasis on techniques that are useful for measuring dynamic

cerebrovascular responses. At the conclusion of this chapter, the rationale for this thesis, and associated research questions and hypotheses are presented.

Overview of Cerebrovascular Control

Adequate cerebral blood flow is essential for normal brain function, as it delivers oxygen and nutrients, and clears metabolic by-products. Despite comprising only ~2-3% of the total body mass, the brain requires ~15-20% of cardiac output ($\dot{Q}$) at rest (Williams & Leggett, 1989; Xing *et al.*, 2017) due to its high metabolic rate [~20% of an individual's resting oxygen uptake (Rolfe & Brown, 1997)] and limited substrate storage (Brown & Ransom, 2007). Blood flow to the brain is supplied simultaneously via the anterior and posterior vessels that connect in the Circle of Willis, with internal carotid arteries contributing ~72% to total cerebral blood flow and vertebral arteries contributing ~28% (Zarrinkoob *et al.*, 2015).

Cerebral blood flow is a tightly regulated variable, under the control of multiple integrated and redundant mechanisms acting together to maintain flow near its set point (Claassen *et al.*, 2021). Cerebral blood flow can be characterized by Ohm's Law, with dependencies on both cerebral perfusion pressure and resistance:

$$\text{Cerebral Blood Flow} = \frac{(\text{Mean Arterial Pressure} - \text{Critical Closing Pressure})}{\text{Cerebrovascular Resistance}} \quad \text{Eq. 1}$$

where critical closing pressure is the intravascular pressure at which small vessels collapse and flow ceases (Burton, 1951). In healthy individuals, the changes in cerebral perfusion pressure are largely dominated by changes in mean arterial pressure (MAP), but small changes in intracranial pressure, which affects critical closing pressure, do occur during posture transitions (D'Antona *et al.*, 2022). In contrast to the direct effect of MAP on cerebral blood flow, other regulatory factors

primarily exert their effects by altering cerebrovascular tone. Importantly, small changes in the radius of the resistance vessels have large effects on cerebrovascular resistance, and therefore blood flow, as described by Poiseuille's Law:

$$Resistance = \frac{8 \cdot \eta \cdot L}{\pi \cdot r^4} \quad Eq. 2$$

where η is blood viscosity, L is vessel length, and r is the vessel radius. These other factors that affect cerebral blood flow are the rate of cerebral metabolism, cerebral autoregulation, autonomic innervation of the cerebral vasculature, arterial blood gases, and cardiac output (Smith & Ainslie, 2017). Brief descriptions of each regulatory mechanism are provided below.

Metabolic Control of Cerebral Blood Flow

Metabolic control of cerebral blood flow refers to the tight neurovascular coupling between cortical activation and blood flow. Increased regional cerebral blood flow can be elicited in response to cognitive (Sorond *et al.*, 2011), sensory (Aaslid, 1987) or motor tasks (Gross *et al.*, 1980; Linkis *et al.*, 1995), with changes in cerebral blood flow occurring rapidly within seconds to increase flow to the areas of the brain with increased activity. This increase in flow is often much greater than the increase in energy consumption by the neurons (Lin *et al.*, 2010), suggesting that feedforward mechanisms largely contribute to the hyperemic response. Indeed, glutamate from synapses act on both the neurons and astrocytes, leading to release of nitric oxide from neurons, and prostanoids and potassium ions from astrocytes that dilate the cerebrovascular smooth muscle (Attwell *et al.*, 2010). Despite neurovascular coupling being primarily described as a feedforward mechanism to increase cerebral blood flow, vasoactive metabolic signals (i.e., adenosine and lactate) also contribute to the hyperemic response based on the level of O₂ availability (Gordon *et al.*, 2008), suggesting that neurovascular coupling may

rely on a combination of feedforward and feedback mechanisms to increase blood flow (Iadecola, 2017).

Cerebral Autoregulation

Cerebral autoregulation is a myogenic reflex mechanism by which the cerebral vasculature buffers the effect of changes in perfusion pressure on blood flow. This response is intrinsic to the vascular smooth muscle, where changes in transmural pressure elicit compensatory changes in vascular tone, leading to changes in resistance to maintain relatively constant flow (Claassen *et al.*, 2021). This phenomenon was classically characterized by Lassen's autoregulatory curve, where cerebral blood flow was quite stable between MAP of 50 mmHg and 150 mmHg when data from 376 patients with a range of blood pressures were pooled (Lassen, 1959). More recently, it was revealed that the cerebral vasculature is better at buffering the effect of hypertension compared to hypotension on cerebral blood flow (Tzeng *et al.*, 2010; Brassard *et al.*, 2017). The ability of autoregulation to buffer dynamic fluctuations in pressure and maintain flow is also dependent on the rate in which pressure changes, such that slow changes in pressure can be effectively counterbalanced by changes in cerebrovascular resistance, but autoregulation is progressively less effective with more rapid changes in pressure (Claassen *et al.*, 2021). The effectiveness of the myogenic mechanism is also dependent on blood gas tensions, with autoregulation being enhanced by hypocapnia and impaired by hypercapnia (Aaslid *et al.*, 1989; Edwards *et al.*, 2004).

Chemical Control of Cerebral Blood Flow

The cerebral vasculature is highly sensitive to changes in arterial blood gas tensions. Reductions in arterial partial pressure of CO₂ (P_aCO₂) induce cerebral vasoconstriction, whereas

increased $P_a\text{CO}_2$ causes cerebral dilation. The effect of $P_a\text{CO}_2$ on the cerebral vasculature is modulated via the pH of the extracellular fluid (Kontos *et al.*, 1977). The relationship between $P_a\text{CO}_2$ and cerebral blood flow is non-linear across the hypocapnic to hypercapnic range (Markwalder *et al.*, 1984), with hypercapnia increasing flow by ~3-6% per 1 mmHg of $P_a\text{CO}_2$ and hypocapnia decreasing flow ~1-3% per 1 mmHg of $P_a\text{CO}_2$ (Willie *et al.*, 2012). Changes in $P_a\text{CO}_2$ can induce changes in tone throughout the cerebrovascular tree, including in large extracranial arteries (Willie *et al.*, 2012); however, modest changes in $P_a\text{CO}_2$ appear to have minor effects on large artery diameter (Verbree *et al.*, 2014), suggesting an important role of smaller arterioles in modulating cerebrovascular resistance and flow in response to changes in $P_a\text{CO}_2$. Reductions in arterial partial pressure of oxygen ($P_a\text{O}_2$) also affect cerebrovascular tone, leading to dilation and increased flow once below 40-45 mmHg (Ainslie & Ogoh, 2010). Critically though, the effect of hypoxemia on cerebral blood flow is highly dependent on the prevailing $P_a\text{CO}_2$, as hypoxia also induces hyperventilation and hypocapnia, creating conflicting cerebrovascular stimuli (Ainslie & Poulin, 2004).

Neurogenic Control of Cerebral Blood Flow

Sympathetic, parasympathetic and sensory nerves provide a combination of intrinsic and extrinsic sources of innervation to the cerebral vasculature that can affect cerebrovascular tone (Claassen *et al.*, 2021). Isolating the contribution of the autonomic innervation to the cerebral vessels is particularly challenging, as often pharmacological interventions affect systemic cardiovascular control or other important regulators of cerebral blood flow. The strongest evidence supporting the neurogenic control of cerebral blood flow comes from experiments where the sympathetic ganglia that innervate the cerebral vasculature are excised, leading to increased cerebral blood flow (Hafkenschiel *et al.*, 1951; Shenkin *et al.*, 1951; Alborch *et al.*,

1977; Jeng *et al.*, 1999). Sympathetic activity primarily produces constriction in the larger compared to smaller pial vessels (Baumbach & Heistad, 1983). Sympathetically-mediated changes in cerebrovascular tone are important for attenuating blood flow responses associated with changes in arterial pressure (Zhang *et al.*, 2004), but sympathetic control appears to have a relatively minor role in the regulation of cerebral blood flow under normal conditions compared to other regulating factors (ter Laan *et al.*, 2013). Parasympathetic and trigeminal sensory nerves also innervate the cerebral vasculature, and release vasodilatory neurotransmitters (e.g., acetylcholine, or calcitonin gene-related peptide); however, very limited data about these nervous systems are currently available from humans (Willie *et al.*, 2014; Claassen *et al.*, 2021), making it difficult to conclude whether either system has a substantial impact on cerebrovascular regulation.

Effect of Cardiac Output on Cerebral Blood Flow

A correlation between acute changes in cardiac output and cerebral blood flow has been observed in healthy individuals (Meng *et al.*, 2015). This relationship was identified through manipulations of central venous volume and pressure via orthostatic stress or fluid loading at rest and during exercise to induce changes in cardiac output and mean middle cerebral blood velocity (MCAv) (Ogoh *et al.*, 2005a). Mechanistically, a direct, independent effect of cardiac output on cerebral blood flow is challenging to reconcile given that according to Ohm's Law, flow is dependent on the pressure gradient and resistance, not cardiac output. Additionally, it has been reported that there is no relationship between cardiac output and dynamic cerebral autoregulation in healthy adults (Deegan *et al.*, 2010). Accordingly, the precise mechanism underlying this relationship remains to be clearly described, but is suggested to be mediated via the sympathetic innervation of the cerebral vasculature (Meng *et al.*, 2015).

Importantly, cerebrovascular control does not operate in isolation, with many of its key determinants, such as MAP and $P_a\text{CO}_2$, being variables that are reflexively regulated by other organ systems. Respiration and cerebrovascular control are particularly entwined. The rate of cerebral blood flow affects the rate of CO_2 clearance from the brain and central pH, and as a result influences the central chemoreceptor stimulation, ventilation, and consequently, $P_a\text{CO}_2$ (Ainslie & Duffin, 2009). These complex interactions between cardiovascular, respiratory, and cerebrovascular systems can be manipulated by different stressors, including exercise (Ogoh & Ainslie, 2009; Smith & Ainslie, 2017) and orthostatic stress (Serrador *et al.*, 2006; Ogoh *et al.*, 2013; Zuj *et al.*, 2013).

Effect of Exercise on Cerebral Blood Flow

The effect of exercise on cerebral blood flow is intensity dependent, with increased cerebral blood flow observed during moderate exercise, but reduced flow relative to moderate exercise at maximal intensities (Smith & Ainslie, 2017). Multiple factors that affect cerebral blood flow simultaneously change during exercise, creating this intensity-dependent response: cardiac output and arterial blood pressure increase, $P_a\text{CO}_2$ changes, cortical activity increases, and the autonomic nervous system adapts. With all of these changes occurring concurrently, it is difficult to identify how each factor contributes to the overall integrative cerebral blood flow response (Smith & Ainslie, 2017), but new insights may be gained by focusing on cerebrovascular regulation during dynamic changes.

The factors that appear to predominately modulate the cerebrovascular response to incremental exercise are neurovascular coupling, $P_a\text{CO}_2$, and autoregulation. Activation of the

cortical areas associated with the physical activity increases regional cerebral blood flow (Gross *et al.*, 1980; Linkis *et al.*, 1995), while autoregulation buffers the effect of exercise-induced elevations in arterial pressure (Brys *et al.*, 2003). Increased $P_a\text{CO}_2$ during moderate-intensity exercise also contributes to the increased blood flow; however, hypocapnia is induced by hyperventilation at high intensities of exercises, preventing further increases or even reducing cerebral blood flow (Smith & Ainslie, 2017). The potent effect of $P_a\text{CO}_2$ on the global cerebral blood flow response is reinforced by the observation that when $P_a\text{CO}_2$ is clamped to prevent hypocapnia following the respiratory compensation point, MCAv continues to increase until exhaustion (Olin *et al.*, 2011). However, others have challenged the role of $P_a\text{CO}_2$ in during incremental exercise, as increased cerebral blood flow still occurred during isocapnic exercise (Smith *et al.*, 2016). The effects of cardiac output and sympathetic activity on cerebral blood flow during exercise are considerably less established.

Despite Ogoh *et al.* (Ogoh *et al.*, 2005a) identifying a linear relationship between cardiac output and MCAv at rest and during moderate exercise, recent investigations suggest that cardiac output has a negligible effect on MCAv responses to exercise (Smirl *et al.*, 2012; Saito *et al.*, 2022). Heart transplant recipients, who have an impaired ability to increase cardiac output, have the same cerebral hyperemic response as healthy control participants (Smirl *et al.*, 2012), and changes in MCAv and posterior cerebral artery velocity (PCAv) at the onset of exercise were not associated with the rapid changes in cardiac output (Saito *et al.*, 2022). Despite the dynamics of the cerebral blood velocity responses being clearly different from cardiac output at the onset of exercise, Saito *et al.* (Saito *et al.*, 2022) still concluded that the change in cardiac output from rest to end-of-exercise was modestly associated with the changes in MCAv and PCAv. The lack of association between dynamic responses, but association between rest-to-exercise changes,

may suggest that the steady-state relationship between cardiac output and cerebral blood velocity is more correlative, rather than causative during exercise.

The influence of sympathetic activity on the cerebrovascular response to exercise is also debated. Methodological challenges linked to the effectiveness and specificity of neurological blockades (Smith & Ainslie, 2017), as well as the possibility of sympathetic-mediated cerebrovascular responses being masked by other more dominant regulatory mechanisms, such as autoregulation or $P_a\text{CO}_2$ (Ogoh & Ainslie, 2009), makes identifying a distinct role of sympathetic activity on cerebrovascular control during exercise difficult. However, increased sympathetic activity was suggested as a potential mediator of exercise-induced increases in cerebrovascular tone (Ogoh *et al.*, 2010, 2011), which could be viewed as a protective mechanism to limit the effect of increased arterial pressure on the cerebral microcirculation (Cassaglia *et al.*, 2008). However, recent evidence suggests that submaximal dynamic exercise in healthy adults does not cause increased cerebral sympathetic nerve activity (Tymko *et al.*, 2024).

Recent works attempting to model the MCAv response to a step increase in exercise intensity suggested that changes in end-tidal partial pressure of CO_2 (P_{ETCO_2}) did not affect the dynamic cerebrovascular response (Billinger *et al.*, 2017; Witte *et al.*, 2019). This finding was surprising given that changes in $P_a\text{CO}_2$ commonly occur during exercise transitions (Casaburi *et al.*, 1977, 1980; Ward *et al.*, 1983) and that increased $P_a\text{CO}_2$ is a potent stimulus for cerebrovascular vasodilation (Aaslid *et al.*, 1989). Critically, within these works (Billinger *et al.*, 2017; Witte *et al.*, 2019), the potential influence of P_{ETCO_2} was evaluated using bivariate linear regression of changes in MCAv and P_{ETCO_2} from rest to the end of exercise, which does not account for the complex interactions that may occur in a time-dependent manner. Similar limitations also apply to the correlative analysis used to establish relationships between MCAv

and mean arterial pressure or $P_{ET}CO_2$ during high-intensity interval exercise (Whitaker *et al.*, 2022). Investigating mechanisms of cerebrovascular control during high-intensity interval exercise is particularly relevant, as it has been proposed as a training strategy to improve cerebrovascular function and cognition (Lucas *et al.*, 2015; Calverley *et al.*, 2020). Moving forward, more advanced time-series analysis approaches, such as autoregressive moving-average modelling that can account changes in multiple factors and response delays (Edwards *et al.*, 2003, 2004), are needed to disentangle the effects of multiple competing factors on cerebral blood flow that dynamically change during both moderate- and high-intensity exercise.

Effects of Orthostatic Stress on Cerebral Blood Flow

Orthostatic stress challenges the regulation of cerebral blood flow. Upon transitioning to upright posture, mean arterial pressure transiently decreases at the level of the heart, followed by a baroreflex-mediated recovery (Harms *et al.*, 2021). A hydrostatic gradient is also created between the heart and brain in the upright posture, which reduces cerebral perfusion pressure by ~20-30 mmHg relative to the supine posture (Harms *et al.*, 2021). Venous return to the heart is reduced, leading to reduced stroke volume, but it is compensated for with an increase in heart rate to maintain cardiac output (Harms *et al.*, 2021). Mild postural hypocapnia also occurs in upright posture compared to supine (Bjurstedt *et al.*, 1962; Cencetti *et al.*, 1997; van Lieshout *et al.*, 2001; Serrador *et al.*, 2006; Watenpaugh *et al.*, 2007), with hypocapnia attributed to changes in venous pooling and reduced cardiac output (Serrador *et al.*, 2006), as well as increased chemosensitivity while upright (Jordan *et al.*, 2000; Wang *et al.*, 2004). Importantly, in healthy individuals, mechanisms of cardiovascular, respiratory and cerebrovascular control dynamically compensate for these changes to maintain adequate cerebral perfusion. However, if one of the

mechanisms fails, cerebral blood flow rapidly declines and oxygen delivery to the brain is compromised, leading to symptoms of orthostatic intolerance and eventually syncope (Van Lieshout *et al.*, 2003).

The factors that contribute to the development of low cerebral blood flow during orthostatic stress and syncope have been debated. Systemic orthostatic hypotension and hemodynamic collapse (low peripheral vascular resistance and/or cardiac output) (Van Dijk & Wieling, 2013) are often implicated as the culprits causing reduced cerebral perfusion; however, cerebrovascular-specific responses to upright posture may also be important to consider (Grubb *et al.*, 1991; Levine *et al.*, 1994). Reduced $P_a\text{CO}_2$ and associated increases in cerebrovascular tone also induce reductions in cerebral blood flow independent of arterial pressure (Zuj *et al.*, 2013), and may precede the development of orthostatic intolerance. This is consistent with the observation that individuals who experience orthostatic intolerance have lower $P_{\text{ET}}\text{CO}_2$ and cerebral perfusion when upright than those who do not (Novak *et al.*, 1998). However, others argue against the role of $P_a\text{CO}_2$ in mediating changes of cerebral blood flow during orthostatic stress (Immink *et al.*, 2014). Importantly, simultaneous changes in MAP, cardiac output, and $P_{\text{ET}}\text{CO}_2$ during head-up tilt testing to pre-syncope all correlate with reductions in MCAv, making it challenging to identify clear roles of each physiological determinant of cerebral perfusion (Mastrandrea *et al.*, 2023). Due to the integrative nature of cerebrovascular control, it is also possible that reductions in cerebral blood flow attributed to one factor influences the action of other regulatory factors, leading to further adjustment in flow. For example, an orthostatically-induced reduction in vertebral artery blood flow may attenuate the clearance of CO_2 from the chemosensitive area of the brain, prompting an increase in ventilation via the central chemoreflex and a reduction in $P_a\text{CO}_2$ (Ogoh *et al.*, 2013). Accordingly, more work needs to be

done to interrogate the integrative physiological cascade leading to syncope, with a specific interest in understanding consequences of changes in vertebral blood flow.

When examining physiological responses to upright posture, it is also important to consider that cardiovascular and cerebrovascular regulation may differ between sexes and age groups. Younger women typically have lower orthostatic tolerance than younger men (Convertino, 1998; Fu *et al.*, 2004, 2005), which may be attributed to differences in autonomic control of blood pressure (Convertino, 1998), cardiac filling (Fu *et al.*, 2004, 2005), oxygen carrying capacity (Diaz-Canestro *et al.*, 2022), or total peripheral resistance responses (TPR) (Fritsch-Yelle *et al.*, 1996b; Waters *et al.*, 2002). In community dwelling older adults, no sex-based difference in orthostatic tolerance was observed (Romero-Ortuno *et al.*, 2010); however, older women were more likely to present to the emergency department due to syncopal events (Grossman *et al.*, 2005). Interestingly, cerebrovascular reactivity to CO₂ is reduced (Kastrup *et al.*, 1998; Matteis *et al.*, 1998) and autonomic control of blood pressure is altered (Hart *et al.*, 2011; Barnes *et al.*, 2014) following menopause, which may contribute to age-related and sex-specific differences in orthostatic tolerance.

Understanding how mechanisms of cerebrovascular control are altered following a period of bed rest or immobility is of clinical relevance, especially for older adults. Bed rest associated with illness or surgery increases the risk of experiencing orthostatic hypotension and reduced cerebral oxygenation (Jans *et al.*, 2012), putting individuals at higher risk for syncope and falls following hospitalization (Mahoney *et al.*, 2000). In younger adults, periods of bed rest are associated with impaired blood pressure regulation (Hughson *et al.*, 1994; Kamiya *et al.*, 2003; Arbeille *et al.*, 2008), hypovolemia (Levine *et al.*, 1997; Diedrich *et al.*, 2007), cardiac atrophy and stiffening (Levine *et al.*, 1997), and reduced MCAv during orthostatic stress (Zhang *et al.*,

1997), all contributing to markedly reduced orthostatic tolerance immediately following bed rest. The severity of these bed rest effects and their influence on orthostatic tolerance in late-middle-aged and older adults is relatively unknown; however, the incidence of syncope increases with advancing age (Soteriades *et al.*, 2002), suggesting that the risk of orthostatic intolerance associated with bed rest may be exacerbated by older age.

Measurement Techniques for Cerebral Blood Flow

Historically, changes in cerebral blood flow were first described by Angelo Mosso, where changes in brain volume of patients with skull defects were identified during cognitive tasks (Zago *et al.*, 2009). Since this seminal observation of neurovascular coupling, many investigations of cerebrovascular control have followed using either invasive or non-invasive methods, with strengths and weaknesses associated with each method. Invasive tracer infusion methods that rely on the Fick principle (i.e., differences in concentration of a tracer in concurrent arterial and jugular venous samples) were first used to quantify global cerebral blood flow (Kety & Schmidt, 1945, 1948). Invasive tracer techniques can be used for quantitative cerebral blood flow measures during exercise (Thomas *et al.*, 1989; Nybo *et al.*, 2003), but they have very limited temporal resolution and require steady-state conditions during measurement. Similarly, magnetic resonance imaging provides excellent reliability of measures of cerebral blood flow as a non-invasive technique, but it also has poor temporal resolution and is not implementable during dynamic posture transitions or intense whole-body exercise due to movement (Tymko *et al.*, 2018). Conversely, non-invasive ultrasound-based measurement techniques to measure blood flow through extracranial arteries (e.g., vertebral artery or internal carotid artery) or blood velocity as a surrogate index of flow through intracranial arteries (e.g., MCA or PCA) have high

temporal resolution, making ultrasound the most feasible option for studying dynamic changes in cerebral blood flow (Tymko *et al.*, 2018).

Transcranial Doppler ultrasound has been widely used for decades to investigate cerebrovascular responses at rest (Aaslid *et al.*, 1982, 1989; Aaslid, 1987), as well as during exercise (Jorgensen *et al.*, 1992b, 1992a) and orthostatic stress (Zhang *et al.*, 1997). This measurement technique is limited by the fact that the cross-sectional area of the insonated artery is unknown, and only provides an index of flow. Importantly, the cross-sectional area of large intracranial arteries is relatively stable, but they do have measurable changes in response to large changes in P_aCO_2 (Coverdale *et al.*, 2014; Verbree *et al.*, 2014) or MAP (Giller *et al.*, 1993). Only one investigation has directly studied the effect of MAP on MCA diameter during craniotomy, which found less than 4 % change in diameter for a 30 mmHg change in MAP (Giller *et al.*, 1993). Without accounting for changes in vessel cross-sectional area, changes in cerebral blood velocity can underestimate changes in blood flow, with this effect being greater during hypercapnia (18% difference between flow and velocity for a 9 mmHg increase in $P_{ET}CO_2$) compared to hypocapnia (7% difference between flow and velocity for 13 mmHg decrease in $P_{ET}CO_2$) (Coverdale *et al.*, 2014). Critically, smaller magnitude changes in $P_{ET}CO_2$ than those associated with substantial changes in MCA cross-sectional area (Verbree *et al.*, 2014) are typically observed during orthostatic stress (Serrador *et al.*, 2006; Zuj *et al.*, 2013), moderate- (Casaburi *et al.*, 1977, 1980; Ward *et al.*, 1983) or high-intensity exercise (Hedge *et al.*, 2022a), or high-intensity interval exercise (Whitaker *et al.*, 2022). Nevertheless, it remains unknown whether large intracranial artery diameter changes substantially during posture transitions or vigorous whole-body exercise. Small reductions in MCA cross-sectional area (~2%) without a substantial change in $P_{ET}CO_2$ have been observed during isometric handgrip

exercise, suggesting that exercise can induce sympathetic-mediated vasoconstriction of large intracranial arteries (Verbree *et al.*, 2017), but the small magnitude of effect likely does not substantially alter the interpretation of large changes in MCAv.

The limitations of transcranial Doppler measures of blood velocity can be addressed by duplex ultrasound, where blood velocity and vessel diameter can be measured simultaneously to calculate volumetric blood flow. Duplex ultrasound has been widely applied to extracranial vessels (Thomas *et al.*, 2015), and more recently to the MCA (Jarrett *et al.*, 2020); however, this technique is limited by the use of handheld sonography (i.e., no stabilization equipment, but needs to maintain consistent angle of insonation), and is not feasible for continuous collections during rapid posture changes (i.e., cannot hold probe in place while participants transition from supine to standing positions) nor for prolonged periods of time during upright exercise (e.g., 20-30 min of continuous data collection while cycling). Accordingly, in the context of this thesis, where investigations required continuous information about cerebral blood flow during rapid changes in posture, or high-quality signals without gaps during prolonged periods of exercise to facilitate time-series modeling, transcranial Doppler ultrasound of the MCA was used to measure MCAv as an index of flow. Duplex measures of vertebral blood flow were also collected when experimentally feasible, such as during graded simulated orthostatic stress with lower-body negative pressure (LBNP), where participant motion was minimal and the hand of a highly-trained sonographer could be stabilized for prolonged periods of continuous data collection.

Measurement Techniques for Systemic Hemodynamics

Measurement of continuous arterial blood pressure is required for studying cerebrovascular control during dynamic transitions in exercise intensity or posture. Non-invasive, continuous blood pressure monitoring is possible via the volume-clamp method (Penaz, 1973), where an inflatable cuff is wrapped around a finger. The pressure inside the cuff is dynamically regulated in order to match intra-arterial pressure to unload the finger arteries. Finger pressure waveforms can be filtered and calibrated to continuously estimate brachial blood pressures (Bos *et al.*, 1996), with strong agreement with invasive measures of brachial pressure (Guelen *et al.*, 2008).

Stroke volume and $\dot{Q}$ can also be estimated from arterial pressure waveforms using the ModelFlow® algorithm (Wesseling *et al.*, 1993), which is a nonlinear, time-varying, three-element model that characterizes the aortic flow-pressure relationship. The three elements of ModelFlow® include: aortic characteristic impedance, arterial compliance, and systemic vascular resistance (Wesseling *et al.*, 1993). ModelFlow® can provide accurate estimates of changes in stroke volume and $\dot{Q}$ from non-invasive blood pressure waveforms during both exercise (Faisal *et al.*, 2009) and orthostatic stress (Harms *et al.*, 1999) making it an attractive option for non-invasive investigations of systemic hemodynamics. However, ModelFlow® does underestimate changes in stroke volume when arterial pressure and total peripheral resistance rapidly fall, such as during the initial dynamic phase of posture transitions (Gibbons *et al.*, 2019). Changes in stroke volume and $\dot{Q}$ can also be measured non-invasively via Doppler ultrasound, with strong agreement being reported between ultrasound and direct, invasive measures (e.g., thermodilution and Fick method) (Huntsman *et al.*, 1983; Ihlen *et al.*, 1984). Limitations of using Doppler ultrasound from the suprasternal notch to measure stroke volume include not knowing

the angle of insonation, and that the angle may change during interventions, such as LBNP, if the heart's orientation changes relative to the probe. Due to challenges associated with motion during rapid posture transitions and upright cycling, pulse contour analysis of the arterial pressure waveform by the ModelFlow® algorithm was selected over Doppler ultrasound to estimate stroke volume and $\dot{Q}$ during the exercise studies and supine-to-stand tests within this thesis; however, ultrasound was used to measure stroke volume and $\dot{Q}$ during the graded LBNP test.

Measurement Techniques for Arterial PCO₂

Measurement of P_aCO₂, or an estimate of it, is essential in the investigation of cerebrovascular control. P_aCO₂ can be directly measured from arterial blood samples or can be non-invasively estimated by capnography (Huttmann *et al.*, 2014), with P_{ET}CO₂ providing an index of P_aCO₂ at rest for a given posture (Bjurstedt *et al.*, 1962; Williams & Babb, 1997). P_{ET}CO₂ does slightly underestimate P_aCO₂ while supine at rest (Bjurstedt *et al.*, 1962). The P_{ET}CO₂-P_aCO₂ gradient increases from supine to upright posture due to increased physiological deadspace and increased alveolar ventilation/ $\dot{Q}$ ratio (Riley *et al.*, 1959; Bjurstedt *et al.*, 1962), leading to an overestimation of the degree of postural hypocapnia (Immink *et al.*, 2006). Accordingly, when using P_{ET}CO₂ as an index of P_aCO₂, it is important that comparisons are only made within a given posture.

Exercise also affects the P_{ET}CO₂-P_aCO₂ gradient, with changes in P_{ET}CO₂ during exercise overestimating the changes in P_aCO₂ (Jones *et al.*, 1979; Whipp *et al.*, 1989; Williams & Babb, 1997). P_{ET}CO₂ reflects peak alveolar PCO₂, whereas P_aCO₂ more closely matches the mean

alveolar PCO_2 over a respiratory cycle (Whipp *et al.*, 1989). Accordingly, calculating cerebrovascular reactivity to CO_2 during exercise with P_{ETCO_2} may lead to erroneous estimates of reactivity. Critically, a regression equation was developed by Jones *et al.* (Jones *et al.*, 1979) to correct for the overestimation of P_aCO_2 by P_{ETCO_2} during exercise. This empirical equation, which uses P_{ETCO_2} and tidal volume to estimate P_aCO_2 , has shown strong agreement with directly measured P_aCO_2 through levels of submaximal exercise, but does slightly underestimate P_aCO_2 at maximal exercise (Jones *et al.*, 1979; Williams & Babb, 1997).

For this thesis, continuous information about P_aCO_2 was required for dynamic modelling experiments of cerebrovascular control during exercise and serial measurements were needed during orthostatic stress experiments. In order to minimize participant burden, non-invasive estimates of P_aCO_2 were obtained using the Jones equation during exercise (Jones *et al.*, 1979), and P_{ETCO_2} was used as an index of P_aCO_2 for experiments at rest.

Thesis Rationale

As outlined in the preceding literature review, the contributions of different mechanisms of cerebrovascular control to changes in cerebral blood flow during exercise or orthostatic stress are difficult to isolate. Therefore, the purpose of this thesis is to leverage interventions, such as transitions in exercise intensity, bed rest, and graded LBNP, to identify which factors play prominent roles in cerebrovascular regulation in different situations, and determine how the different factors may interact. The following experiments will enhance our fundamental understanding of cerebrovascular control during exercise and the integrative cerebrovascular cascade leading to syncope.

Research Questions and Hypotheses

- 1) What factors mediate the dynamic MCAv response to step changes in exercise intensity?

H: $P_a\text{CO}_2$ is the primary factor driving the dynamic changes in MCAv during transitions in exercise intensity.

- 2) Are the mechanisms regulating MCAv dynamics during exercise affected by the intensity of exercise being performed?

H: $P_a\text{CO}_2$ is the primary regulator of MCAv during both moderate- and high-intensity exercise, and cerebral autoregulation will remain intact.

- 3) Are reductions in MCAv while standing following bed rest mediated by different factors in post-menopausal women and age-matched men?

H: Yes. Sex-based differences in arterial pressure regulation, cerebrovascular reactivity, and orthostatic tolerance exist between post-menopausal women and age-matched men, likely leading to unique sex-based responses to orthostatic stress following bed rest.

- 4) Does orthostatic stress decrease vertebral blood flow, and does that influence respiratory control?

H: Orthostatic stress will reduce vertebral blood flow, which will stimulate the central chemoreceptor via reduced CO_2 clearance, prompting a compensatory increase in alveolar ventilation causing a reduction in $P_{\text{ET}}\text{CO}_2$.

To address these research questions, I conducted 4 studies that are described in chapters 2-5.

Chapter 2. Competing influences of arterial pressure and carbon dioxide on the dynamic cerebrovascular response to step transitions in exercise intensity

Abstract

Recent investigations of middle cerebral artery blood velocity (MCAv) kinetics at the onset of exercise have not accounted for potential dynamic changes in arterial partial pressure of carbon dioxide ($P_a\text{CO}_2$) during the transient phase of exercise transitions when modeling MCAv kinetics, despite $P_a\text{CO}_2$ having known effects on cerebrovascular tone. The purpose of our study was to determine the independent effects of mean arterial pressure (MAP) and estimated $P_a\text{CO}_2$ ($eP_a\text{CO}_2$) on mean MCAv during repeated exercise transitions. We hypothesized that cerebral autoregulation would minimize the effects of exercise-induced changes in MAP on mean MCAv, and that dynamic changes in $eP_a\text{CO}_2$ would contribute to changes in mean MCAv. Eighteen young healthy adults (7 women, age: 28 ± 5 yr) performed three exercise transitions from 25 W to 90% of the ventilatory threshold in sequence with 5 min stages. Autoregressive moving-average analysis isolated the independent effects of dynamic changes in MAP_{MCA} and $eP_a\text{CO}_2$ on MCAv. Mean MCAv increased ($p < 0.001$) from 25 W ($60.8 \pm 12.9 \text{ cm} \cdot \text{s}^{-1}$) to 90% of ventilatory threshold ($67.4 \pm 13.7 \text{ cm} \cdot \text{s}^{-1}$). MAP_{MCA} ($\Delta = 9 \pm 6 \text{ mmHg}$, $p < 0.001$) and $eP_a\text{CO}_2$ ($\Delta = 2.8 \pm 1.3 \text{ mmHg}$, $p < 0.001$) also increased with exercise intensity. The ARMA MAP step response stabilized at $0.12 \pm 0.14 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$, whereas the $eP_a\text{CO}_2$ step response plateaued at $1.98 \pm 0.85 \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}$. These data suggest that dynamic changes in $eP_a\text{CO}_2$, and not MAP, drive the MCAv response during transitions in exercise intensity.

Introduction

Regulation of cerebral blood flow is complex, with arterial blood gases, central hemodynamics, neurogenic activity, cerebral metabolism, and autoregulation all acting in an integrated fashion to facilitate optimal delivery of oxygen and nutrients to the brain while removing waste products (Claassen *et al.*, 2021). Whole-body exercise is a strong stimulus that affects many of the factors that influence cerebral blood flow: arterial pressure increases, arterial partial pressure of carbon dioxide ($P_a\text{CO}_2$) changes based on exercise intensity, and cerebral metabolism is elevated (Ogoh & Ainslie, 2009). Middle cerebral artery blood velocity (MCAv) response kinetics to a step increase in exercise intensity has recently been proposed as a tool to investigate differences in cerebrovascular function and health (Billinger *et al.*, 2017, 2021; Ward *et al.*, 2018; Kaufman *et al.*, 2019; Kempf *et al.*, 2019; Witte *et al.*, 2019). At the onset of transitions to higher intensities of exercise, increases in $P_a\text{CO}_2$ are commonly observed due to the slightly slower dynamics of minute ventilation compared to carbon dioxide output (Casaburi *et al.*, 1977, 1980; Ward *et al.*, 1983). Given that $P_a\text{CO}_2$ is a potent regulator of cerebrovascular tone (Aaslid *et al.*, 1989), dynamic changes in $P_a\text{CO}_2$ at the onset of exercise transitions may be contributing to the observed MCAv response kinetics.

The potential influence of end-tidal PCO_2 (P_{ETCO_2}) on MCAv dynamics in response to a step increase in exercise intensity was previously evaluated using bivariate linear correlation of the calculated changes from rest to the end of exercise, which suggested that the change in P_{ETCO_2} was not associated with any of the exponential model parameters used to characterize MCAv kinetics (Billinger *et al.*, 2017; Witte *et al.*, 2019). To gain insight into the simultaneous contributions of different regulatory mechanisms to the increase in MCAv during transitions in exercise intensity, a causal, multi-input time-series analysis method such as autoregressive

moving average (ARMA) analysis could be used. ARMA analysis has previously been used to characterize the independent effects of arterial pressure and $P_{ET}CO_2$ on cerebrovascular control at rest and during hyper- and hypocapnia (Panerai *et al.*, 2000; Edwards *et al.*, 2003, 2004).

Therefore, the purpose of this study was to investigate the independent effect of arterial pressure and estimated P_aCO_2 (eP_aCO_2) on mean MCAv during dynamic transitions in exercise intensity. It was hypothesized that intact cerebral autoregulation would minimize the effects of exercise-induced changes in arterial pressure on mean MCAv during exercise, and that dynamic changes in eP_aCO_2 would contribute to dynamic changes in mean MCAv.

Methods

Participants

Twenty young healthy adults volunteered to participate in the study. The final analyzed sample only included 18 participants [11 men and 7 women, age: 28 ± 5 yr; height: 1.73 ± 0.06 m; mass: 73.7 ± 10.9 kg; peak oxygen uptake ($\dot{V}O_2$): 43.6 ± 8.7 mL $\cdot$ min $^{-1}\cdot$ kg $^{-1}$; mean $\pm$ SD] as a result of poor data quality during interval exercise for two participants. We calculated a minimum required sample size of 7 participants ($\alpha = 0.05$, power = 0.80) to observe exercise-related changes in cerebrovascular control based on previously reported large effect sizes for the effect of exercise intensity on cerebrovascular tone (Ogoh *et al.*, 2011). However, we recruited 20 participants in order to account for potential data loss and demonstrate the applicability of autoregressive moving-average (ARMA) modeling to analyze dynamic cerebrovascular responses to exercise in a larger sample of participants.

Participants had no known cardiovascular or respiratory conditions, and none was taking medications that could influence the cardiovascular response to exercise. Participants were instructed to arrive to the laboratory for testing at least two hours post-prandial, abstain from alcohol consumption and vigorous exercise in the 24 hours preceding each test, and avoid caffeine consumption on the day of the tests. The study was conducted according to the Declaration of Helsinki, except for registration in a trial database, and all procedures were approved by University of Waterloo's Clinical Research Ethics Committee (ORE #41551). All participants signed an informed consent before participating in the study and were made aware of their right to withdraw at any time.

Data Collection

Participants visited the laboratory on two occasions at the same time of day to perform ramp-incremental and moderate-intensity interval exercise tests, with the two sessions separated by either 24 or 48 hr. All tests were performed in an environmentally controlled laboratory on an electrically braked cycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, Netherlands), with participants cycling at a cadence of 60 revolutions per minute.

During the first visit, participants performed a ramp-incremental exercise test. Participants cycled at 25 W for 4 min, followed by a linear increase in work rate of $25 \text{ W} \cdot \text{min}^{-1}$ until exercise intolerance. The test was terminated when the cadence dropped below 55 revolutions per minute despite strong verbal encouragement. Throughout testing, breath-by-breath gas exchange and ventilation were measured using a portable metabolic system (MetaMax 3B-R2, CORTEX Biophysik, Leipzig, Germany), with participants breathing through a mask

(7450 SeriesV2™ Mask, Hans Rudolph, Inc., Shawnee, USA). The system's flow sensor was calibrated using a 3-L syringe, and O₂ and CO₂ sensors were calibrated with precision analyzed gas mixtures prior to each test. Heart rate was also recorded during exercise (Polar H7, Polar Electro Oy, Kempele, Finland). The results from the ramp-incremental test were used to estimate each participant's ventilatory threshold (VT), peak $\dot{V}O_2$, and the work rate used during the participant's second visit.

During the second visit, participants cycled at 25 W for 11 min, immediately followed by 30 min of low-to-moderate-intensity interval cycling where the work rate alternated between 25 W and the work rate associated with 90% of the $\dot{V}O_2$ at VT, with each stage lasting for 5 min. Breath-by-breath gas exchange and ventilation data were time synchronized with lead II electrocardiography (ECG module, Finapres Medical Systems, Enschede, Netherlands), MCAv, and continuous arterial blood pressure, which were recorded at 1000 Hz (PowerLab and Labchart, version 7.3.7; AD Instruments, Colorado Springs, USA). MCAv data were collected using a 2 MHz transcranial Doppler ultrasound probe (WAKIe 2TC, Atys Medical, Soucieu-en-Jarrest, France), which was held in place over the right temporal insonation window using a headset (Spencer Technologies, Seattle, USA). Finger arterial blood pressure was measured by the volume-clamp method (Finapres NOVA, Finapres Medical Systems, Enschede, Netherlands), and values were reconstructed to brachial pressure, height corrected to the level of the heart, and calibrated using two brachial pressure measures obtained with an arm cuff while seated at rest. Throughout exercise, the arm and hand used to measure blood pressure rested on an elevated platform at the level of the heart to prevent measurement artifacts. Stroke volume was estimated for each cardiac cycle via pulse contour analysis of the finger arterial waveform using the Modelflow® algorithm.

Data Analysis

Ramp-incremental exercise response. Peak $\dot{V}O_2$ was calculated as the highest $\dot{V}O_2$ computed from a 20-s moving average during the ramp exercise test. $\dot{V}O_2$ at VT was estimated by visual inspection using standard ventilatory and gas exchange indices (and their ratios), as previously described (Beaver *et al.*, 1986). The work rate associated with 90% of the $\dot{V}O_2$ at VT was estimated after left-shifting the $\dot{V}O_2$ response by the mean response time, which was calculated using segmented regression (Hughson & Inman, 1986).

Interval exercise responses. The electrocardiogram R-spikes were used to gate beat-by-beat analysis of heart rate, arterial pressure, stroke volume, and MCAv. Systolic, diastolic, and mean values for the brachial pressure waveform and outer envelope of the transcranial Doppler spectrum were calculated for each cardiac cycle. Arterial pressure at the level of the MCA was subsequently estimated after accounting for hydrostatic pressure gradient between the level of the transcranial Doppler ultrasound probe and the heart when cycling in the upright position [correction factor = distance (in cm) $\cdot$ 0.78 mmHg $\cdot$ cm⁻¹]. Resistance-area product (RAP) and critical closing pressure (CrCP) were calculated beat-by-beat using the two-point mean/diastolic method (Panerai *et al.*, 2011). Estimated cardiac output ($\dot{Q}$) was calculated as the product of heart rate and stroke volume. Total peripheral resistance (TPR) was also calculated as the quotient of brachial MAP and $\dot{Q}$. eP_aCO_2 was estimated from $P_{ET}CO_2$ and tidal volume for each breath using the equation developed under exercise conditions by Jones *et al.* (Jones *et al.*, 1979).

Beat-by-beat and breath-by-breath data were linearly interpolated to 1 Hz (MATLAB R2020b, The MathWorks Inc., Natick, USA). MAP at the level of the MCA (MAP_{MCA}) and

ePaCO₂ throughout the entire 30 min of exercise were used as inputs for an ARMA modelling procedure to predict mean MCAv, as previously described (Edwards *et al.*, 2003, 2004). Briefly, ARMA models are linear, time-invariant systems that account for multiple inputs and delays when determining input-output relationships, with model parameters estimated following an autoregressive parameter reduction algorithm (Perrott & Cohen, 1996). The model orders for each individual was selected based on: i) minimum description length criterion, ii) minimum error terms associated with each input, and iii) minimal residuals with a Gaussian distribution. The model parameters for each participant were used to generate simulated mean MCAv responses to a 1 mmHg step increase in MAP_{MCA} or ePaCO₂ in order to determine how MCAv dynamically responds to each input independently. The steady-state changes in mean MCAv were calculated for both MAP_{MCA} ePaCO₂ step responses (i.e., $\Delta \text{mean MCAv} / \Delta \text{MAP}_{\text{MCA}}$ or $\Delta \text{mean MCAv} / \Delta \text{ePaCO}_2$). Cross-correlation functions between MAP_{MCA} or ePaCO₂ and mean MCAv were also calculated to identify the lag at the peak of the function. The three on-transient transitions (i.e., 25W to 90% of VT) were also ensemble averaged, and means were calculated during the last minute of cycling at each work rate.

Statistical Analysis

Statistical analyses were conducted in R (v. 4.0.3). Paired t-tests were used to compare variables between 25 W and 90% of VT. Statistical significance was set to $p \leq 0.05$, and data are reported as means $\pm$ standard deviation.

Results

Ramp-incremental exercise responses. Group mean peak $\dot{V}O_2$ was $3.24 \pm 0.96 \text{ L}\cdot\text{min}^{-1}$, which corresponded with a mean peak work rate of $295 \pm 80 \text{ W}$. The mean $\dot{V}O_2$ at VT was $1.83 \pm 0.44 \text{ L}\cdot\text{min}^{-1}$, and occurred at a work rate of $129 \pm 35 \text{ W}$ after accounting for a mean response time of $49 \pm 12 \text{ s}$. The work rate estimated to correspond to 90% of the VT $\dot{V}O_2$ was $112 \pm 31 \text{ W}$.

Interval exercise responses. Group mean time-series responses for MAP_{MCA} , eP_aCO_2 , and mean MCAv throughout the 30 min of interval exercise are presented in Figure 1. Mean MCAv ($\Delta = 6.5 \pm 3.1 \text{ cm}\cdot\text{s}^{-1}$, $p < 0.001$), MAP_{MCA} ($\Delta = 9 \pm 6 \text{ mmHg}$, $p < 0.001$), $\dot{Q}$ ($\Delta = 5.23 \pm 1.35 \text{ L}\cdot\text{min}^{-1}$, $p < 0.001$), and eP_aCO_2 ($\Delta = 2.8 \pm 1.3 \text{ mmHg}$, $p < 0.001$) all increased from 25 W to 90% of VT, whereas TPR was decreased ($\Delta = -3.56 \pm 1.13 \text{ mmHg}\cdot\text{min}\cdot\text{L}^{-1}$, $p < 0.001$; Table 1). The ability of ARMA models to predict dynamic changes in mean MCAv during exercise with MAP_{MCA} and eP_aCO_2 as the only inputs is clearly demonstrated by the small residuals in Figure 2. The ARMA model solutions used to determine how mean MCAv responds to a step increase in MAP_{MCA} or eP_aCO_2 are shown in Figure 3. A sustained 1 mmHg increase in MAP_{MCA} resulted in a rapid increase in mean MCAv , but mean MCAv returned close to baseline levels in $\sim 7\text{s}$ of increasing MAP_{MCA} , indicating intact cerebral autoregulation. The MAP_{MCA} step response stabilized at $0.12 \pm 0.14 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$. Conversely, the 1 mmHg step increase in eP_aCO_2 resulted in a step response with a steady-state of $1.98 \pm 0.85 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$. The temporal characteristics of the ARMA solutions (i.e., an immediate spike in MCAv due to an increase in MAP_{MCA} followed by an autoregulatory reduction, or the delayed rise in MCAv following an increase in eP_aCO_2) were supported by the lags at the peak of the cross-correlation functions between MAP_{MCA} ($-1 \pm 1 \text{ s}$) or eP_aCO_2 ($7 \pm 1 \text{ s}$) and mean MCAv . After accounting for

differences in baseline mean MCAv while cycling at 25 W, these ARMA solutions suggest a 3.20 ± 1.07 % increase in mean MCAv per unit increase in eP_aCO_2 . In order to highlight the differences in the time-courses of the different regulators of MCAv and cerebrovascular tone during dynamic transitions in exercise intensity, ensemble averaged on- and off-transient group mean responses are depicted in Figure 4. The effect of eP_aCO_2 versus MAP_{MCA} on mean MCAv was especially evident during the off-transient, as the rapid reduction in MAP_{MCA} was dissociated from the MCAv response, which instead followed the decrease in eP_aCO_2 . The effect of the rapid reduction in MAP on MCAv during the off-transient was largely buffered by rapid changes in cerebrovascular tone and resistance (i.e., CrCP and RAP).

Table 1. Summary of responses to cycling at 25 W and 90 % of the ventilatory threshold.

Variable	25 W	90% of VT	p-value
$\dot{V}O_2$ (L·min ⁻¹)	0.76 ± 0.10	1.66 ± 0.39	<0.001
$\dot{V}CO_2$ (L·min ⁻¹)	0.66 ± 0.09	1.52 ± 0.30	<0.001
$\dot{V}_E$ (L·min ⁻¹)	21.6 ± 2.6	44.2 ± 8.0	<0.001
$\dot{V}_E/\dot{V}O_2$	24.5 ± 1.5	24.6 ± 2.3	0.777
$\dot{V}_E/\dot{V}CO_2$	28.0 ± 1.8	26.7 ± 2.2	<0.001
RER	0.87 ± 0.03	0.92 ± 0.04	<0.001
ePaCO ₂ (mmHg)	38.8 ± 2.3	41.6 ± 2.9	<0.001
Heart Rate (beats·min ⁻¹)	93 ± 10	128 ± 11	<0.001
Stroke Volume (mL)	101 ± 18	115 ± 21	<0.001
$\dot{Q}$ (L·min ⁻¹)	9.3 ± 1.6	14.6 ± 2.1	<0.001
TPR (mmHg·min·L ⁻¹)	11.57 ± 1.61	8.01 ± 1.30	<0.001
MAP _{Brachial} (mmHg)	105 ± 10	114 ± 10	<0.001
SBP _{Brachial} (mmHg)	140 ± 14	155 ± 13	<0.001
DBP _{Brachial} (mmHg)	82 ± 8	87 ± 9	0.002
PP _{Brachial} (mmHg)	58 ± 11	69 ± 12	<0.001
MAP _{MCA} (mmHg)	75 ± 10	84 ± 10	<0.001
SBP _{MCA} (mmHg)	110 ± 13	125 ± 13	<0.001
DBP _{MCA} (mmHg)	52 ± 8	56 ± 10	0.002
Mean MCAv (cm·s ⁻¹)	60.8 ± 12.9	67.4 ± 13.7	<0.001
Systolic MCAv (cm·s ⁻¹)	103.1 ± 18.0	124.7 ± 18.8	<0.001
Diastolic MCAv (cm·s ⁻¹)	37.5 ± 9.0	37.9 ± 9.6	0.390
RAP (mmHg·s·cm ⁻¹)	1.05 ± 0.26	0.97 ± 0.23	0.004
CrCP (mmHg)	14 ± 11	21 ± 11	<0.001

Data are mean ± SD. $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, carbon dioxide output; $\dot{V}_E$, expired minute ventilation; RER, respiratory exchange ratio; ePaCO₂, estimated arterial partial pressure of carbon dioxide; $\dot{Q}$, cardiac output; TPR, total peripheral resistance; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MCAv, middle cerebral blood velocity; RAP, resistance-area product; CrCP, critical closing pressure.

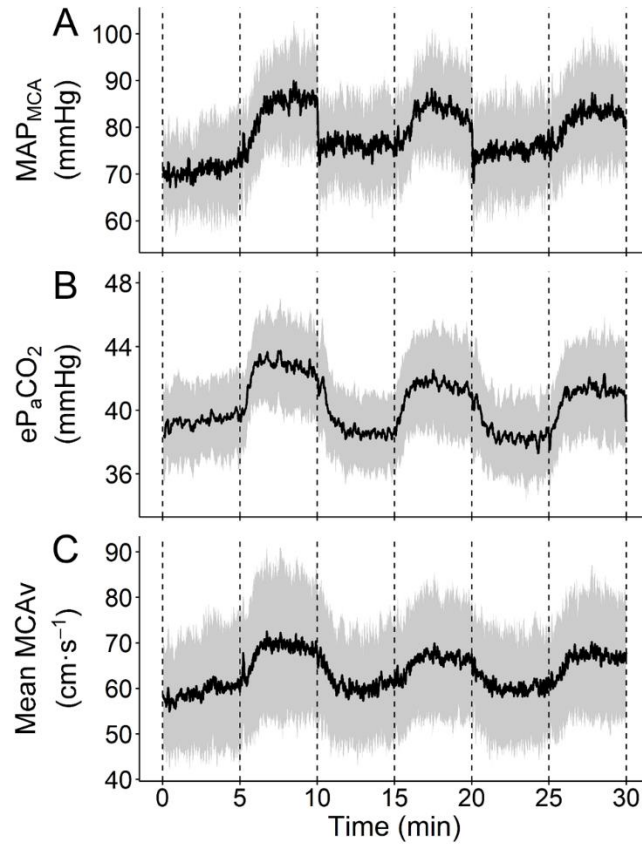


Figure 1. Group mean ($n = 18$) moderate-intensity interval exercise responses for mean arterial pressure corrected to the level of the middle cerebral artery (MAP_{MCA} ; A), estimated arterial partial pressure of carbon dioxide (eP_aCO_2 ; B), and mean middle cerebral artery blood velocity (MCAV; C). The solid lines represent the means and the shaded areas represent ± 1 standard deviation. The vertical dashed lines represent the start or end of an exercise interval.

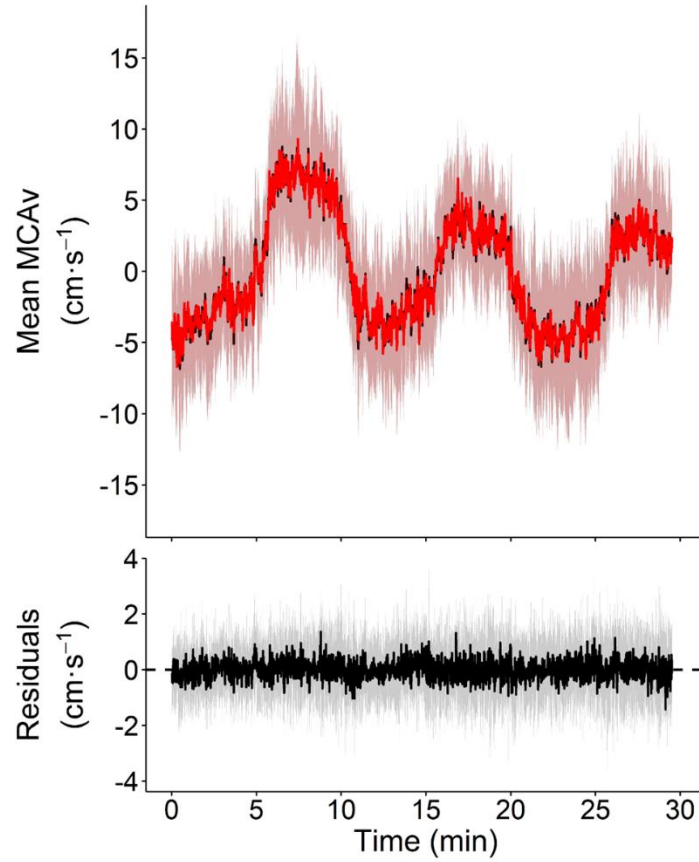


Figure 2. Comparison of group mean ($n = 18$) directly measured (black) and predicted (red) changes in mean middle cerebral artery blood velocity (MCAv) during exercise, with calculated residuals shown below. The measured and modeled mean MCAv responses oscillate around 0 $\text{cm}\cdot\text{s}^{-1}$, as the measured mean MCAv and inputs to predict MCAv were linearly detrended during analysis. The solid lines represent the means and the shaded areas represent ± 1 standard deviation.

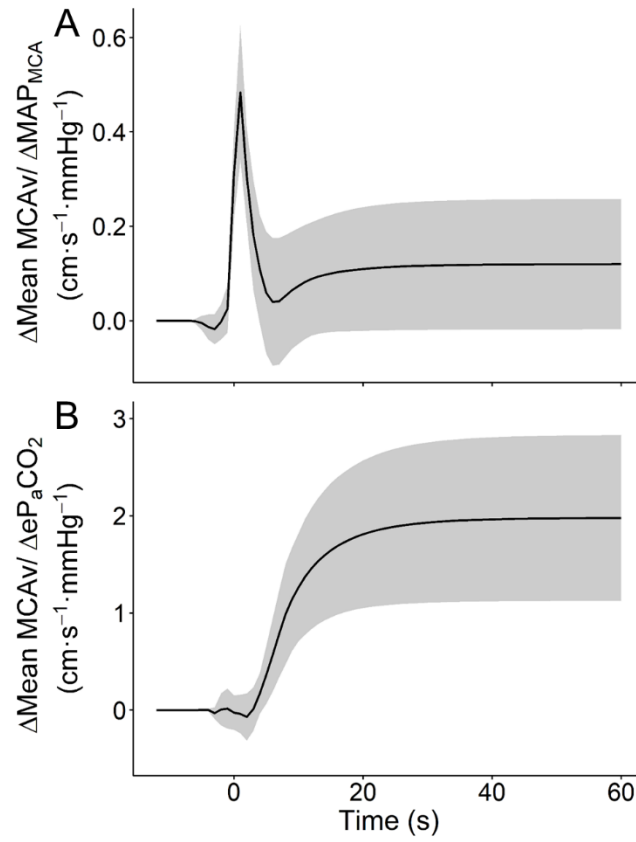


Figure 3. Group mean ($n = 18$) solutions from autoregressive moving average analysis showing autoregulatory recovery of middle cerebral artery blood velocity (MCAv) to a 1 mmHg step increase in mean arterial pressure (MAP; A) or increase in MCAv for a 1 mmHg step increase in the estimated arterial partial pressure of carbon dioxide (ePaCO_2 ; B). The solid lines represent the means and the shaded areas represent ± 1 standard deviation.

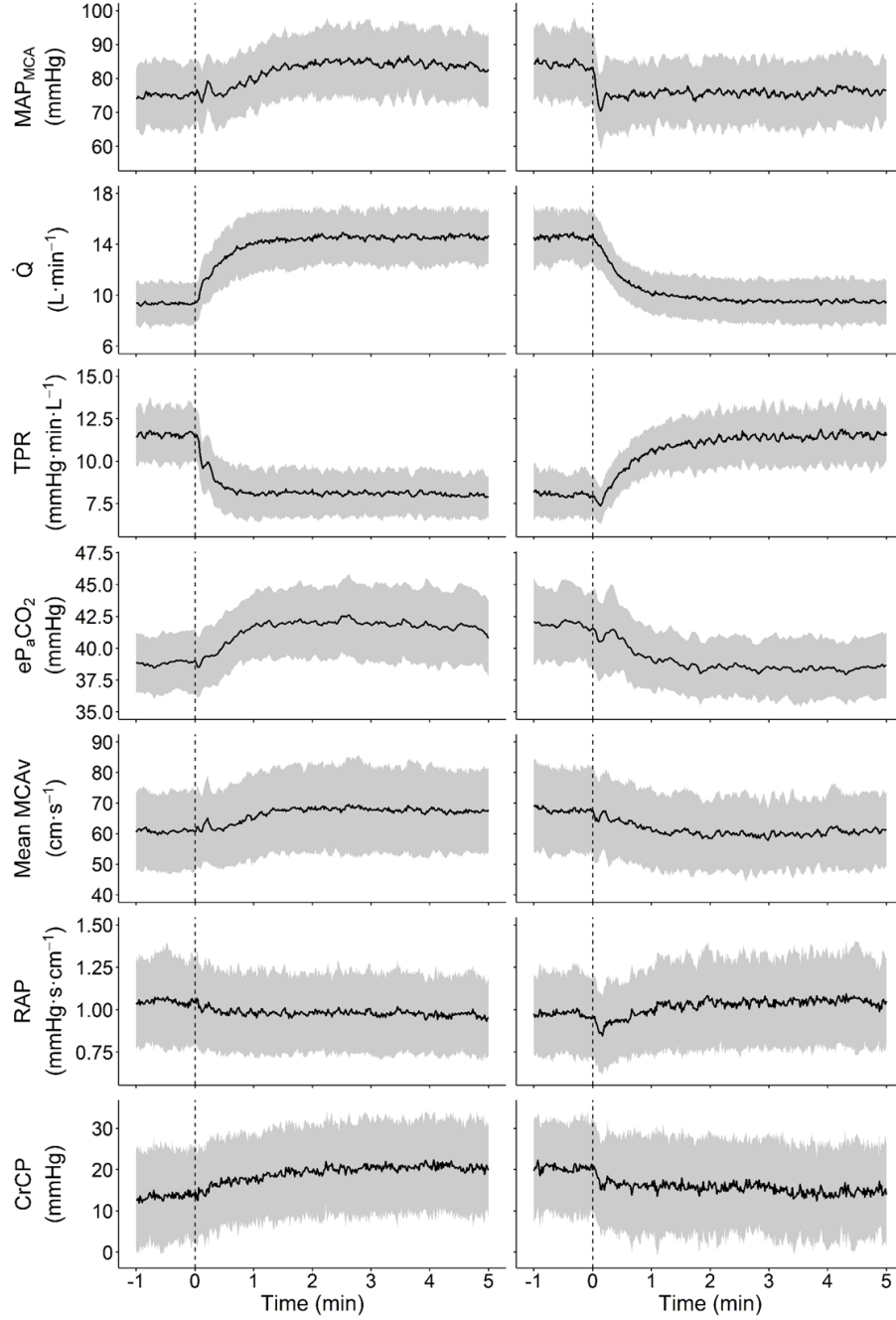


Figure 4. Group mean ($n = 18$) on- (left) and off- (right) transient responses for mean arterial pressure corrected to the level of the middle cerebral artery (MAP_{MCA}), cardiac output ($\dot{Q}$), total peripheral resistance (TPR), estimated arterial partial pressure of carbon dioxide (eP_aCO_2), middle cerebral artery blood velocity (MCAv), resistance-area product (RAP), and critical closing pressure (CrCP). The dashed vertical line at time = 0 s demarcates when the work rate either increased or decreased. The solid lines represent means and the shaded areas represent ± 1 standard deviation. Three transitions from each participant were averaged together for the on-transient response, and two transitions for each participant were averaged together for the off-transient response.

Discussion

This is the first investigation to use a causal, multi-input model to assess the impact of simultaneous changes in arterial blood pressure and eP_aCO_2 on cerebral blood velocity during exercise transients. Our ARMA analyses go beyond the simple correlation-type analyses that compare changes in variables from rest to end of exercise that have been previously reported (Billinger *et al.*, 2017; Witte *et al.*, 2019; Saito *et al.*, 2022). ARMA analysis precisely modeled mean MCAv dynamics, and enabled identification of the independent effects of dynamic changes in MAP_{MCA} and eP_aCO_2 on mean MCAv during transitions in exercise intensity. MAP, eP_aCO_2 and MCAv all increased during transitions to higher intensity exercise. In support of our hypothesis, cerebral autoregulation minimized the effect of exercise-induced changes in MAP on mean MCAv, with simulated MAP step responses showing return of mean MCAv back towards baseline following a transient spike. Conversely, the simulated eP_aCO_2 step response identified a ~3% per unit change in eP_aCO_2 during exercise, suggesting that changes in eP_aCO_2 largely explain the dynamic changes in MCAv during exercise transitions.

Many factors can influence cerebral blood flow, such as arterial pressure, P_aCO_2 , $\dot{Q}$, autonomic outflow, and cerebral metabolism (Claassen *et al.*, 2021). Given that all these factors change simultaneously during exercise, it is challenging to determine how much each factor contributes to the overall change in cerebral blood flow. Recent work demonstrated that large acute changes in $\dot{Q}$ at the onset of exercise do not affect the dynamic MCAv response (Saito *et al.*, 2022). The negligible effect of $\dot{Q}$ on MCAv during exercise is also supported by the observation that heart transplant recipients have a similar MCAv response to exercise as age-matched control participants, despite their impaired ability to increase heart rate and $\dot{Q}$ (Smirl *et al.*, 2012). In our experimental design, we also minimized the influence of exercise-associated

changes in cortical activation (Gross *et al.*, 1980; Linkis *et al.*, 1995) that could affect MCAv via neurovascular coupling by using exercise-to-exercise transitions rather than rest-to-exercise transitions. Indeed, mean MCAv exhibited much slower dynamics during the on- and off-transients (>1 min for mean MCAv to stabilize following transition in work rate; Figure 4) than the large and rapid changes in cerebral blood velocity that are typically associated with cortical activation (~ 5 s) (Aaslid, 1987), suggesting changes in cerebral metabolism between the two intensities of exercise had minimal effects on our response dynamics. After minimizing the effects of these other factors, we proceeded with time-series analysis to identify the independent effects of MAP_{MCA} and ePaCO_2 on mean MCAv during exercise.

In our ARMA analysis, we identified that cerebral autoregulation remained intact during exercise, limiting the effect of MAP on MCAv by increasing cerebrovascular tone. These findings contrast with a report suggesting that increased MAP during exercise substantially contributed to increased MCAv during steady-state exercise, as assessed with a bivariate linear correlation (Saito *et al.*, 2022). MAP and mean MCAv also both increased with exercise intensity in the present study, but it was revealed through our causal modelling technique that changes in MAP were not primarily responsible for the gross changes in mean MCAv. However, our ARMA solution for the dynamic effect of MAP on mean MCAv, where MCAv transiently increases for less than 10 s following an increase in MAP, is consistent with the high-pass filter characteristic identified in studies of dynamic cerebrovascular autoregulation (Zhang *et al.*, 1998) and with Saito *et al.*'s observation that rapid fluctuations in MCAv in the first ~ 30 s of exercise are due to rapid oscillations in MAP (Saito *et al.*, 2022), which occur too quickly for cerebral autoregulation to completely buffer. The ARMA analysis also identified that cerebrovascular reactivity during exercise was 3.2% per mmHg of ePaCO_2 , with similar temporal

response characteristics to step changes in $P_{ET}CO_2$ performed at rest (Poulin *et al.*, 1996, 1998). This level of reactivity to CO_2 is consistent with values previously reported at rest (Markwalder *et al.*, 1984; Kastrup *et al.*, 1997). Since autoregulation minimized the effect of MAP on sustained changes in mean MCAv when transitioning from low- to moderate-intensity exercise, changes in eP_aCO_2 appeared to largely dictate the observed changes in mean MCAv. Simple calculations using the group average increases in MAP (8.7 mmHg) and eP_aCO_2 (2.8 mmHg) coupled with the steady-states of the simulated MAP ($0.12 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$) and eP_aCO_2 ($1.98 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$) step responses support this interpretation, as the calculation suggest that changes in MAP only contributed ~16% to the overall change in MCAv while changes in P_aCO_2 contributed ~84%. The strong effect of P_aCO_2 and hyperventilation-induced hypocapnia during high-intensity exercise on MCAv has been widely reported (Ogoh & Ainslie, 2009; Ogoh *et al.*, 2010), but our work extends these previous findings by demonstrating that P_aCO_2 also has important effects on MCAv during dynamic transitions in exercise intensity. However, our observed effect of eP_aCO_2 on MCAv responses during exercise transients while cycling in upright posture does contrast with the findings of Smith *et al.* (Smith *et al.*, 2016) during steady-state recumbent cycling, where similar submaximal changes in cerebral blood flow were observed during poikilocapnic and isocapnic exercise. Instead, they suggested that increased cerebral blood flow during normoxic exercise may be due to increased cerebral metabolism and/or shear stress-mediated vasodilation (Smith *et al.*, 2016).

Following the increase in work rate from 25 W to 90% of VT, RAP decreased and CrCP increased. The mean decrease in RAP of $\sim 0.08 \text{ mmHg}\cdot\text{s}\cdot\text{cm}^{-1}$ and mean increase in CrCP of $\sim 7 \text{ mmHg}$ from low- to moderate-intensity cycling exercise were comparable to changes induced by dynamic knee extension (Ogoh *et al.*, 2010) and cycling (Ogoh *et al.*, 2011) exercises in similar

age adults. The observations of increased CrCP and reduced RAP during moderate-intensity exercise, where both eP_aCO_2 and MAP were elevated, are in contrast to what would be expected based on autoregulatory investigations performed at rest (Panerai, 2003; Panerai *et al.*, 2020, 2021). RAP is expected to increase in order to limit the effect of rising MAP on MCAv (Panerai *et al.*, 2021), whereas increased P_aCO_2 is expected to reduce CrCP (Panerai *et al.*, 1999, 2020). The beat-to-beat autoregulatory relationship between RAP and MAP was still observed during exercise, just on small time scales, which was most apparent during the rapid reduction in MAP during the off-transient, where RAP rapidly decreased as well; however, the global trends in CrCP and RAP over the course of several minutes following an exercise transition suggest that the regulation of cerebrovascular tone and resistance during exercise is more complex than at rest. Perhaps the simultaneous changes in arterial pressure and eP_aCO_2 during exercise interacted to modify how cerebrovascular tone and resistance were regulated over time. Increased sympathetic activation of the cerebral vasculature at the higher intensity of exercise may also contribute to the increase in cerebrovascular tone and help minimize the impact of elevated MAP (Willie *et al.*, 2014); however, we were unable to isolate neurogenic control's influence on cerebrovascular responses in the present study, and its role during exercise continues to be debated (Smith & Ainslie, 2017) with recent evidence suggesting that submaximal dynamic exercise in healthy adults does not cause increases in cerebral sympathetic nerve activity (Tymko *et al.*, 2024). Future mechanistic work using isocapnic exercise transitions is needed to resolve how changing MAP and eP_aCO_2 affect RAP and CrCP following increases in exercise intensity.

Exponential modelling following a time delay has recently been suggested as a novel method to characterize MCAv dynamics after an increase in work rate (Billinger *et al.*, 2017). This application was inspired by the modeling technique that has been used for decades to study

$\dot{V}O_2$ kinetics (Hughson & Morrissey, 1982; Whipp *et al.*, 1982; Poole & Jones, 2012; Hedge & Hughson, 2020). However, there are important assumptions that must be considered when translating such a model of analysis to cerebrovascular responses. In modelling $\dot{V}O_2$ responses to a step increase in work rate with an exponential function, it is assumed $\dot{V}O_2$ is increasing directly in proportion to the increase in work rate and will reach a steady-state, with the kinetics being determined by how quickly convective oxygen delivery and oxygen utilization can be upregulated to meet this new metabolic demand, which even then has notable limitations (Hughson, 2009). That being said, the MCAv response to an increase in exercise intensity is considerably more complex, with interactive and redundant regulatory mechanisms regulating the overall change in MCAv. Critically, MCAv is not responding directly to the increase in exercise work rate (unlike $\dot{V}O_2$), but rather it is the net effect of multiple competing factors that are influenced by exercise. This point is reinforced by the observation that MCAv does not necessarily exponentially rise and stabilize at a new steady-state, but can decrease (see Figure 4) relative to its peak change during moderate-intensity constant-work rate exercise (Weston *et al.*, 2022). There is certainly useful and important information about cerebrovascular function that can be gleaned from exercise responses, especially for those with a history of stroke (Kaufman *et al.*, 2019; Kempf *et al.*, 2019; Billinger *et al.*, 2021); however, different modelling approaches that account for complex interactions between multiple mechanisms that regulate cerebral blood flow need to be utilized in the future.

Methodological Considerations

Transcranial Doppler ultrasound was used to measure MCAv as an index of cerebral blood flow, which requires assuming that MCA diameter is constant in order to interpret the

data. The benefits of transcranial Doppler technique in the current investigation outweighed this limitation, as it provided stable and continuous signals throughout a prolonged period of whole-body exercise, which was essential for the ARMA analysis. Nevertheless, small changes in MCA diameter have been reported following large changes in $P_{ET}CO_2$ (Coverdale *et al.*, 2014; Verbree *et al.*, 2014). In the present study, the mean increase in eP_aCO_2 observed from 25 W to the end of the 90% VT stage was 2.8 ± 1.3 mmHg, which is within the range of hypercapnia (7.5 mmHg) where MCA diameter has been reported to be stable (Verbree *et al.*, 2014). Importantly, if small changes in MCA diameter did occur with our observed increases in eP_aCO_2 from low- to moderate-intensity exercise, then it would only mean that our results underestimate the importance of P_aCO_2 on dynamic MCAv responses to exercise.

In the present study, a regression equation developed during exercise (Jones *et al.*, 1979) was used to estimate P_aCO_2 from $P_{ET}CO_2$ and tidal volume. It has been previously demonstrated that this equation (Jones *et al.*, 1979) provided reasonable estimates of P_aCO_2 in young healthy adults over the range of exercise intensities included in this study (Williams & Babb, 1997). Directly measuring P_aCO_2 , if feasible, or estimating it with a regression equation during exercise is essential. Simply using $P_{ET}CO_2$ as an indicator of P_aCO_2 overestimates the change in P_aCO_2 during exercise (Jones *et al.*, 1979), which could have implications on how cerebrovascular reactivity to CO_2 is interpreted to contribute to MCAv dynamics during exercise. Additionally, we used pulse contour analysis of the finger arterial pressure waveform with the ModelFlow® algorithm to estimate stroke volume and $\dot{Q}$. Importantly, estimated $\dot{Q}$ using the ModelFlow® algorithm was highly correlated (range of correlation coefficients: 0.90-0.96) with measures of $\dot{Q}$ obtained via the acetylene rebreathing method during both moderate- and high-intensity exercise

(Faisal *et al.*, 2009), suggesting that the Modelflow® algorithm provided a valid estimate of the changes in $\dot{Q}$ during exercise.

Our ARMA modeling technique only utilized two inputs, MAP_{MCA} and eP_aCO_2 , to predict dynamic MCAv responses. We acknowledge that other factors that could influence MCAv are also changing during these exercise transitions. Based on the minimal residuals between the ARMA-modeled and measured MCAv responses, with no pattern of bias in the residuals (Figure 2), we infer that the combination of MAP_{MCA} , autoregulation, and eP_aCO_2 explain most of the dynamic changes in MCAv during step transitions in exercise intensity. If another factor, such as $\dot{Q}$ or cerebral metabolism via neurovascular coupling, were having a substantial independent effect on the MCAv response during these exercise-to-exercise transitions, it would be expected to produce a distinct pattern in the residuals of our ARMA analysis (i.e., systematic under- or over-estimation of MCAv during different phases of the transitions).

Conclusion

Multiple factors that influence MCAv dynamically change during exercise, making it difficult to isolate the contribution of the different factors to the overall MCAv response. Accordingly, we utilized a causal, multi-input ARMA modelling technique to determine the independent effects of simultaneous changes in arterial blood pressure and eP_aCO_2 on MCAv during exercise. Solutions from the ARMA analysis and the small residuals with no distinguishing patterns suggested that cerebral autoregulation minimized the effect of exercise-induced changes in MAP on mean MCAv, while changes in eP_aCO_2 largely explained the

dynamic changes in mean MCAv during exercise transitions. Therefore, P_aCO_2 , and not MAP, predominantly drives the MCAv response during transitions in exercise intensity.

Chapter 3: Effects of dynamic changes in arterial pressure and carbon dioxide on middle cerebral artery blood velocity during moderate- and high-intensity interval exercise

Abstract

Rapid changes in work rate challenge the regulation of cerebral blood flow through dynamic changes in mean arterial pressure (MAP), arterial partial pressure of carbon dioxide ($P_a\text{CO}_2$), cerebral metabolism, and cardiac output. The magnitude of change for each factor depends on the exercise intensity. Larger increases in MAP and cardiac output occur during high- compared to moderate-intensity exercise, and $P_a\text{CO}_2$ changes in an intensity-dependent manner. Therefore, we investigated whether cerebral autoregulation and cerebrovascular reactivity to CO_2 were different during moderate- and high-intensity interval exercise. We hypothesized that autoregulation and cerebrovascular reactivity would not differ between exercise intensities. Fourteen young healthy adults (5 women) performed moderate- and high-intensity interval exercises, and autoregressive moving-average (ARMA) analysis quantified indices of cerebral autoregulation and cerebrovascular reactivity for each test. A wider range of MAP, estimated $P_a\text{CO}_2$, and middle cerebral artery blood velocity (MCAv) were measured during high-intensity exercise. The MAP step response was higher ($p=0.004$) during the first half ($0.23 \pm 0.14 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$) compared to the second half ($0.15 \pm 0.11 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$) of the high-intensity protocol, suggesting changes in cerebral autoregulation within a bout of high-intensity exercise. Cerebrovascular reactivity to CO_2 was not different between moderate- and high-intensity tests (moderate: 1.87 ± 0.72 vs. high first half: 1.94 ± 0.89 vs. high second half: $1.76 \pm 0.70 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$; main effect $p=0.368$). Autoregulation was also unable to prevent large transient increases in MCAv at the onset of exercise transitions that were associated with spikes in MAP,

which were exacerbated by shorter recovery intervals during high-intensity exercise. These findings suggest that exercise intensity has important effects on cerebrovascular control.

Introduction

Rapid changes in work rate challenge the regulation of cerebral blood flow through dynamic changes in mean arterial pressure (MAP), arterial partial pressure of carbon dioxide ($P_a\text{CO}_2$), cerebral metabolism, and cardiac output ($\dot{Q}$) (Smith & Ainslie, 2017). The magnitude of change for each of these factors depends on exercise intensity. Larger increases in MAP and $\dot{Q}$ occur during high-intensity exercise compared to moderate exercise, whilst $P_a\text{CO}_2$ rises during moderate exercise, but tends to decrease during high-intensity exercise (Smith & Ainslie, 2017). Given that cerebral autoregulation (Aaslid *et al.*, 1989) and cerebrovascular reactivity to CO_2 (Markwalder *et al.*, 1984) are affected by hypo- and hypercapnia, it is possible that differences in $P_a\text{CO}_2$ while performing moderate or high-intensity exercise may affect mechanisms of cerebrovascular control.

Previous work has identified that dynamic cerebral autoregulation is maintained during both constant-load moderate-intensity exercise (Brys *et al.*, 2003) and high-intensity interval exercise (Tsukamoto *et al.*, 2019), but it was reported that autoregulation was impaired during exhaustive exercise (Ogoh *et al.*, 2005b). Cerebrovascular reactivity to CO_2 was also enhanced during submaximal exercise compared to rest (Rasmussen *et al.*, 2006; Ogoh *et al.*, 2008; Murrell *et al.*, 2013), but was reported to be reduced following high-intensity interval exercise (Burma *et al.*, 2020). Critically, no cerebrovascular reactivity assessments have been performed during a bout of high-intensity interval exercise, where cerebrovascular reactivity to hyperventilation-induced hypocapnia may be an important mechanism to increase cerebrovascular resistance and protect the brain from large increases in MAP (Lucas *et al.*, 2015).

Recently, linear relationships between changes in end-tidal partial pressure of CO₂ (P_{ET}CO₂) or mean arterial pressure (MAP) and middle cerebral artery blood velocity (MCAv) during high-intensity interval exercise have been reported (Whitaker *et al.*, 2022). Since MAP tends to increase and P_{ET}CO₂ tends to decrease over multiple intervals of high-intensity exercise (Whitaker *et al.*, 2022), interpretation of the bivariate linear relationships between MAP or P_{ET}CO₂ and MCAv may be influenced by changes in the other variable (e.g., MAP or P_{ET}CO₂). Time-series analysis may help clarify the independent effects of P_{ET}CO₂ and MAP on MCAv by including both variables as simultaneous inputs into an autoregressive moving-average (ARMA) model to predict changes in mean MCAv. ARMA analysis has previously been utilized to isolate the independent effects of MAP and PCO₂ on cerebrovascular control (Panerai *et al.*, 2000; Edwards *et al.*, 2003, 2004), and can capitalize on the inherent fluctuations in estimated arterial partial pressure of CO₂ (eP_aCO₂), MAP, and MCAv during interval-type exercise to characterize cerebral autoregulation and cerebrovascular reactivity to CO₂.

Therefore, the purpose of this experiment was to quantify indices of cerebral autoregulation and cerebrovascular reactivity to CO₂ during both moderate- and high-intensity interval exercise. It was hypothesized that the MAP and eP_aCO₂ step responses of young healthy adults as indicators of cerebral autoregulation and cerebrovascular reactivity to CO₂, respectively, would not differ between moderate- and high-intensity interval exercises.

Methods

Participants

Seventeen young healthy adults volunteered to participate in the study; however, 2 participants' responses were excluded from analysis due to poor signal quality and 1 additional participant was excluded because of high correlation without a time lag between the measured MAP and eP_aCO_2 signals that prevented reliable time-series analysis. Accordingly, the final analyzed sample included 14 young healthy adults (9 men, 5 women; age: 27 ± 5 yr; height: 1.71 ± 0.09 m; mass: 72 ± 12 kg; peak oxygen uptake ($\dot{V}O_2$): 42 ± 5 mL $\cdot$ min $^{-1}\cdot$ kg $^{-1}$; mean $\pm$ SD). A minimum sample size of 7 participants ($\alpha = 0.05$, power = 0.80) was calculated for this study based on previously reported large effect sizes for the effect of different cycling intensities on cerebrovascular tone (Ogoh *et al.*, 2011). However, a larger sample was recruited with a final sample size of $n = 14$ as a conservative estimate to account for potential differences in exercise intensities between studies.

For all laboratory visits, participants were instructed to: i) arrive for testing at least 2 h post-prandial, ii) not consume alcohol nor perform vigorous exercise in the preceding 24 h, and iii) and avoid caffeine consumption on the day of the tests. The study was conducted according to the Declaration of Helsinki, except for registration in a trial database, and all procedures were approved by University of Waterloo's Clinical Research Ethics Committee (ORE #32164). All participants signed an informed consent before participating and were made aware of their right to withdraw at any time.

Exercise Testing

Participants visited the laboratory on three separate occasions to perform different exercise tests, with each session separated by at least 48 h. Exercise testing was performed in an environmentally controlled laboratory on an electrically braked cycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, Netherlands), with participants cycling at a cadence of 60 revolutions per minute.

During the first visit, participants completed a ramp-incremental exercise test to symptom-limited tolerance, where the test began with 4 min of baseline cycling at 25 W followed by a 25 W·min⁻¹ ramp. The incremental exercise test was terminated when cycling cadence dropped below 55 revolutions per minute despite strong verbal encouragement. The results from the incremental test were used to estimate each participant's ventilatory threshold (VT) $\dot{V}O_2$, peak $\dot{V}O_2$, and prescribe the work rates for the moderate- and high-intensity interval exercise tests.

In the second and third visits, participants performed one of two different interval exercise tests in a random order. The interval exercise tests followed a pseudorandom binary sequence for the changes in work rate, where work rate alternated between 25 W and the work rate associated with 90% of the $\dot{V}O_2$ at VT for moderate-intensity exercise, or 25 W and the work rate that corresponded with 50% of the difference between VT and peak $\dot{V}O_2$ ($\Delta 50\%$) for high-intensity exercise. The interval exercise tests were 18.5 min in duration (Figure 5), with the time-series for changes in work rate generated by a digital shift register with adder module feedback (Bennett *et al.*, 1981; Hughson *et al.*, 1990; Hedge & Hughson, 2020). The $\Delta 50\%$ stages were sufficient to elicit heart rate responses >80% of participants maximal heart rate achieved during the incremental exercise test. These pseudorandom changes in work rate, with

intervals ranging between 30 s and 120 s, are particularly useful for assessing dynamic exercise responses (Beltrame & Hughson, 2017; Hedge & Hughson, 2020).

Data Collection

Gas exchange and ventilation were collected breath-by-breath during all exercise tests using a portable metabolic system (MetaMax 3B-R2, CORTEX Biophysik, Leipzig, Germany), with participants breathing through a mask (7450 SeriesV2™ Mask, Hans Rudolph, Inc., Shawnee, USA). The system's flow sensor, and O₂ and CO₂ sensors were calibrated prior to each test using a 3-L syringe and precision analyzed gas mixtures, respectively. During testing, participants also wore a heart rate monitor (Polar H7, Polar Electro Oy, Kempele, Finland).

During the interval exercise tests, respiratory data collected using the portable metabolic system were time synchronized with continuous measures of arterial blood pressure and MCAv, which were recorded at 1000 Hz (PowerLab and Labchart, version 7.3.7; AD Instruments, Colorado Springs, USA). Blood velocity data were collected from the right middle cerebral artery using a 2 MHz transcranial Doppler ultrasound probe (WAKIe 2TC, Atys Medical, Soucieu-en-Jarrest, France), which was securely held in place with a headset (Spencer Technologies, Seattle, USA). Finger arterial blood pressure was measured by the volume-clamp method (Finapres NOVA, Finapres Medical Systems, Enschede, Netherlands), and values were reconstructed to brachial pressure, height corrected to the level of the heart, and calibrated using two brachial pressure measures obtained with an arm cuff while seated at rest. Stroke volume was estimated for each cardiac cycle via pulse contour analysis of the finger arterial waveform using the Modelflow® algorithm. During exercise, the arm and hand used to measure blood

pressure was supported on an elevated platform at the level of the heart to prevent measurement artifacts.

Data Analysis

Ramp-incremental exercise responses. Peak $\dot{V}O_2$ was calculated as the highest $\dot{V}O_2$ computed using a 20-s moving mean, and $\dot{V}O_2$ at VT was estimated by visual inspection using standard ventilatory and gas exchange indices (and their ratios), as previously described (Beaver *et al.*, 1986). The work rate associated with the $\dot{V}O_2$ at 90% VT, VT, and $\Delta 50\%$ were estimated after left-shifting the $\dot{V}O_2$ response by each participant's mean response time to align the $\dot{V}O_2$ and work rate profiles. The mean response time was determined using the segmented regression method (Hughson & Inman, 1986).

Interval exercise responses. Heart rate, estimated stroke volume, arterial blood pressure, and MCAv were extracted beat-by-beat, with MAP and mean MCAv calculated from the brachial pressure waveform and outer envelope of the transcranial Doppler spectrum, respectively. MAP at the level of the MCA (MAP_{MCA}) was subsequently estimated after accounting for hydrostatic pressure gradient between the level of the transcranial Doppler ultrasound probe and the heart when cycling in the upright position [correction factor = distance (in cm) $\cdot 0.78 \text{ mmHg} \cdot \text{cm}^{-1}$]. Estimated cardiac output ($\dot{Q}$) was calculated as the product of heart rate and stroke volume. Resistance-area product (RAP) and critical closing pressure (CrCP) were also calculated beat-by-beat using the two-point mean/diastolic method (Panerai *et al.*, 2011). P_aCO_2 was estimated (eP_aCO_2) from $P_{ET}CO_2$ and tidal volume for each breath using the equation developed by Jones *et al.* (Jones *et al.*, 1979).

Cerebrovascular and respiratory data were linearly interpolated to 1 Hz (MATLAB R2020b, The MathWorks Inc., Natick, USA). ARMA analysis was conducted as previously described (Edwards *et al.*, 2003, 2004), using MAP_{MCA} and eP_aCO_2 to model mean $MCAv$ during moderate- and high-intensity interval exercise in order to determine how $MCAv$ dynamically responds to independent changes in MAP_{MCA} or eP_aCO_2 . ARMA models are useful for characterizing linear, time-invariant systems with multiple inputs and delays (Perrott & Cohen, 1996). The model orders for each individual test were selected based on: i) minimum description length criterion, ii) minimum error terms associated with each input, and iii) minimal residuals with a Gaussian distribution. A model for each participant and exercise intensity was used to generate simulated mean $MCAv$ responses to a 1 mmHg step increase in MAP_{MCA} or eP_aCO_2 . The steady-state changes were calculated for both MAP_{MCA} and eP_aCO_2 simulated step responses (i.e., $\Delta mean\ MCAv / \Delta MAP_{MCA}$ or $\Delta mean\ MCAv / \Delta eP_aCO_2$). Due to an emerging pattern in the residuals for the ARMA predictions of mean $MCAv$ during the first half of the high-intensity protocol but not the moderate-intensity protocol (Figure 6), the high-intensity interval protocols were separated into two pieces for analysis (0-9 min, and 9-18.5 min) whereas the moderate intensity tests were analyzed as one piece. The high-intensity tests were split near the mid-point based on the observation that eP_aCO_2 was consistently higher during the first half of the test compared to the second half. The calculated steady-state changes from the simulated MAP and eP_aCO_2 step responses were subsequently normalized to each participant's average mean $MCAv$ value during each respective exercise test, or section of the exercise test.

Based on the visual observation that large transient spikes occurred during the high-intensity exercise bout for some but not all on-transients (Figure 7), the immediate dynamic responses to increases in work rate were determined after different recovery intervals at the

lower work rate. The peak change in MAP_{MCA} and mean $MCAv$ during the first 10 s of on-transient responses were extracted following 30-s (4 transitions in the 18.5 min protocol), 60-s (2 transitions in the 18.5 min protocol), and 90-s (2 transitions in the 18.5 min protocol) recovery periods, with the average peak change calculated for each participant for a given recovery period. The transition following the first 30-s recovery interval (1.5 min into the test with only a single 30-s interval of higher intensity exercise preceding it) was not included in the analysis.

Statistical Analysis

Statistical analyses were conducted in R (v. 4.0.3). Paired t-tests were used to compare ARMA solutions and mean protocol responses between the first and second halves of the high-intensity interval protocol to determine if mechanisms of cerebrovascular control changed within a single exercise test. Subsequently, a linear mixed model [R library: lmerTest (Kuznetsova *et al.*, 2017)] with a fixed effect of intensity and random effect of participant was used to compare moderate-intensity responses to the two halves of the high-intensity protocol. A linear mixed model with fixed effects of intensity (moderate vs. high) and recovery interval (30 s vs. 60 s vs. 90 s), and a random effect of participant was also used to test for differences in initial peak changes in MAP_{MAP} and $MCAv$ during the first 10 s of transitions to higher work rates. Significant interactions were investigated using pairwise comparisons of estimated marginal means with Holm correction for multiple comparison [R library: emmeans (Lenth *et al.*, 2022)]. Statistical significance was set to $p \leq 0.05$, and data are reported as means $\pm$ standard deviation.

Results

Ramp-incremental exercise responses

The mean peak work rate achieved at the end of the incremental exercise test was 272 ± 32 W, which corresponded with a peak $\dot{V}O_2$ of 3.06 ± 0.50 L $\cdot$ min $^{-1}$. The mean $\dot{V}O_2$ at VT was 1.69 ± 0.15 L $\cdot$ min $^{-1}$, and occurred at a work rate of 120 ± 6 W after accounting for a mean response time of 49 ± 10 s. The work rates estimated to correspond to 90% of the VT $\dot{V}O_2$ and $\Delta 50\%$ were 108 ± 6 W and 188 ± 23 W, respectively.

High-intensity interval response

The high-intensity protocol elicited heart rate responses $>80\%$ of maximal heart rate (Figure 5). Mean heart rate (126 ± 12 vs. 143 ± 14 beats $\cdot$ min $^{-1}$; $p < 0.001$) and estimated $\dot{Q}$ (11.9 ± 1.5 vs. 13.5 ± 1.9 beats $\cdot$ min $^{-1}$; $p < 0.001$) were higher during the second half of the test, but MAP_{MCA} (89 ± 9 vs. 83 ± 10 mmHg; $p < 0.001$) and total peripheral resistance (9.94 ± 1.51 vs. 8.39 ± 1.62 mmHg $\cdot$ min $\cdot$ L $^{-1}$; $p < 0.001$) were lower. eP_aCO_2 (40.9 ± 2.9 vs. 38.4 ± 3.3 mmHg; $p < 0.001$) and mean $MCAv$ (61.1 ± 16.1 vs. 56.5 ± 15.8 mmHg; $p < 0.001$) were higher during the first half of the test (Figure 7). ARMA models using MAP_{MCA} and eP_aCO_2 as inputs predicted the mean $MCAv$ response to both halves of the high-intensity interval exercise test, with small residuals (Figure 8). ARMA solutions for both halves of the high-intensity exercise test demonstrated intact autoregulatory responses, with mean $MCAv$ decreasing back towards baseline within ~ 10 s of a step increase in MAP (Figure 9). The MAP step response was higher ($p = 0.004$) during the first half (0.23 ± 0.14 cm $\cdot$ s $^{-1}\cdot$ mmHg $^{-1}$) compared to the second half (0.15 ± 0.11 cm $\cdot$ s $^{-1}\cdot$ mmHg $^{-1}$) of the high-intensity protocol, but the eP_aCO_2 step response was not

different (first half: 1.94 ± 0.89 vs. second half: $1.76 \pm 0.70 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$; $p = 0.242$). After normalizing for differences in mean MCAv during each section of the high-intensity test, the steady-states of the MAP step responses for the two halves were $0.37 \pm 0.17 \text{ }\cdot\text{mmHg}^{-1}$ and $0.25 \pm 0.14 \text{ }\cdot\text{mmHg}^{-1}$ (difference $p = 0.007$), while reactivity to CO_2 were $3.09 \pm 1.05 \text{ }\cdot\text{mmHg}^{-1}$ and $3.10 \pm 0.76 \text{ }\cdot\text{mmHg}^{-1}$ (difference $p = 0.967$), respectively.

Comparison of moderate- and high-intensity interval responses

The average mean MCAv during the two 18.5 min exercise protocols were not different (moderate: 56.9 ± 15.2 vs. high: $58.7 \pm 15.9 \text{ cm}\cdot\text{s}^{-1}$; $p = 0.424$) despite heart rate (moderate: 111 ± 11 vs. high: $135 \pm 13 \text{ beat}\cdot\text{min}^{-1}$; $p < 0.001$), MAP_{MCA} (moderate: 76 ± 11 vs. high: $86 \pm 9 \text{ mmHg}$; $p < 0.001$) and $\dot{Q}$ (moderate: 10.2 ± 1.3 vs. high: $12.7 \pm 1.7 \text{ L}\cdot\text{min}^{-1}$; $p < 0.001$) being considerably lower during the moderate-intensity interval protocol (Figure 7). A smaller range of MAP_{MCA} (moderate max-min: 49 ± 9 vs. high max-min: $66 \pm 12 \text{ mmHg}$, $p < 0.001$), ePaCO_2 (moderate max-min: 10.9 ± 3.8 vs. high max-min: $13.2 \pm 2.0 \text{ mmHg}$, $p = 0.048$), and mean MCAv (moderate max-min: 31.7 ± 11.2 vs. high max-min: $38.4 \pm 12.9 \text{ cm}\cdot\text{s}^{-1}$, $p = 0.015$) were measured during moderate-intensity exercise. In contrast to the high-intensity interval test, where ePaCO_2 increased to a peak within the first ~ 3 min of exercise and then subsequently declined, ePaCO_2 oscillated consistently around its mean of $40.4 \pm 3.2 \text{ mmHg}$ throughout the test (Figure 7).

ARMA models precisely predicted the mean MCAv response to moderate-intensity interval exercise, with small residuals (Figure 6). The MAP and ePaCO_2 step responses were $0.14 \pm 0.13 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$ and $1.87 \pm 0.72 \text{ cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$, respectively. When normalized to mean

MCAv during the moderate intensity test, the MAP step response was $0.26 \pm 0.22 \text{ \%} \cdot \text{mmHg}^{-1}$ and CO₂ reactivity was $3.24 \pm 0.76 \text{ \%} \cdot \text{mmHg}^{-1}$. The group mean ARMA solutions for step changes in MAP_{MCA} and eP_aCO₂ during moderate-intensity exercise are compared to the solutions calculated using the first or second halves of the high-intensity interval protocol in Figure 10. No statistical differences were identified between moderate- and high-intensity exercises for the MAP (main effect: $p = 0.073$, $\eta_p^2 = 0.18$) or eP_aCO₂ (main effect: $p = 0.368$, $\eta_p^2 = 0.07$) step responses.

The mean CrCP during the high-intensity interval protocol ($23 \pm 6 \text{ mmHg}$) was higher ($p = 0.047$) than during the moderate-intensity interval protocol ($16 \pm 10 \text{ mmHg}$), but mean RAP was not different (moderate: 1.11 ± 0.29 vs. high: $1.15 \pm 0.31 \text{ mmHg} \cdot \text{s} \cdot \text{cm}^{-1}$; $p = 0.444$). Despite the mean RAP over the course of the whole session not being different, high-intensity interval exercise induced much greater swings in RAP than moderate-intensity interval exercise to compensate for the greater absolute increases in MAP_{MCA} during $\Delta 50\%$ intervals (Figure 7). At the onset of an increase in work rate, abrupt increases in MAP_{MCA} caused spikes in mean MCAv, followed by autoregulatory recovery as RAP increased (Figure 11). The magnitude of these rapid increases in MAP_{MCA} and mean MCAv at the onset of an increase in work rate differed based on exercise intensity and the preceding recovery interval duration (intensity $\times$ recovery interval interactions for MAP_{MCA}: $p = 0.002$, $\eta_p^2 = 0.18$ and MCAv: $p < 0.001$, $\eta_p^2 = 0.24$). The increases in both MAP_{MCA} and MCAv at the onset of transitions following 30-s recovery intervals were greater (all $p \leq 0.002$) than the peak changes in MAP_{MCA} and MCAv following both 60-s and 90-s recovery intervals during the high-intensity exercise protocol (Table 2). However, there were no differences in peak changes in MAP_{MCA} or MCAv at the onset of transitions from 25 W to 90% of the VT based on the preceding recovery intervals length (all $p > 0.916$).

Table 2. Comparison of the magnitude of rapid changes in mean arterial pressure and middle cerebral blood velocity at the onset of step increases in work rate following different recovery interval durations.

	Moderate Intensity			High Intensity			Recovery	Intensity	Interaction
	30-s	60-s	90-s	30-s	60-s	90-s	Interval		
$\Delta\text{MAP}_{\text{MCA}}$ (mmHg)	7 $\pm$ 3	6 $\pm$ 3	7 $\pm$ 4	17 $\pm$ 4*†	11 $\pm$ 5†	9 $\pm$ 5	<0.001	<0.001	0.002
$\Delta\text{Mean MCAv}$ (cm·s ⁻¹)	3.8 $\pm$ 3.2	4.0 $\pm$ 2.2	4.9 $\pm$ 3.4	10.7 $\pm$ 3.3*†	6.4 $\pm$ 2.7	4.9 $\pm$ 3.4	0.007	<0.001	<0.001

Means $\pm$ SD. MAP_{MCA}, mean arterial pressure at the level of the middle cerebral artery; MCAv, middle cerebral artery blood velocity. *, different ($p \leq 0.05$) from 60-s and 90-s for a given exercise intensity. †, different ($p \leq 0.05$) from moderate intensity for a given recovery interval.

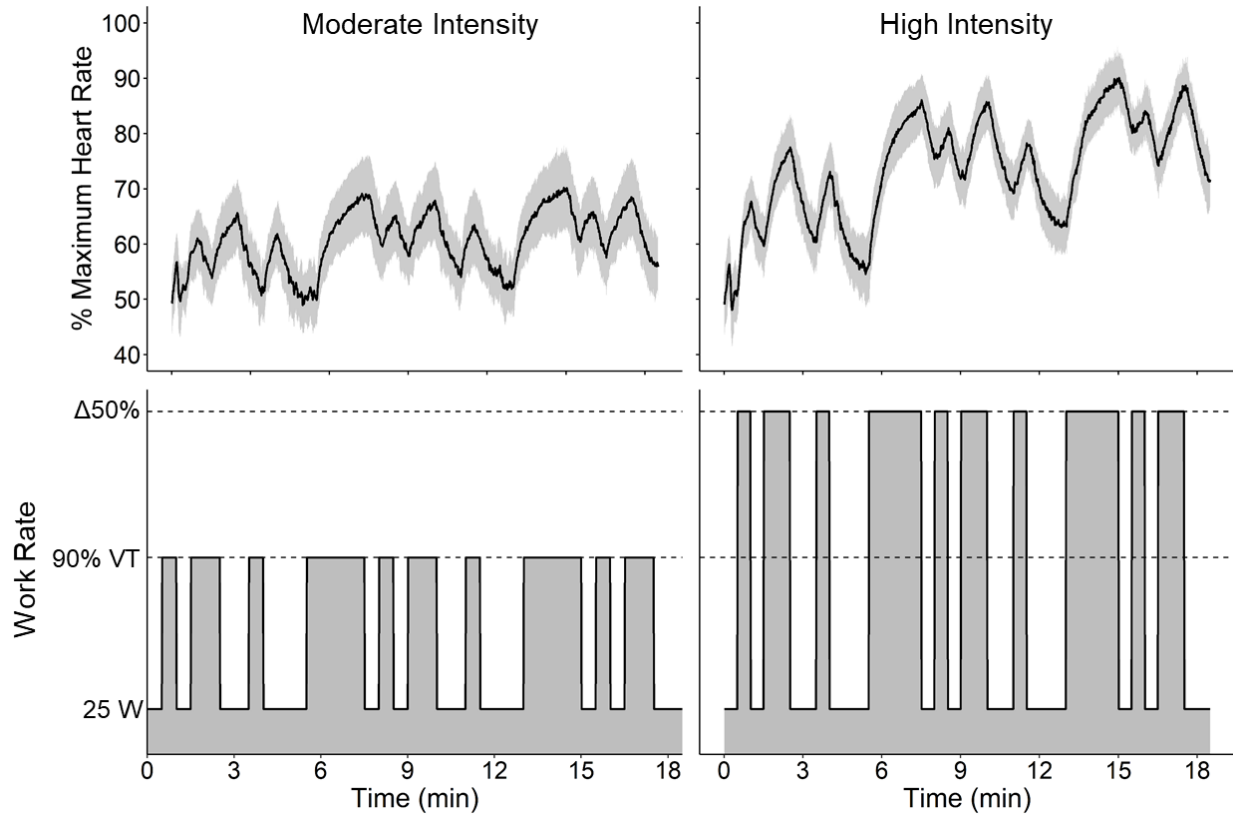


Figure 5. Group mean ($n = 14$) heart rate responses and schematic for the changes in work rate during the 18.5 min moderate- and high-intensity pseudorandom interval exercise protocols. Heart rate is expressed as the percentage of the maximum heart rate achieved during the incremental exercise test, with the shaded area around the solid line representing ± 1 SD. $\Delta 50\%$, 50% of the difference between ventilatory threshold and peak oxygen uptake; VT, ventilatory threshold.

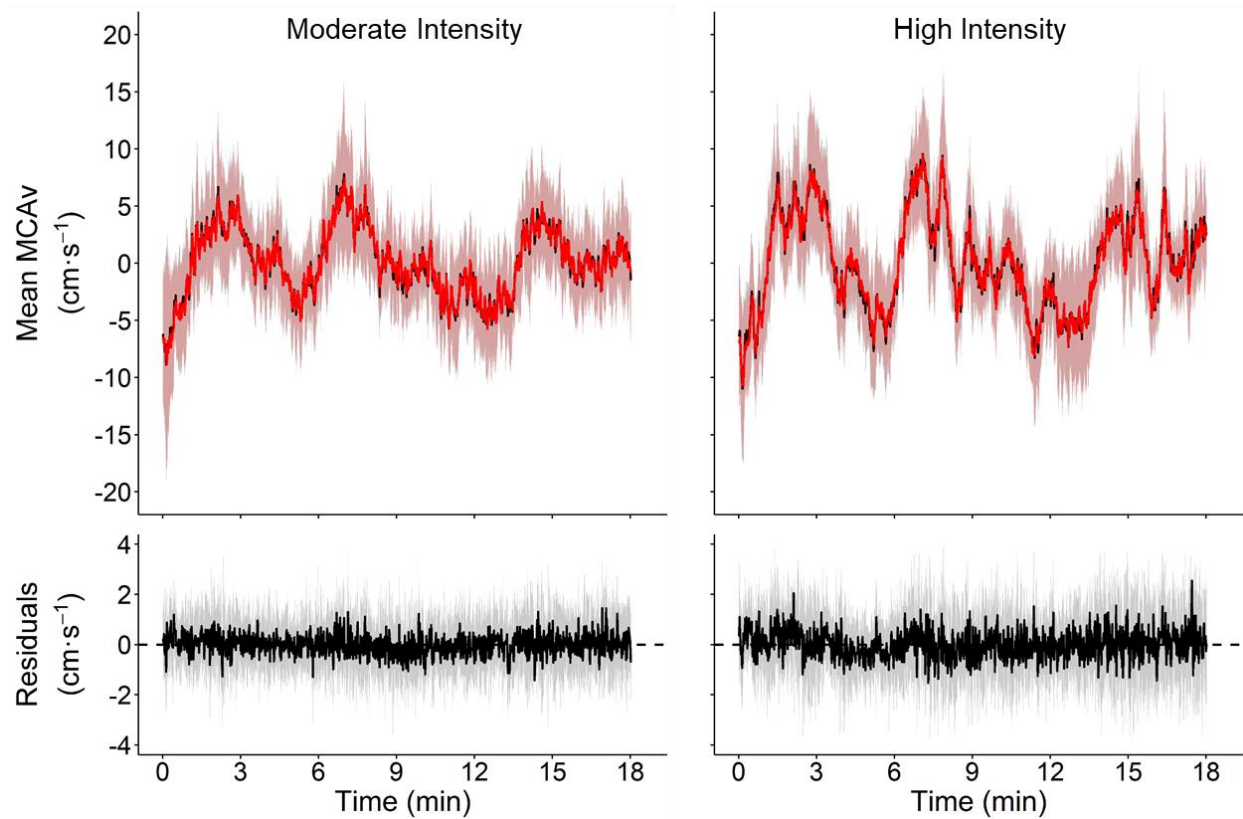


Figure 6. Comparison of group mean ($n = 14$) directly measured (black) and predicted (red) changes in mean middle cerebral artery blood velocity (MCAv) using autoregressive moving-average analysis during moderate- (left) and high-intensity (right) interval exercise, with calculated residuals shown below. A pattern in the residuals during the first ~ 9 min of high-intensity exercise was observed, leading to analyzing the high-intensity exercise response as two separate sections (see text for details). The measured and modeled mean MCAv responses oscillate around $0 \text{ cm}\cdot\text{s}^{-1}$, as the measured mean MCAv and inputs to predict MCAv were linearly detrended during analysis. The solid lines represent the means and the shaded areas represent ± 1 standard deviation.

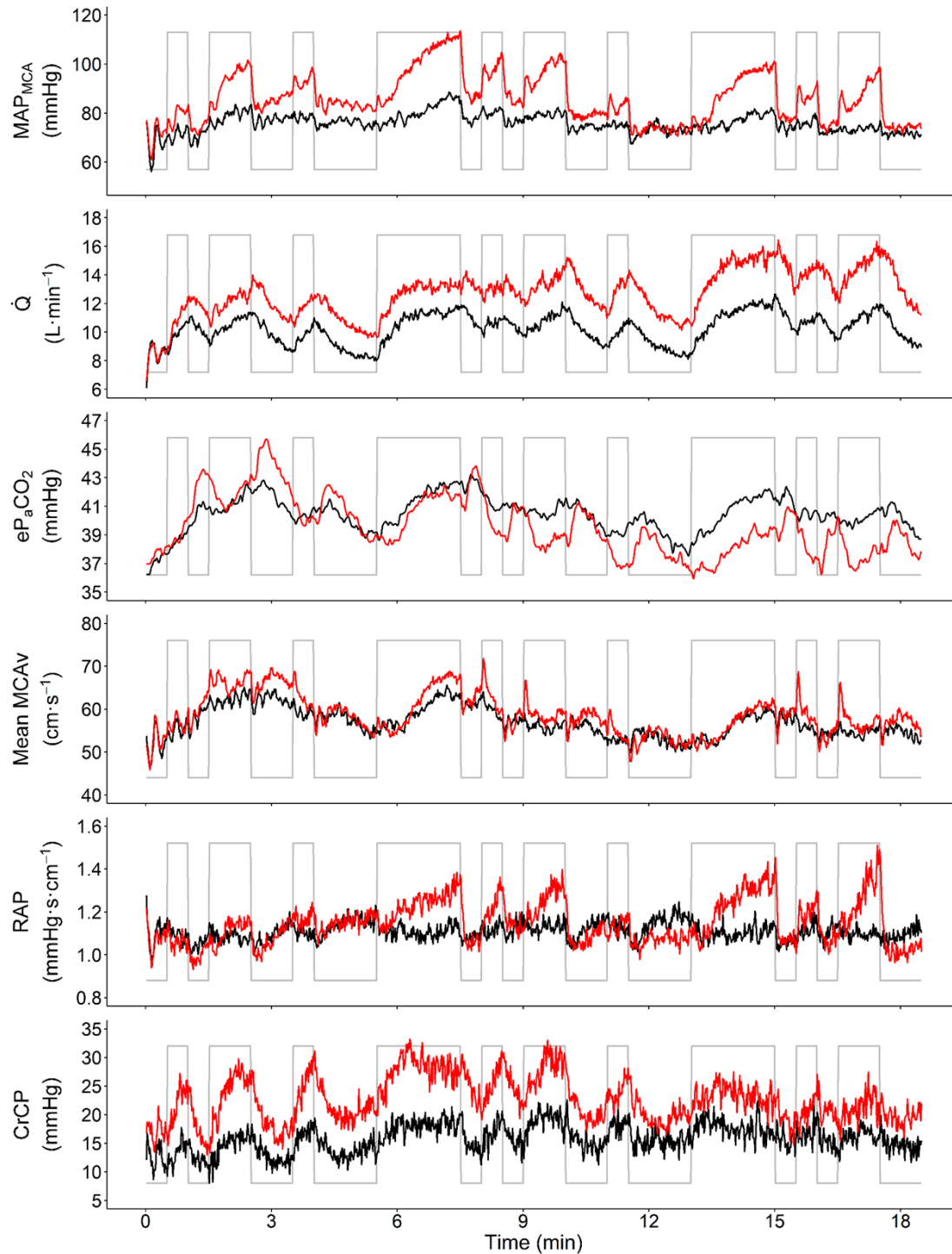


Figure 7. Group mean ($n = 14$) moderate- (black) and high-intensity (red) interval exercise responses for mean arterial pressure corrected to the level of the middle cerebral artery (MAP_{MCA}), cardiac output ($\dot{Q}$), estimated arterial partial pressure of carbon dioxide (eP_aCO_2), mean middle cerebral artery blood velocity (MCAv), resistance-area product (RAP), and critical closing pressure (CrCP). The grey line represents the timing of changes in work rate. The visualization of the standard deviations around the group means were omitted for visual clarity.

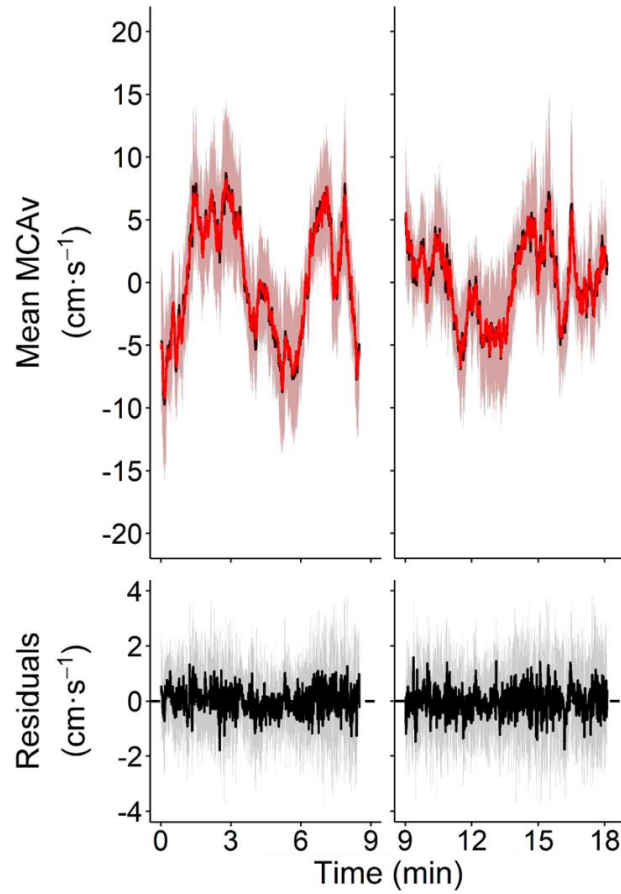


Figure 8. Comparison of group mean ($n = 14$) directly measured (black) and predicted (red) changes in mean middle cerebral artery blood velocity (MCAv) for the first and second halves of high-intensity interval exercise, with calculated residuals shown below. The measured and modeled mean MCAv responses oscillate around $0 \text{ cm} \cdot \text{s}^{-1}$, as the measured mean MCAv and inputs to predict MCAv were linearly detrended for each section during analysis. The solid lines represent the means and the shaded areas represent ± 1 standard deviation.

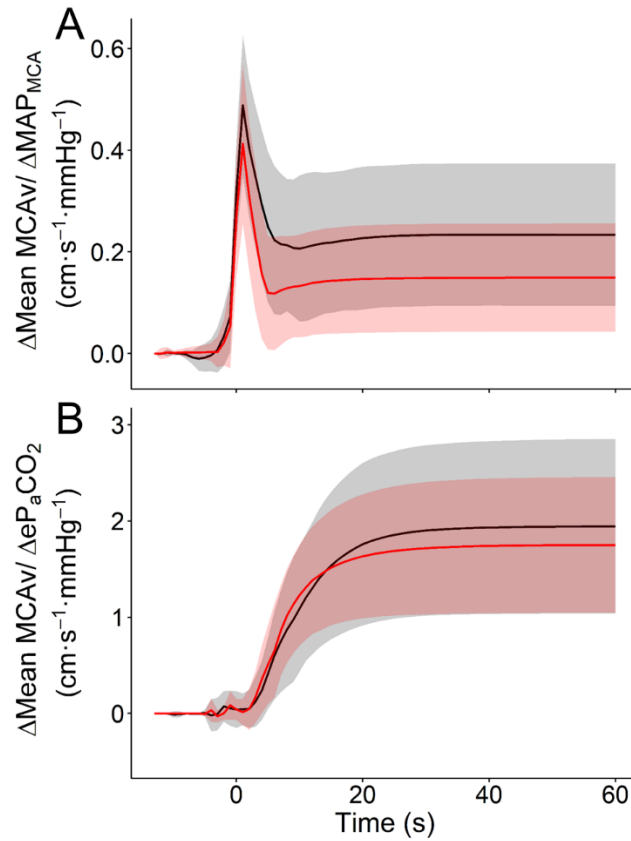


Figure 9. Group mean ($n = 14$) autoregressive moving average analysis solutions obtained from the first (black) and second (red) halves of the high-intensity interval exercise protocol. Mean middle cerebral artery blood velocity (MCAv) responses were generated in response a 1 mmHg step increase in mean arterial pressure (MAP; A) or a 1 mmHg step increase in the estimated arterial partial pressure of carbon dioxide (ePaCO_2 ; B) at time = 0 s. The solid lines represent the means and the shaded areas represent ± 1 standard deviation. The mean MCAv response to an increase in MAP_{MCA} was different between sections of the high-intensity exercise test ($p = 0.004$), but the response to an increase in ePaCO_2 was not different ($p = 0.242$).

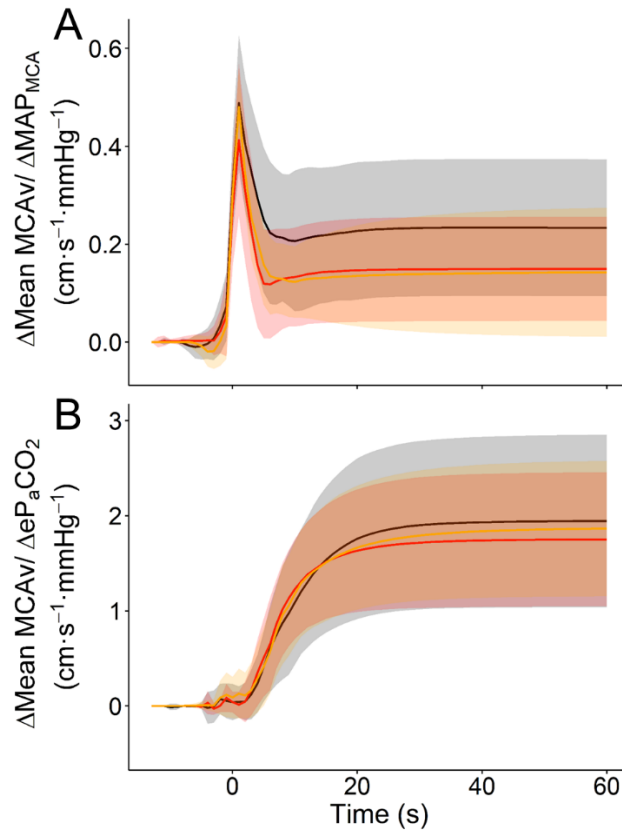


Figure 10. Group mean ($n = 14$) autoregressive moving average analysis solutions obtained from the first (black) and second (red) halves of the high-intensity interval exercise protocol, and the whole moderate-intensity interval exercise test (orange). Mean middle cerebral artery blood velocity (MCAv) responses were generated in response a 1 mmHg step increase in mean arterial pressure (MAP; A) or a 1 mmHg step increase in the estimated arterial partial pressure of carbon dioxide (eP_aCO_2 ; B) at time = 0 s. The solid lines represent the means and the shaded areas represent ± 1 standard deviation.

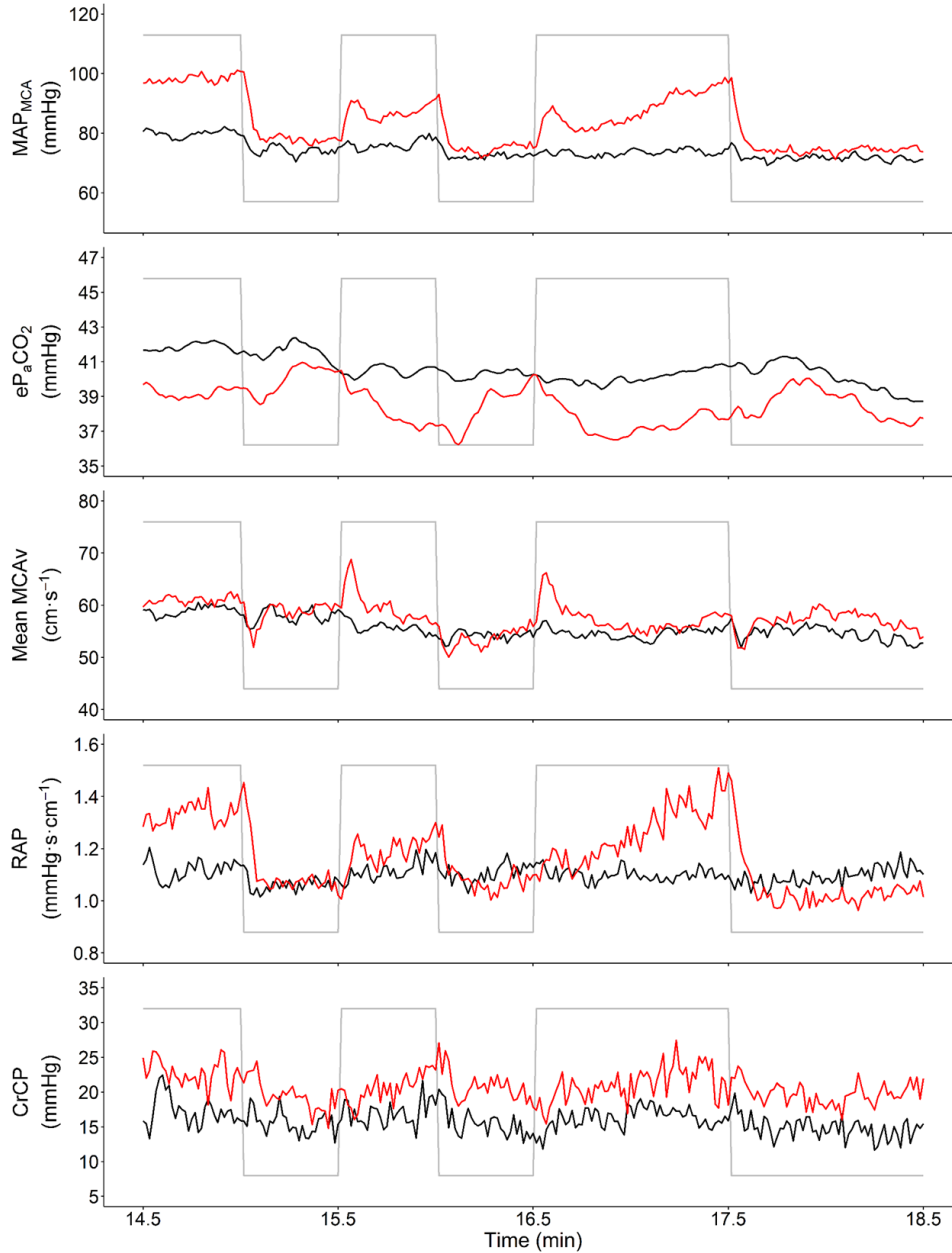


Figure 11. Group mean ($n = 14$) moderate- (black) and high-intensity (red) interval exercise responses highlighting the rapid increase in mean middle cerebral artery blood velocity (MCAv) at the onset of high-intensity intervals linked to an abrupt increase in mean arterial pressure (MAP_{MCA}). The abrupt increase in MAP_{MCA} at the onset of these high-intensity intervals occurred too quickly for autoregulation to buffer the increase in pressure with an increase in cerebrovascular resistance. Thereafter, cerebrovascular resistance increased to minimize the effect of elevated MAP_{MCA} on mean MCAv. This large spike in MCAv was not observed at the onset of transitions from 25 W to moderate-intensity intervals. These displayed responses were extracted from 14 min 30 s to 18 min 30 s during the exercise protocols, with the grey line representing the timing of changes in work rate. $\text{eP}_{\text{a}}\text{CO}_2$, estimated arterial partial pressure of carbon dioxide; RAP, resistance-area product; CrCP, critical closing pressure.

Discussion

This study provides detailed descriptions of cerebrovascular responses to acute bouts of moderate- and high-intensity interval exercise. High-intensity exercise elicited a wider range of MCAv, MAP, and ePaCO₂ responses than moderate-intensity exercise. Mean MCAv responses during both protocols were well characterized by ARMA models with inputs of MAP_{MCA} and ePaCO₂, suggesting that dynamic autoregulation and changes in MAP_{MCA} and ePaCO₂ largely explained the MCAv dynamics during both intensities of exercise. A single ARMA model characterized moderate exercise responses well, but two ARMA models were needed to address a pattern of bias in the residuals of predicted mean MCAv responses during high-intensity exercise. By modelling the first and second half of high-intensity interval exercise separately, we identified higher MAP step responses at the beginning of high-intensity exercise compared to the end of the test, suggesting that autoregulation was less effective at buffering changes in MAP during the early stages of high-intensity interval exercise. Importantly, ePaCO₂ was elevated throughout the first half of the high-intensity interval protocol, and hypercapnia is known to impair cerebral autoregulation (Aaslid *et al.*, 1989). Cerebrovascular reactivity to CO₂ was not different between the first or second half of high-intensity interval exercise and moderate-intensity interval exercise. Contrary to our hypothesis, these findings suggest that mechanisms of cerebrovascular control are affected by the intensity of exercise, with changes in ePaCO₂ during a bout of high-intensity exercise potentially contributing to observed changes in cerebral autoregulation.

Cerebral autoregulation is an important regulatory mechanism that buffers the effect of changing arterial blood pressure on cerebral blood flow (Claassen *et al.*, 2021). Effective autoregulation during high-intensity exercise is particularly important, as large increases in

pressure occur (Wright *et al.*, 1999; Sabbahi *et al.*, 2018). If cerebral autoregulation were to be substantially impaired during high-intensity exercise, then marked elevations in arterial blood pressure could be transmitted to the brain, potentially increasing the risk for cerebral hyperperfusion and disruption of the blood-brain barrier (Lucas *et al.*, 2015; Calverley *et al.*, 2020). Previously, impaired dynamic autoregulation was identified during exhaustive exercise (Ogoh *et al.*, 2005b), and was associated with increased blood-brain barrier permeability (Bailey *et al.*, 2011).

In the present study, the MAP step response was reduced during the second half of our high-intensity interval exercise protocol, suggesting that autoregulation improved during the later stages of high-intensity interval exercise. Our observed change in autoregulation revealed by ARMA analysis of repeated transitions between low- and high-intensity exercise conflicts with a report of maintained autoregulation gain assessed by transfer function analysis of the MCAv response to spontaneous fluctuations in MAP during the last 3 minutes of 4-min high-intensity intervals at a constant work rate (Tsukamoto *et al.*, 2019). Differences in P_aCO_2 during interval exercise may contribute to the divergent results between our study and those of Tsukamoto *et al.* (Tsukamoto *et al.*, 2019). In the present study, participants had a relatively large increase in eP_aCO_2 (~8 mmHg) over the first ~3 min of interval exercise prior to decreasing over the remainder of the test, whereas the participants in Tsukamoto *et al.*'s study were considerably hypocapnic compared to rest during all of their high-intensity intervals (mean reduction of 5-9 mmHg depending on the interval) (Tsukamoto *et al.*, 2019). Since hypercapnia impairs cerebral autoregulation (Aaslid *et al.*, 1989), improved autoregulation during the later stages of our high intensity interval test are likely attributable, at least in part, to the reduction in eP_aCO_2 observed later in the test. Interestingly though, the group means for the moderate-intensity protocol and

second half of the high-intensity protocol were very close ($\Delta\text{mean MCAv}/\Delta\text{MAP}_{\text{MCA}}$: 0.14 vs. 0.15 $\text{cm}\cdot\text{s}^{-1}\cdot\text{mmHg}^{-1}$, respectively), despite the mean eP_aCO_2 being lower (~ 2 mmHg) for the second half of the high-intensity exercise protocol. Accordingly, other factors in addition to P_aCO_2 may also influence the effectiveness of cerebral autoregulation during exercise.

The dynamics of the autoregulation response predicted by ARMA analysis to an increase in MAP_{MCA} (i.e., rapid increase followed by a decrease towards baseline) is consistent with the transient spikes in MCAv that were associated with rapid changes in MAP at the onset or offset of each higher-intensity exercise interval. Our observation of these MCAv spikes during interval exercise is in agreement with previous reports of transient increases in mean MCAv at the onset of moderate- (Saito *et al.*, 2022) or high-intensity (Labrecque *et al.*, 2020) exercise that were linked to changes in the MAP. Although autoregulation could not effectively buffer the rapid changes in MAP immediately following transitions in work rate, it did effectively buffer the effect of sustained changes in MAP on MCAv through modification of cerebrovascular tone and resistance during both intensities of exercise. CrCP was consistently higher throughout the entire high-intensity interval protocol compared to the moderate-intensity protocol, and CrCP appeared follow the changes in MAP over time. RAP was similar between the two protocols during the recovery intervals while cycling at 25 W but increased by up to $\sim 40\%$ during the on-transients of the high-intensity interval protocol to compensate for an increase in MAP of 20-30 mmHg (Figure 7). Together, these data suggest that both CrCP and RAP increase in response to increased MAP during high-intensity exercise to oppose the effect of pressure on MCAv. Interestingly, this rise in RAP during on-transients was not apparent during the moderate-intensity interval protocol, where RAP instead tended to decrease slightly (Figure 7).

The influence of eP_aCO_2 on MCAv dynamics in the present study is consistent with reported reductions in MCAv across sequential intervals of high-intensity exercise that were linked to reductions in $P_{ET}CO_2$ (Whitaker *et al.*, 2022). Previous work identified that cerebrovascular reactivity to CO_2 was enhanced during exercise compared to rest (Rasmussen *et al.*, 2006; Ogoh *et al.*, 2008; Murrell *et al.*, 2013), but was reduced acutely following high-intensity interval exercise (Burma *et al.*, 2020). Our results build on these findings by demonstrating that CO_2 reactivity was similar at $\sim 3\% \cdot mmHg^{-1}$ of eP_aCO_2 during moderate- and high-intensity exercise. The causal role of eP_aCO_2 on dynamic changes in mean MCAv identified by ARMA analysis during both moderate and high-intensity exercise in the present study does conflict with the report of increased mean MCAv during incremental isocapnic exercise (Smith *et al.*, 2016). There are important differences between the present study and the investigation by Smith *et al.* (Smith *et al.*, 2016), as they focused on investigating the influence of $P_{ET}CO_2$ on steady-state measures of cerebral blood flow and velocity whereas we focused on the dynamic changes during exercise transients. Given that the cerebral vasculature has multiple integrative and redundant mechanisms acting simultaneously to ensure adequate supply of oxygen and nutrients and clearance of metabolic waste products (Claassen *et al.*, 2021), it is plausible that in the absence of an exercise-associated increase in P_aCO_2 , such as during exercise with isocapnic clamping, that an increase in MCAv may occur via a different mechanism (e.g., neurovascular coupling). Importantly, the time course for the change in MCAv in response to a change in eP_aCO_2 during exercise determined by ARMA analysis (Figure 8) exhibited a similar magnitudes of change and temporal response delay as the MCAv response to a step change in $P_{ET}CO_2$ at rest (Poulin *et al.*, 1996, 1998), reinforcing that changes in eP_aCO_2 are contributing to the observed MCAv dynamics. Future work should investigate whether cerebral blood flow

dynamics are different in response to step changes in work rate performed during poikilocapnia versus isocapnia.

A wide variety of interval training protocols are commonly used in real-life, which have various combinations of work and rest intervals (Ito, 2019) that can induce different training adaptations (Kavaliauskas *et al.*, 2015). Pseudorandom binary exercise used in the present study provided a unique exercise stimulus that enabled exploration of the interaction between different intensities of exercise and recovery intervals (30 s, 60 s, and 90 s) on cerebrovascular responses to an increase in work rate. As previously discussed, autoregulation is unable to buffer rapid changes in MAP at the onset of exercise (Labrecque *et al.*, 2020; Saito *et al.*, 2022), which has raised concern about the risk of hyperperfusion and cerebrovascular injury during high-intensity interval exercise in individuals with already compromised cerebrovascular health (Lucas *et al.*, 2015; Calverley *et al.*, 2020). In the present study, we determined that the change in MCAv immediately following an increase in work rate was dependent on both exercise intensity and the preceding duration of recovery, leading to the greatest transient increase in MAP_{MCA} and mean MCAv during high-intensity intervals initiated after only 30 s of active recovery. Interestingly, the recovery interval duration did not influence the magnitude of initial MAP_{MCA} and mean MCAv responses during moderate-intensity interval exercise transitions, which were similar to the response following 90 s of recovery during the high-intensity protocol. Accordingly, short recovery periods appear to exacerbate the pressure stimulus leading to amplified spikes in MCAv at the onset of high-intensity intervals. Moving forward, duration of recovery intervals during high-intensity interval exercise should be systematically investigated to identify whether there are optimal active recovery periods between intervals in order to minimize the magnitude of the transient spike in pressure and MCAv to decrease the possibility of a cerebrovascular event in

potentially at-risk populations. Additional research is also needed to determine whether this initial transient response does actually confer increased risk.

Methodological Considerations

We acknowledge that our ARMA models only utilized MAP_{MCA} and ePaCO_2 to predict mean MCAv responses to interval exercise, and that other factors that can influence MCAv change during exercise, such as cerebral metabolism, $\dot{Q}$, and autonomic outflow (Ogoh & Ainslie, 2009; Smith & Ainslie, 2017). Importantly, ARMA analysis predicted dynamic mean MCAv with small residuals between modeled and measured responses suggesting that changes in MAP_{MCA} and ePaCO_2 explain most of the dynamic changes in MCAv during both moderate- and high-intensity interval exercise, but we cannot rule out potential modifying effects of other factors. However, recent evidence suggests that submaximal dynamic exercise in healthy adults does not cause increased cerebral sympathetic nerve activity (Tymko *et al.*, 2024), and that changes in cardiac output at the onset of exercise do not influence MCAv dynamics (Saito *et al.*, 2022). We also tried to minimize the influence of changes in cerebral metabolism and neurovascular coupling on our measured MCAv responses by using exercise-to-exercise transitions.

In the present study, transcranial Doppler ultrasound was used to measure MCAv as a surrogate index for cerebral blood flow during interval exercise. Interpretation of data obtained using this measurement technique is limited by the unknown MCA diameter and the subsequent assumption that the diameter remains constant during exercise. However, this technique was selected as it enabled stable, continuous measurements of blood velocity during prolonged

periods of upright cycling, which was essential for time-series analysis. We recognize that small changes in MCA diameter have been reported in response to large changes in $P_{ET}CO_2$ (Coverdale *et al.*, 2014; Verbree *et al.*, 2014), and that it is possible that small changes in MCA diameter could occur during interval exercise, meaning that our reported magnitudes of change in MCAv during exercise may be underestimating the change in volumetric cerebral blood flow. However, if our measured changes in eP_aCO_2 during exercise did induce a change in MCA diameter, this would only mean that we are underestimating the importance of fluctuations in P_aCO_2 on cerebral blood flow dynamics.

We estimated P_aCO_2 from measures of $P_{ET}CO_2$ and tidal volume using the Jones equation (Jones *et al.*, 1979), and used pulse contour analysis of the finger arterial pressure waveform with the ModelFlow® algorithm to estimate stroke volume and $\dot{Q}$ during exercise. Importantly, the Jones equation to estimate P_aCO_2 was developed during exercise (Jones *et al.*, 1979) and shows strong agreement with directly measured P_aCO_2 through a wide range of exercise intensities in young healthy adults (Williams & Babb, 1997). The Jones equation does slightly over estimate P_aCO_2 at maximal intensities (Williams & Babb, 1997); however, our $\Delta 50\%$ intervals did not induce a maximal exercise response. Similarly, $\dot{Q}$ estimated by the ModelFlow® algorithm was highly correlated (range of r : 0.90-0.96) with $\dot{Q}$ measured via acetylene rebreathing during both moderate- and high-intensity exercise (Faisal *et al.*, 2009), suggesting that the Modelflow® algorithm provided a valid estimate of the changes in $\dot{Q}$ during exercise.

Lastly, the effect size used to estimate the minimum sample size required for the present study ($n = 7$) was based on previously reported large effect sizes for the effect of different cycling intensities on cerebrovascular tone (Ogoh *et al.*, 2011). We recruited a larger sample ($n = 14$) as a conservative estimate to account for potential differences in exercise intensities between

studies. However, previous studies have never used multiple input ARMA analysis to compare MAP and eP_aCO_2 step responses between intensities of exercise, so it is possible that the present study was underpowered to detect differences between exercise tests using our novel analysis method.

Conclusion

Time-series analysis identified that MCAv dynamics during moderate- and high-intensity interval exercise were largely explained by the combination of dynamic changes in MAP_{MCA} , eP_aCO_2 , and cerebral autoregulation. High-intensity interval exercise induced a wider range of mean MCAv, MAP_{MCA} and eP_aCO_2 than moderate-intensity interval exercise. Cerebral autoregulation was less effective at buffering changes in MAP_{MCA} during the early stages of high-intensity interval exercise, which corresponded to when eP_aCO_2 was elevated, but CO_2 reactivity was not different between exercise intensities. Large transient increases in MCAv due to rapid increases in MAP at the onset of exercise transitions appeared to be exacerbated by shorter recovery intervals during high-intensity interval exercise. Therefore, these findings suggest that mechanisms of cerebrovascular control are affected by the intensity of exercise. Future work should investigate how clamping $P_{ET}CO_2$ affects MCAv dynamics in response to exercise transitions, and identify optimal recovery interval durations for high-intensity interval programs in order to minimize the risk of transmitting rapid and large increases in MAP to the brain.

Chapter 4: Sex differences between post-menopausal women and similar age men in response to orthostatic stress following two weeks of bed rest

Abstract

Reduced orthostatic tolerance is common following periods of bed rest that are associated with illness or surgery, putting individuals at higher risk for syncope and falls following hospitalization. Following menopause, mechanisms of female cardiovascular regulation change, which may be associated with sex-specific responses to orthostatic stress following bed rest. The purpose of our experiment was to investigate sex differences between healthy post-menopausal women and similar age men (age: 55-65 years) for their orthostatic tolerance and cerebrovascular responses to standing following bed rest. Twenty-two late-middle-aged adults (11 women) completed 14 days of head-down bed rest, with half of the participants being randomized into an exercise group that performed high-intensity exercise during bed rest. Supine-to-stand tests were performed before and ~5 h after bed rest. Women had lower orthostatic tolerance than men after bed rest (bed rest x sex interaction: $p=0.004$), without a protective effect of daily exercise. Both men and women were mildly hypocapnic while supine (main effect: $p=0.019$) following bed rest and had lower middle cerebral artery blood velocity (MCAv) nadirs upon standing (main effect: $p=0.027$). During the third minute of standing, both men and women had lower end-tidal PCO_2 (mean effect: $p<0.001$) and MCAv (main effect: $p=0.002$) after bed rest, but only men had increased cerebrovascular resistance index (bed rest x sex interaction: $p=0.005$) and only women were hypotensive (bed rest x sex interaction: $p=0.020$) compared to pre-bed rest. Accordingly, lower MCAv of post-menopausal women and men while standing after bed rest was mediated by different factors.

Introduction

Orthostatic intolerance is a common issue caused by periods of low cerebral perfusion (Van Lieshout *et al.*, 2003), and is often identified when symptoms such as light-headedness, dizziness or syncope occur while standing. Bed rest associated with illness or surgery increases the risk of orthostatic hypotension, decreased cerebral oxygenation and orthostatic intolerance (Jans *et al.*, 2012), putting individuals at higher risk for syncope and falls following hospitalization (Mahoney *et al.*, 2000). Experimental investigations with young healthy men and women reveal profound reductions in orthostatic tolerance and middle cerebral artery blood velocity (MCAv) during orthostatic stress following bed rest (Levine *et al.*, 1997; Zhang *et al.*, 1997; Guinet *et al.*, 2009); however, experimental data about orthostatic tolerance and cerebrovascular responses to upright posture following supine or 6° head-down bed rest [HDBR; a common microgravity analogue model (Hargens & Vico, 2016)], from late-middle-aged and older adults are scarce. Given that the risk of experiencing cerebral hypoperfusion and syncope increases with advancing aging (Soteriades *et al.*, 2002), it is plausible that the effects of bed rest are exacerbated in older individuals. Investigating the effects of HDBR in combination with high-intensity countermeasure exercise in a late middle-aged-cohort may also be particularly relevant for space medicine, as many active astronauts fall within this age group (Kovacs & Shadden, 2017).

Periods of bed rest may induce age- and sex-specific systemic and cerebrovascular hemodynamic responses to orthostatic stress. Younger women typically have lower orthostatic tolerance than younger men (Convertino, 1998; Fu *et al.*, 2004, 2005), with the difference accentuated by hypovolemia (Fu *et al.*, 2005), and pre-menopausal women had lower orthostatic tolerance than men following spaceflight (Fritsch-Yelle *et al.*, 1996b; Waters *et al.*, 2002).

Suggested mechanisms underlying lower orthostatic tolerance of women compared to men include differences in autonomic control of blood pressure (Convertino, 1998), blood volume (Diaz-Canestro *et al.*, 2022), cardiac filling (Fu *et al.*, 2004, 2005), oxygen carrying capacity (Diaz-Canestro *et al.*, 2022), and lower total peripheral resistance responses (TPR) of women following spaceflight (Fritsch-Yelle *et al.*, 1996b; Waters *et al.*, 2002). In contrast to younger healthy adults, no sex difference in orthostatic tolerance of community dwelling older adults was reported (Romero-Ortuno *et al.*, 2010); however, older women are still more likely to present to the emergency department due to syncope (Grossman *et al.*, 2005). Following menopause, female cerebrovascular function (Kastrup *et al.*, 1998; Matteis *et al.*, 1998) and autonomic control of blood pressure (Hart *et al.*, 2011; Barnes *et al.*, 2014) are altered, which could contribute to potential age-related differences in orthostatic tolerance following bed rest between men and women.

Our group previously identified marked reductions in 80° head-up tilt tolerance time immediately after 14 days of bed rest in late-middle-aged adults, without a sex difference between men and women and no protective effects of daily, in-bed high-intensity countermeasure exercise on orthostatic tolerance (Mastrandrea *et al.*, 2023). In this study, we report results from the same cohort during supine-to-stand tests performed pre-bed rest and ~5 h after first being re-introduced to upright posture in order to evaluate effects that may persist in the hours after returning to upright posture and ambulation. Our supine-to-stand test results are likely more clinically relevant and reflective of orthostatic challenges experienced by recently discharged patients compared to typical post-bed rest head-up tilt tests (Kulkarni *et al.*, 2020), which are conventionally performed as the first instance of upright posture (Angerer *et al.*, 2014).

Therefore, the purpose of this study was to investigate sex differences between post-menopausal women and similar age men for their orthostatic tolerance and cerebrovascular responses to standing following two weeks of bed rest, and to determine whether daily countermeasure exercise affected these responses. It was hypothesized that women would have lower orthostatic tolerance than men while standing following bed rest, and that in-bed exercise would not affect the cardiovascular or cerebrovascular responses to orthostatic stress following bed rest. Sex-based effects of bed rest on systemic and cerebrovascular hemodynamics, as well as comparison between stand and tilt tolerance times were explored.

Methods

Participants

Twenty-two healthy 55- to 65-year-old adults completed 14 days of HDBR as part of multi-investigator clinical trial (see Table 3 for participant characteristics). Detailed participant inclusion and exclusion criteria for this study were described by Hajj-Boutros et al. (Hajj-Boutros *et al.*, 2024), and changes in bed rest standard measures (e.g., tilt tolerance, cardiorespiratory fitness, anthropometric data, body composition, muscle strength, select blood biomarkers, etc.) have been previously published (Hajj-Boutros *et al.*, 2023; Hedge *et al.*, 2023; Mastrandrea *et al.*, 2023). The sample size and design of study, with 11 men and 11 women being randomized into either an exercise or control group, was set by the funding agency. It was anticipated that the study design including 11 men and 11 women would be sufficient to identify sex-specific effects of bed rest on orthostatic tolerance based on: i) large effect sizes for pre- to post-bed rest reductions in orthostatic tolerance (Levine *et al.*, 1997; Watenpaugh *et al.*, 2007;

Hastings *et al.*, 2012), ii) the lack of effect of exercise countermeasures without artificial gravity or fluid loading on post-bed rest orthostatic tolerance (Watenpaugh *et al.*, 2007; Shibata *et al.*, 2010; Hastings *et al.*, 2012), and iii) a large effect size for sex-differences between men and women for orthostatic tolerance under hypovolemic conditions (Fu *et al.*, 2005).

Briefly, all participants were regularly active (≥ 2.5 h of self-reported moderate-to-vigorous physical activity per week), free of physical or mental illness, and had no history of smoking within at least 6 months of starting the study. Female participants were at least 1-year post-menopausal and had measured follicle-stimulating hormone levels >30 IU/L. For all data collection sessions, participants were at least 2 h post-prandial, and had not consumed caffeine on the day of the test prior to the session. This study was conducted according to the declaration of Helsinki, and all procedures were approved by the University of Waterloo's Clinical Research Ethics Committee (ORE No. 40420) and McGill University Research Ethics Board (IRB00010120), as well as registered as a clinical trial (NCT04964999). All participants signed an informed consent form prior to participating in the study and were made aware of their right to withdraw at any time.

Table 3. Pre-bed rest participant characteristics.

Characteristic	<u>Control</u>		<u>Exercise</u>	
	Men (n=6)	Women (n=5)	Men (n=5)	Women (n=6)
Age, years	61 $\pm$ 4	57 $\pm$ 1	58 $\pm$ 2	61 $\pm$ 3
Height, m	1.71 $\pm$ 0.13	1.61 $\pm$ 0.05	1.75 $\pm$ 0.04	1.61 $\pm$ 0.04
Mass kg	75.8 $\pm$ 15.1	58.1 $\pm$ 7.9	84.2 $\pm$ 5.6	62.8 $\pm$ 9.5
BMI, kg·m⁻²	25.7 $\pm$ 2.4	22.2 $\pm$ 2.0	27.5 $\pm$ 1.2	24.3 $\pm$ 3.5
BSA, m²	1.88 $\pm$ 0.26	1.61 $\pm$ 0.12	2.00 $\pm$ 0.09	1.65 $\pm$ 0.12

Means $\pm$ SD. BMI, body mass index; BSA, body surface area.

Bed Rest and Exercise Countermeasures

Participants stayed at the McGill University Health Centre in Montreal, Canada for 26 consecutive days. This period consisted of: a) 5 days of baseline data collection, where participants were free to get out of bed and ambulate, b) 14 days of strict 6° head-down bed rest (pillows allowed), and c) 1 week of post-bed rest data collection as well as supervised recovery and rehabilitation. Importantly, during the bed rest phase of the study, participants completed all activities, including showering, eating, and toileting in the head-down tilt position. Participants were randomly allocated into either the exercise intervention (5 men, 6 women) or sedentary control (6 men, 5 women) groups.

Participants in the exercise group performed 3 exercise sessions per day throughout the 14 days of HDBR, with sessions occurring in the morning, afternoon and evening in order to break-up prolonged sedentary periods, as previously described in detail (Hedge *et al.*, 2022b, 2023). Briefly, the total exercise time per day was ~1 h, with exercise sessions consisting of a mixture of high-intensity interval cycling, constant-load cycling, as well as upper- and lower-body resistance exercises. All exercise sessions were completed while remaining in bed, including cycling on a supine ergometer (ergoselect 8, ergoline GmbH, Bitz, Germany) that was tilted to -6°, and resistance exercises, which were performed using a machine with weighted cables (Model P246, Atlantis Inc., Laval, Canada), resistance bands (THERABAND, Akron, USA or COREFX, Oakville, Canada), or body weight. Conversely, participants in the control group remained inactive in bed for the entire 14 days, but did receive daily passive manual therapy from a physiotherapist.

Supine-to-Stand Test

Supine-to-stand tests were performed near mid-day on both the second day of baseline data collection (PRE) and the first day after bed rest (POST), with testing occurring ~5 h after participants' first instance of upright posture. Prior to the post-bed rest supine-to-stand test, participants completed the head-up tilt test to pre-syncope at ~7:30am (Mastrandrea *et al.*, 2023). Following the tilt test, participants laid down for recovery, and were transferred to another room for further postural testing that they completed ~9:30am. After this, participants were free to ambulate under supervision, ate breakfast, and performed a battery of cognitive assessments while seated in the upright position. Participants then walked to the testing location where the post-bed rest supine-to-stand test occurred at ~1pm. Two women (1 exercise, 1 control) were unable to attempt the supine-to-stand on the first day following bed rest out of caution after they experienced severe symptoms of orthostatic intolerance during their first instance of upright posture, and instead completed their post-bed rest supine-to-stand tests on the third day of recovery. The supine-to-stand test consisted of ~10 min of supine rest during the instrumentation phase followed by a 3-min period of quiet, supine baseline data collection. Participants were then instructed to transition from the supine position to standing with their arms crossed in front of their chest for up to a maximum of 8 min facing towards a plain wall. During the standing phase of the test, participants were asked to stand quietly, with their feet flat on the floor. The standing phase of the test was stopped prior to reaching 8 min if any of the following situations occurred: a) participant request to stop due to symptoms of orthostatic intolerance, or b) cardiovascular measurements falling outside of the predefined safety parameters (systolic brachial blood pressure < 90 mmHg, or a reduction in heart rate > 15 beats·min⁻¹).

Throughout the supine and standing periods, lead-II electrocardiography (ECG module, Finapres Medical Systems, Enschede, Netherlands), MCAv, arterial blood pressure, and expired concentrations of CO₂ were recorded at 1000 Hz (PowerLab and Labchart, version 7.3.7; AD Instruments, Colorado Springs, USA). MCAv data were collected using a 2 MHz transcranial Doppler ultrasound probe (WAKIe 2TC, Atys Medical, Soucieu-en-Jarrest, France), which was held in place over the right temporal insonation window using a headset (Spencer Technologies, Seattle, USA). The Doppler ultrasound probe angle, depth, power, and gain were consistent from pre- to post-bed rest, with the same testing team performing all of the measures. Continuous finger arterial blood pressure was measured using the volume-clamp method (Finapres NOVA, Finapres Medical Systems, Enschede, Netherlands), and values were reconstructed to brachial pressure, corrected to the level of the heart using a height correction unit, and calibrated using two brachial pressure measures obtained using an arm cuff. Arterial pressure at the level of the MCA was subsequently estimated after accounting for the orthostatic pressure gradient between the transcranial Doppler ultrasound probe and the heart when in the upright posture [correction factor = distance (in cm)·0.78 mmHg·cm⁻¹]. Stroke volume was estimated for each cardiac cycle via pulse contour analysis of the finger arterial waveform using the Modelflow® algorithm (Wesseling *et al.*, 1993), and normalized for body surface area (Du Bois & Du Bois, 1916). Expired air was sampled via nasal cannula, and CO₂ concentrations were measured using an infrared carbon dioxide sensor (model 17501, Vacumed, Ventura, USA or CD-3A, AMETEK AEI Technologies, Pittsburgh, USA). End-tidal CO₂ concentrations were identified for each breath and converted into partial pressures (P_{ET}CO₂) based on the ambient temperature and pressure recorded during data collection.

Beat-by-beat analysis of heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), estimated stroke volume, and blood velocity were gated using the electrocardiogram's R-wave. MAP and MCAv were calculated as the average pressure and outer envelope of the transcranial Doppler spectrum over each cardiac cycle, respectively. Cerebrovascular resistance index (CVRI) was calculated as $MAP_{MCA} / \text{mean MCAv}$. Estimated cardiac output ($\dot{Q}$) was the product of the estimated stroke volume and heart rate, and also normalized for body surface area. TPR was calculated as the quotient of $MAP_{Brachial} / \dot{Q}$. The foot of the expired CO_2 waveform was used to identify the onset of expiration and calculate breathing frequency.

Beat-by-beat and breath-by-breath data were linearly interpolated to 1 Hz (MATLAB 2020b, The MathWorks Inc., Natick, USA). One-min averages were calculated for all cardiovascular and respiratory variables while supine, and 30-s averages were used while standing. The supine average was calculated from 30-90 s prior to attaining standing posture in order to avoid contaminating it with the transition period between supine and standing posture. The 30-s standing average was calculated from 150-180 s after being upright, as this is a typical time point for clinical tests of orthostatic hypotension (Shibao *et al.*, 2013), and it is also the shortest duration of time which was tolerated by all participants post-bed rest. Mean arterial pressure nadir was identified as the minimum 1 Hz value once standing upright, with the values of other variables recorded at this time point. The magnitudes of the initial drop upon standing was calculated as the difference between supine and nadir values for arterial blood pressure, MCAv, and CVRI.

Statistical Analysis

Statistical analyses were conducted in R (v. 4.0.3). Linear mixed models with fixed effects of bed rest (PRE vs. POST), sex (male vs. female), and group (control vs. exercise), and a random effect of participant were used to evaluate how bed rest, sex, and exercise affected orthostatic tolerance, and cardiovascular and respiratory responses in each posture [library: lmerTest (Kuznetsova *et al.*, 2017)]. In the case of significant effects, pairwise comparisons of the estimated marginal means with Holm correction for multiple comparisons were used for *post hoc* analysis [library: emmeans (Lenth *et al.*, 2022)]. Effect sizes for main effects/interactions and pairwise comparisons were calculated as partial eta-squared (η_p^2) and Cohen's *d*, respectively. Statistical significance was set to $p \leq 0.05$, and data are reported as means $\pm$ standard deviation.

Results

Orthostatic Tolerance

Tilt tolerance responses from pre-bed rest and immediately post-bed rest (i.e., the first instance of upright posture after bed rest) for this cohort have previously been published (Mastrandrea *et al.*, 2023), and identified reduced tilt tolerance times after bed rest for both men and women without a protective effect of the exercise countermeasure. For the supine-to-stand test, all participants were able to complete 8 min of standing pre-bed rest, but 7 women and 1 man were unable to complete it post-bed rest. Two of the women who were able to complete the full 8 min of standing post-bed rest were unable to be tested until recovery day-3. Accordingly, a bed rest x sex interaction ($p = 0.004$, $\eta_p^2 = 0.33$) was observed for orthostatic tolerance time

during the stand test performed ~5 h after the initial tilt test, with women having shorter standing tolerance times than men after bed rest ($p < 0.001$; Figure 12). Importantly, the exercise countermeasure did not protect orthostatic tolerance during the stand test (bed rest x sex x group interaction: $p = 0.822$, $\eta_p^2 = 0.002$; bed rest x group interaction: $p = 0.574$, $\eta_p^2 = 0.01$). Two tests were terminated by participant request due to feeling symptoms of intolerance prior to a fall in blood pressure, and the other six tests were terminated due to a reduction in blood pressure; however, most participants who had the test terminated due to low blood pressure still reported feeling symptoms of intolerance during the test (e.g., feeling hot, sweaty, shaking), albeit after the test was stopped.

The exercise countermeasure had minimal effects on cardiovascular or cerebrovascular responses during the supine-to-stand test (Tables 2, 3, 5), apart from the magnitude of drop in arterial blood pressure and MCAv from supine to nadir (Table 6). For visualization purposes only, exercise and control group participants' data were pooled to illustrate the bed rest x sex interactions for the average cardiovascular and cerebrovascular time-series responses for men and women during first 3 min of the supine-to-stand transition (Figure 13). Two women in the control group had poor temporal insonation windows, preventing measurement of their MCAv responses. One of these two women also intentionally manipulated her breathing throughout the standing portion of the supine-to-stand test, leading to exclusion of her data from further analysis. Artifact during the posture transition also precluded identification of nadirs in 3 other participants.

Supine Response

A summary of supine responses split by group, sex, and session is presented in Table 4. The exercise countermeasure had no effect on variables during supine rest (all $p > 0.05$). Following bed rest, both men and women had elevated supine heart rates (main effect: $p < 0.001$, $\eta_p^2 = 0.65$) and reduced stroke volume index (SVi; main effect: $p = 0.011$, $\eta_p^2 = 0.32$), without a change in cardiac index ($\dot{Q}_i$; main effect: $p = 0.846$, $\eta_p^2 = 0.002$) or TPR (main effect: $p = 0.970$, $\eta_p^2 < 0.001$). Interactions between the effects of bed rest and sex were observed for supine MAP_{Brachial} ($p = 0.030$, $\eta_p^2 = 0.25$) and SBP_{Brachial} (main effect: $p = 0.026$, $\eta_p^2 = 0.26$); however, pairwise comparisons only identified that supine SBP_{Brachial} ($p = 0.027$, $d = 1.40$), but not MAP_{Brachial} ($p = 0.235$, $d = 0.93$), was reduced for women following bed rest. Supine mean MCAv was not different from pre- to post-bed rest (main effect: $p = 0.271$, $\eta_p^2 = 0.08$), but P_{ETCO₂} was reduced for both men and women (main effect: $p = 0.019$, $\eta_p^2 = 0.28$). There was a sex x bed rest interaction for CVRi ($p = 0.034$, $\eta_p^2 = 0.25$), with men tending to have higher supine CVRi than women post-bed rest ($p = 0.060$, $d = 1.85$).

Initial Orthostatic Response

Upon standing, MAP_{Brachial} reached its nadir an average of ~5s after standing before bed rest, and ~6s after standing after bed rest. No sex differences or interactions between the effects of bed rest, sex, and group were identified for any of the measured cardiovascular and respiratory variables at the timing of the MAP nadir (Table 5). The absolute values of brachial blood pressure nadirs for MAP (main effect: $p = 0.554$, $\eta_p^2 = 0.03$), SBP (main effect: $p = 0.083$, $\eta_p^2 = 0.20$), and DBP (main effect: $p = 0.762$, $\eta_p^2 = 0.007$) were not different after bed rest. At the time

of the MAP nadir, heart rate was increased (main effect: $p < 0.001$, $\eta_p^2 = 0.79$) and SVi was reduced (main effect: $p < 0.001$, $\eta_p^2 = 0.60$) post-bed rest. In men and women, the post-bed rest reduction in $\dot{Q}_i$ (main effect: $p = 0.004$, $\eta_p^2 = 0.46$) was compensated for by an increase in TPR (main effect: $p = 0.016$, $\eta_p^2 = 0.35$). Despite the absolute value of the MAP_{MCA} nadir being unchanged by bed rest, a lower mean MCAv nadir was measured in men and women following bed rest (main effect: $p = 0.027$, $\eta_p^2 = 0.32$), with men having lower MCAv nadirs than women throughout the study (sex main effect: $p = 0.039$, $\eta_p^2 = 0.29$). CVRi at the MAP_{MCA} nadir was not different from pre- to post-bed rest (main effect: $p = 0.507$, $\eta_p^2 = 0.03$). P_{ETCO₂} at the MAP nadir was reduced following bed rest for all combinations of group and sex (main effect: $p < 0.001$, $\eta_p^2 = 0.70$); however, a bed rest x group interaction was observed ($p = 0.011$, $\eta_p^2 = 0.38$), identifying much lower P_{ETCO₂} at the MAP nadir in the exercise group following bed rest ($p < 0.001$, $d = 3.00$).

Examination of the magnitude of reduction (i.e., nadir – supine) in SBP_{Brachial} ($p = 0.029$, $\eta_p^2 = 0.30$) and MCAv ($p = 0.032$, $\eta_p^2 = 0.31$) revealed bed rest x group interactions (Table 6). The magnitude of reduction in SBP_{Brachial} initially upon standing after bed rest was greater in the exercise group than the control group ($p = 0.003$, $d = 2.26$). Although there was no bed rest x group x sex interaction ($p = 0.148$, $\eta_p^2 = 0.14$), the bed rest x group interaction appeared to be driven by men of the exercise group having twice as large a drop in SBP_{Brachial} upon standing following bed rest, and smaller magnitude drops in SBP_{Brachial} after bed rest in the control group (Table 6). A similar tendency was also observed for the drop in MAP_{Brachial} ($p = 0.067$, $\eta_p^2 = 0.22$). The exercise group also experienced larger magnitude drops in MCAv than the control group following bed rest ($p = 0.018$, $d = 1.56$).

Standing Response

After standing for 3 min, heart rate was increased ($p < 0.001$, $\eta_p^2 = 0.80$) and SVi was reduced ($p < 0.001$, $\eta_p^2 = 0.69$) for both men and women post-bed rest, but $\dot{Q}_i$ ($p = 0.076$, $\eta_p^2 = 0.17$) and TPR ($p = 0.415$, $\eta_p^2 = 0.04$) were not different (Table 7). Bed rest x sex interactions were observed for $MAP_{Brachial}$ ($p = 0.020$, $\eta_p^2 = 0.28$), $SBP_{Brachial}$ ($p = 0.020$, $\eta_p^2 = 0.28$), and $DBP_{Brachial}$ ($p = 0.032$, $\eta_p^2 = 0.24$). Women had lower $MAP_{Brachial}$ ($p = 0.026$, $d = 1.42$) and $SBP_{Brachial}$ ($p = 0.001$, $d = 2.059$) during standing after bed rest, while men's $MAP_{Brachial}$ ($p > 0.99$, $d = 0.19$) and $SBP_{Brachial}$ ($p = 0.613$, $d = 0.451$) responses to standing were not different from pre- to post-bed rest. No *post hoc* pairwise comparisons were identified as different for $DBP_{Brachial}$ (all $p > 0.293$). Mean MCAv while standing was reduced in both men and women after bed rest (main effect: $p = 0.002$, $\eta_p^2 = 0.47$), with both sexes also having reduced P_{ETCO_2} (main effect: $p < 0.001$, $\eta_p^2 = 0.61$). Despite both MCAv and P_{ETCO_2} being reduced for men and women after bed rest, a bed rest x sex interaction was observed for standing CVRi ($p = 0.005$, $\eta_p^2 = 0.39$). CVRi of men was higher than women after bed rest ($p = 0.013$, $d = 2.35$), with the CVRi of male participants increasing from pre- to post-bed rest ($p = 0.042$, $d = 1.18$), and the CVRi of female participants not changing ($p = 0.160$, $d = 0.934$).

Table 4. Summary of cardiovascular and respiratory variables while supine before and after 14 days of head-down bed rest.

Supine	Sex	Control		Exercise		Bed Rest	Sex	Bed Rest	Bed Rest	Bed Rest			
		6M / 4W, 3W for MCAv + CVRi		5M / 6W							Sex	Group	Group
		PRE	POST	PRE	POST								
Heart rate, beats·min ⁻¹	Men	64 ± 11	80 ± 11	55 ± 7	69 ± 10	<0.001	0.801	0.226	0.718	0.493			
	Women	64 ± 14	72 ± 3	62 ± 6	74 ± 9								
SVi, mL·m ⁻²	Men	43.2 ± 13.0	34.1 ± 10.0	57.5 ± 13.9	42.4 ± 7.6	0.011	0.996	0.363	0.775	0.236			
	Women	52.0 ± 14.0	41.1 ± 4.6	42.6 ± 6.6	41.4 ± 18.8								
Qi, L·min ⁻¹ ·m ⁻²	Men	2.7 ± 0.7	2.7 ± 0.6	3.1 ± 0.6	2.9 ± 0.7	0.846	0.750	0.653	0.479	0.242			
	Women	3.2 ± 0.3	2.9 ± 0.3	2.6 ± 0.3	3.0 ± 1.1								
MAP _{Brachial} , mmHg	Men	100 ± 13	102 ± 19	92 ± 12	99 ± 12	0.562	0.581	0.030	0.360	0.864			
	Women	99 ± 4	89 ± 8	99 ± 7	95 ± 12								
SBP _{Brachial} , mmHg	Men	131 ± 20	130 ± 22	129 ± 17	132 ± 18	0.056	0.219	0.026	0.226	0.501			
	Women	133 ± 7	113 ± 10*	127 ± 8	119 ± 15*								
DBP _{Brachial} , mmHg	Men	81 ± 10	85 ± 18	71 ± 10	79 ± 9	0.669	0.493	0.058	0.511	0.833			
	Women	77 ± 6	72 ± 6	80 ± 7	77 ± 11								
TPR, mmHg·min·L ⁻¹	Men	21.4 ± 7.2	21.4 ± 6.6	15.3 ± 3.9	17.6 ± 4.1	0.970	0.332	0.352	0.860	0.473			
	Women	19.9 ± 2.2	19.4 ± 1.6	23.5 ± 5.5	21.6 ± 7.9								
Mean MCAv, cm·s ⁻¹	Men	49.9 ± 11.3	42.7 ± 11.4	45.7 ± 9.5	44.3 ± 10.7	0.271	0.136	0.434	0.423	0.620			
	Women	54.5 ± 4.8	53.1 ± 8.2	49.4 ± 9.4	49.4 ± 4.0								
CVRi, mmHg·s·cm ⁻¹	Men	2.11 ± 0.59	2.60 ± 0.96	2.07 ± 0.42	2.28 ± 0.30	0.509	0.102	0.034	0.658	0.461			
	Women	1.87 ± 0.21	1.64 ± 0.15	2.09 ± 0.48	1.93 ± 0.31								
f _b , breaths·min ⁻¹	Men	13 ± 4	12 ± 4	16 ± 8	15 ± 3	0.825	0.986	0.268	0.851	0.654			
	Women	14 ± 6	14 ± 4	14 ± 3	15 ± 3								
P _{ET} CO ₂ , mmHg	Men	39.8 ± 3.0	37.3 ± 3.9	39.6 ± 2.1	37.3 ± 2.8	0.019	0.281	0.555	0.832	0.716			
	Women	40.2 ± 2.9	39.2 ± 4.5	41.2 ± 2.9	39.3 ± 4.4								

Means±SD. M/W, number of men and women in each group; PRE, pre-head-down bed rest; POST, post-head down bed rest; SVi, stroke volume index; Qi, cardiac index; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; TPR, total peripheral resistance; MCAv, middle cerebral artery blood velocity; CVRi, cerebrovascular resistance index; fb, breathing frequency; PETCO₂, end-tidal partial pressure of carbon dioxide. Blood pressure measures with subscripts of brachial or MCA indicate the location of measurement. *, different ($p \leq 0.05$) from PRE for a given sex.

Table 5. Summary of initial orthostatic response to standing before and after 14 days of head-down bed rest.

Value at MAP Nadir	Sex	Control		Exercise		Bed Rest	Sex	Bed Rest Sex	Bed Rest Group	Bed Rest Group Sex
		5M, 4M for MCAv + CVRi / 3W		4M / 6W						
		PRE	POST	PRE	POST					
Heart rate, beats·min ⁻¹	Men	84 ± 13	95 ± 12	81 ± 5	96 ± 6	<0.001	0.546	0.139	0.267	0.741
	Women	81 ± 14	89 ± 5	83 ± 10	92 ± 8					
SVi, mL·m ⁻²	Men	44.3 ± 12.1	26.9 ± 4.7	53.8 ± 16.8	33.4 ± 8.4	<0.001	0.939	0.320	0.885	0.570
	Women	50.4 ± 14.9	36.0 ± 8.2	40.0 ± 7.4	30.5 ± 15.9					
Qi, L·min ⁻¹ ·m ⁻²	Men	3.6 ± 0.6	2.6 ± 0.7	4.3 ± 1.3	3.2 ± 0.8	0.004	0.618	0.478	0.940	0.846
	Women	4.0 ± 0.5	3.2 ± 0.8	3.3 ± 0.5	2.6 ± 1.2					
MAP _{Brachial} , mmHg	Men	77 ± 17	89 ± 19	72 ± 9	62 ± 17	0.554	0.419	0.497	0.381	0.141
	Women	81 ± 8	72 ± 13	66 ± 11	63 ± 17					
SBP _{Brachial} , mmHg	Men	105 ± 19	110 ± 20	103 ± 8	80 ± 18	0.083	0.140	0.830	0.435	0.082
	Women	106 ± 15	90 ± 17	83 ± 14	78 ± 20					
DBP _{Brachial} , mmHg	Men	62 ± 15	78 ± 18	57 ± 10	52 ± 17	0.762	0.391	0.378	0.287	0.220
	Women	63 ± 6	60 ± 10	55 ± 8	53 ± 17					
MAP _{MCA} , mmHg	Men	47 ± 17	58 ± 18	44 ± 8	33 ± 20	0.487	0.676	0.480	0.345	0.158
	Women	53 ± 7	44 ± 13	39 ± 10	35 ± 17					
SBP _{MCA} , mmHg	Men	75 ± 19	79 ± 19	74 ± 7	51 ± 20	0.068	0.238	0.820	0.404	0.092
	Women	79 ± 15	63 ± 17	56 ± 13	50 ± 19					
DBP _{MCA} , mmHg	Men	32 ± 15	47 ± 18	29 ± 10	23 ± 21	0.828	0.673	0.360	0.253	0.248
	Women	35 ± 5	32 ± 11	28 ± 8	25 ± 16					
TPR, mmHg·min·L ⁻¹	Men	11.8 ± 4.2	19.8 ± 6.9	9.0 ± 3.4	10.0 ± 3.4	0.016	0.423	0.631	0.425	0.113
	Women	13.0 ± 1.3	15.0 ± 4.7	12.4 ± 2.2	16.8 ± 7.8					
Mean MCAv, cm·s ⁻¹	Men	38.3 ± 4.3	34.2 ± 5.3	33.5 ± 8.3	26.9 ± 7.7	0.027	0.039	0.566	0.128	0.390
	Women	44.0 ± 9.8	45.0 ± 8.9	39.0 ± 5.6	31.3 ± 5.3					
CVRi, mmHg·s·cm ⁻¹	Men	1.33 ± 0.59	1.90 ± 1.20	1.33 ± 0.23	1.36 ± 0.34	0.507	0.115	0.188	0.691	0.172
	Women	1.22 ± 0.11	0.97 ± 0.09	1.06 ± 0.37	1.10 ± 0.49					
P _{ET} CO ₂ , mmHg	Men	36.6 ± 3.4	34.6 ± 3.1	37.0 ± 1.6	29.7 ± 3.8*	<0.001	0.759	0.223	0.011	0.299
	Women	34.6 ± 0.8	32.8 ± 2.5	36.5 ± 3.7	32.4 ± 2.0*					

Means±SD. M/W, number of men and women in each group; PRE, pre-head-down bed rest; POST, post-head down bed rest; SVi, stroke volume index; Qi, cardiac index; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure;

TPR, total peripheral resistance; MCAv, middle cerebral artery blood velocity; CVRi, cerebrovascular resistance index; f_b , breathing frequency; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide. Blood pressure measures with subscripts of brachial or MCA indicate the location of measurement. *, different ($p \leq 0.05$) from PRE for a given sex.

Table 6. Summary of magnitude of change in cardiovascular variables from supine to nadir during the initial orthostatic response to standing before and after 14 days of head-down bed rest.

Nadir – Supine Value	Sex	Control		Exercise		Bed Rest	Sex	Bed Rest Sex	Bed Rest Group	Bed Rest Group Sex
		5M, 4M for MCAv + CVRi / 3W		4M / 6W						
		PRE	POST	PRE	POST					
MAP _{Brachial} , mmHg	Men	-22 ± 5	-13 ± 9	-20 ± 14	-37 ± 8	0.986	0.679	0.313	0.067	0.171
	Women	-20 ± 10	-14 ± 12	-33 ± 13	-31 ± 15					
SBP _{Brachial} , mmHg	Men	-26 ± 7	-21 ± 13	-25 ± 14	-52 ± 10‡	0.564	0.660	0.051	0.029	0.148
	Women	-30 ± 12	-20 ± 15	-44 ± 17	-41 ± 18‡					
DBP _{Brachial} , mmHg	Men	-18 ± 7	-6 ± 9	-16 ± 15	-27 ± 9	0.689	0.744	0.755	0.093	0.204
	Women	-14 ± 8	-9 ± 10	-25 ± 12	-24 ± 14					
Mean MCAv, cm·s ⁻¹	Men	-11.1 ± 7.1	-12.2 ± 9.0	-9.4 ± 7.0	-16.7 ± 4.5*	0.066	0.835	0.638	0.032	0.565
	Women	-10.5 ± 5.1	-8.0 ± 6.2	-10.4 ± 6.7	-18.1 ± 6.0*					
CVRi, mmHg·s·cm ⁻¹	Men	-0.79 ± 0.29	-0.58 ± 0.36	-0.87 ± 0.23	-0.96 ± 0.52	0.449	0.973	0.888	0.830	0.190
	Women	-0.65 ± 0.10	-0.67 ± 0.23	-1.03 ± 0.41	-0.83 ± 0.36					
P _{ET} CO ₂ , mmHg	Men	-3.8 ± 1.7	-3.4 ± 1.8	-2.2 ± 3.3	-7.9 ± 2.6	0.045	0.380	0.522	0.057	0.268
	Women	-4.5 ± 1.4	-5.1 ± 4.9	-4.7 ± 3.0	-7.0 ± 3.4					

Means±SD. M/W, number of men and women in each group; PRE, pre-head-down bed rest; POST, post-head down bed rest; SVi, stroke volume index; Qi, cardiac index; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; TPR, total peripheral resistance; MCAv, middle cerebral artery blood velocity; CVRi, cerebrovascular resistance index; f_b, breathing frequency; P_{ET}CO₂, end-tidal partial pressure of carbon dioxide. Blood pressure measures with subscripts of brachial or MCA indicate the location of measurement. *, different ($p \leq 0.05$) from PRE for a given sex. ‡, different ($p \leq 0.05$) from control group for a session.

Table 7. Summary of cardiovascular and respiratory variables while standing before and after 14 days of head-down bed rest.

Standing at 3 min	Sex	Control		Exercise		Bed Rest	Sex	Bed Rest Sex	Bed Rest Group	Bed Rest Group Sex
		6M / 4W, 3W for MCAv + CVRi		5M / 6W						
		PRE	POST	PRE	POST					
Heart rate, beats·min ⁻¹	Men	73 ± 14	102 ± 16	71 ± 13	110 ± 16	<0.001	0.405	0.138	0.473	0.511
	Women	80 ± 20	102 ± 12	85 ± 12	108 ± 19					
SVi, mL·m ⁻²	Men	37.3 ± 7.6	23.3 ± 5.7	39.9 ± 10.2	26.6 ± 9.5	<0.001	0.204	0.574	0.228	0.294
	Women	38.5 ± 12.8	22.4 ± 5.7	27.8 ± 7.0	21.3 ± 9.8					
Q̇i, L·min ⁻¹ ·m ⁻²	Men	2.7 ± 0.5	2.3 ± 0.4	2.8 ± 0.7	2.9 ± 1.0	0.076	0.306	0.429	0.113	0.805
	Women	2.9 ± 0.4	2.3 ± 0.4	2.3 ± 0.2	2.2 ± 0.8					
MAP _{Brachial} , mmHg	Men	113 ± 17	115 ± 24	105 ± 15	108 ± 21	0.067	0.416	0.020	0.714	0.740
	Women	110 ± 6	90 ± 19*	117 ± 9	102 ± 20*					
SBP _{Brachial} , mmHg	Men	146 ± 20	141 ± 27	140 ± 21	132 ± 31	<0.001	0.165	0.020	0.666	0.470
	Women	142 ± 11	106 ± 23*	143 ± 13	118 ± 24*					
DBP _{Brachial} , mmHg	Men	93 ± 15	101 ± 22	87 ± 12	95 ± 17	0.786	0.492	0.032	0.879	0.903
	Women	90 ± 7	79 ± 16	100 ± 8	91 ± 18					
MAP _{MCA} , mmHg	Men	83 ± 18	84 ± 23	77 ± 11	79 ± 21	0.050	0.641	0.018	0.736	0.845
	Women	83 ± 5	63 ± 20*	90 ± 9	74 ± 19*					
SBP _{MCA} , mmHg	Men	116 ± 21	110 ± 27	111 ± 18	103 ± 30	<0.001	0.248	0.020	0.685	0.541
	Women	115 ± 10	79 ± 24*	116 ± 14	89 ± 23*					
DBP _{MCA} , mmHg	Men	63 ± 15	70 ± 22	58 ± 7	66 ± 19	0.683	0.787	0.029	0.906	0.981
	Women	63 ± 7	52 ± 18	73 ± 8	63 ± 17					
TPR, mmHg·min·L ⁻¹	Men	23.9 ± 8.1	28.1 ± 9.7	20.0 ± 6.8	20.9 ± 7.6	0.415	0.117	0.721	0.683	0.709
	Women	24.6 ± 3.1	25.7 ± 4.3	31.4 ± 6.6	32.3 ± 14.1					
Mean MCAv, cm·s ⁻¹	Men	43.2 ± 10.2	35.0 ± 10.6	37.9 ± 5.9	33.3 ± 11.5	0.002	0.103	0.280	0.634	0.081
	Women	45.4 ± 2.3	45.0 ± 6.1	44.9 ± 5.3	38.3 ± 3.8					
CVRi, mmHg·s·cm ⁻¹	Men	2.06 ± 0.78	2.59 ± 1.06*†	2.05 ± 0.28	2.43 ± 0.27*†	0.714	0.059	0.005	0.403	0.162
	Women	1.88 ± 0.06	1.22 ± 0.17	2.03 ± 0.29	1.97 ± 0.59					
f _b , breaths·min ⁻¹	Men	13 ± 6	14 ± 4	11 ± 5	13 ± 4	0.172	0.116	0.458	0.605	0.485
	Women	13 ± 7	19 ± 12	16 ± 5	17 ± 5					
P _{ET} CO ₂ , mmHg	Men	37.8 ± 4.4	33.9 ± 5.5	35.9 ± 2.1	29.1 ± 5.4	<0.001	0.974	0.642	0.478	0.560
	Women	36.6 ± 7.4	30.3 ± 5.8	38.0 ± 3.2	31.5 ± 4.7					

Means $\pm$ SD. M/W, number of men and women in each group; PRE, pre-head-down bed rest; POST, post-head down bed rest; SVi, stroke volume index; $\dot{Q}_i$, cardiac index; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; TPR, total peripheral resistance; MCAv, middle cerebral artery blood velocity; CVRi, cerebrovascular resistance index; f_b , breathing frequency; $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide. Blood pressure measures with subscripts of brachial or MCA indicate the location of measurement. *, different ($p\leq 0.05$) from PRE for a given sex. †, different ($p\leq 0.05$) from other sex at a given session.

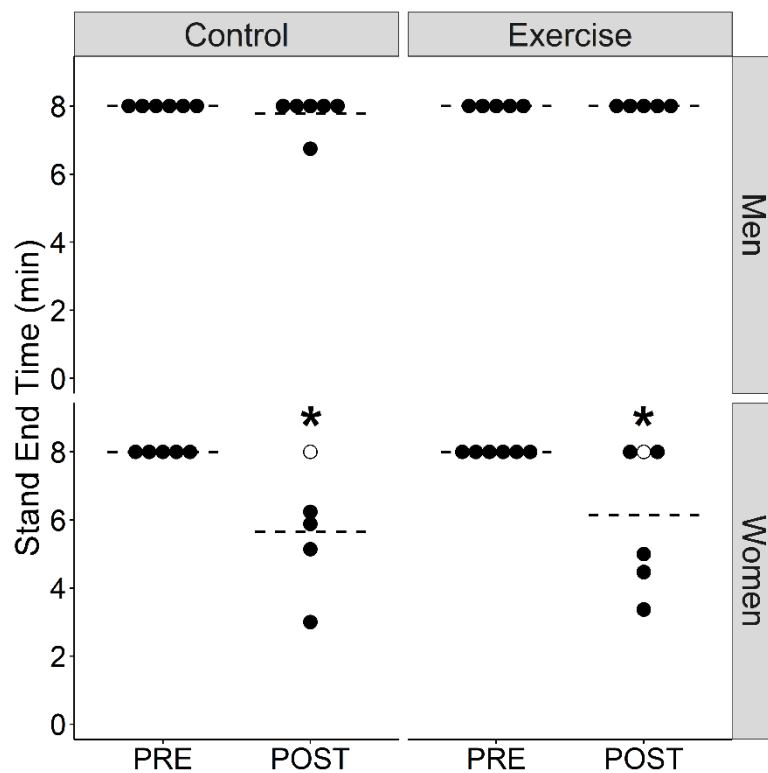


Figure 12. Comparison of stand test tolerance times between men and women, with tests performed before (PRE) and after (POST) bed rest. The maximum standing time achieved during the test was 8 min. Two female participants are identified in white, as they were tested on the third day after bed rest. Dashed lines represent means. *, different ($p \leq 0.05$) from men during a given session.

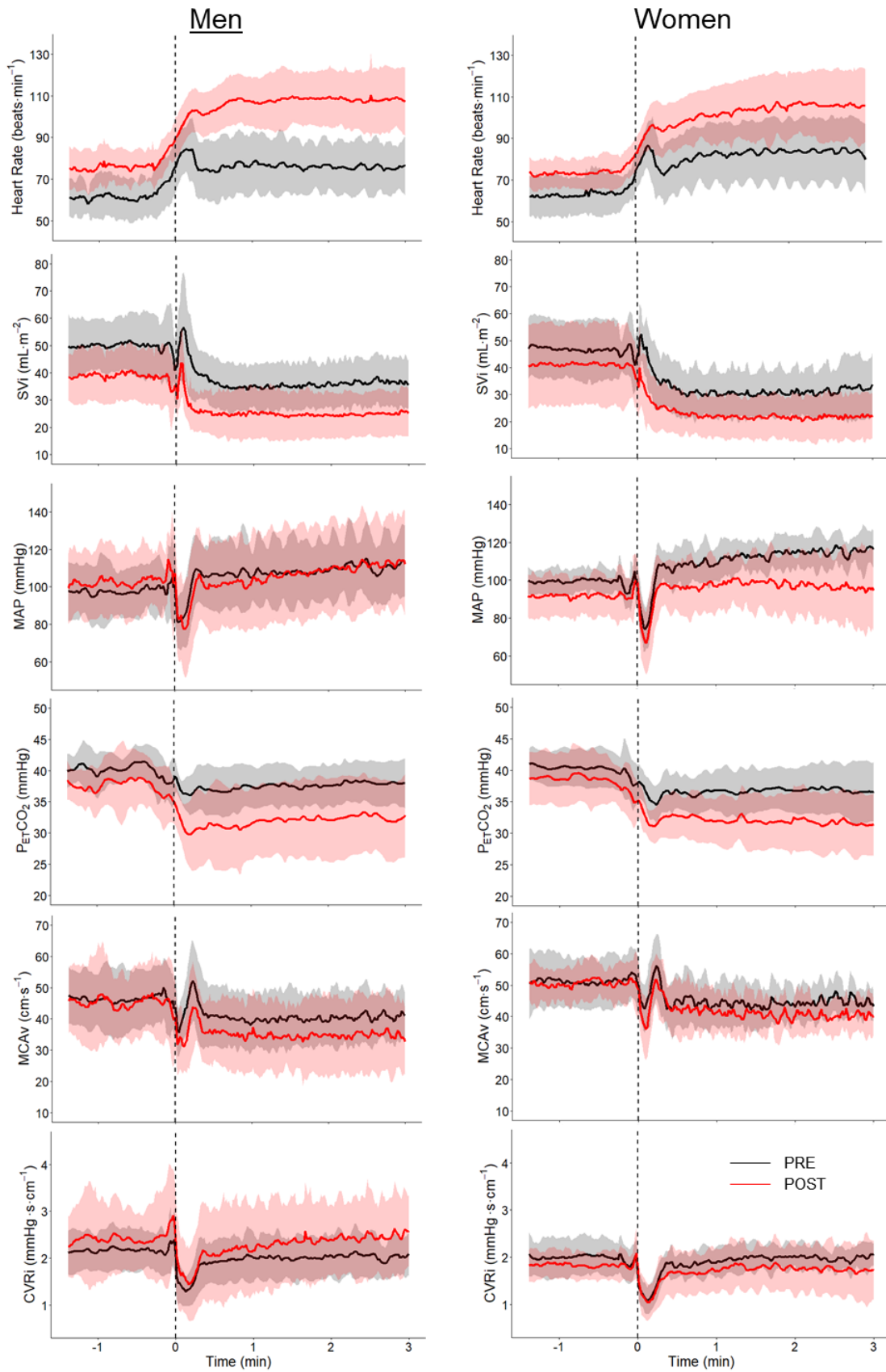


Figure 13. Mean cardiovascular and cerebrovascular time-series responses of men (left) and women (right) to the first 3 min of the supine-to-stand test. Data from both control and exercise groups were collapsed to aid in visualizing the bed rest x sex interactions identified in Tables 2 and 5. The dashed vertical line at time = 0s represents when the participants were standing upright. The black and red colours represent the pre- and post-bed rest responses, with the lines indicating the mean response and shaded areas representing ± 1 standard deviation around the mean. SVi, stroke volume index; MAP_{Brachial}, brachial mean arterial pressure; P_{ET}CO₂, end-tidal partial pressure of carbon dioxide; MCAv, middle cerebral artery blood velocity; CVRi, cerebrovascular resistance index.

Discussion

Signs of orthostatic intolerance in post-menopausal women and similar age men were highly prevalent during an 8-min stand test conducted ~5 h after completing two weeks of bed rest, with no protective effect of high-intensity exercise on orthostatic tolerance. Early termination of post-bed rest stand tests predominantly occurred for female participants, identifying a sex-based difference in orthostatic tolerance that was not evident during tilt tolerance testing performed ~5 h earlier as the first instance of upright posture after bed rest. Both men and women of this 55- to 65-year-old age group were hypocapnic following bed rest and had lower MCAv nadirs upon standing that occurred independent of a change in the MAP nadir. The initial magnitude of drop (i.e., nadir – supine value) in arterial blood pressure and MCAv upon standing after bed rest appeared to be exacerbated by the exercise countermeasures. Sex-specific effects of bed rest were apparent during the third minute of standing. Despite both men and women having lower $P_{ET}CO_2$ and MCAv while standing after bed rest, only men had increased CVRi and only women were hypotensive compared to pre-bed rest. Accordingly, post-menopausal women and similar age men appear to have different factors (e.g., arterial pressure regulation and PCO_2) underlying their reductions in MCAv while standing following a prolonged period of bed rest. That is, a decrease in MAP is not the only risk factor for reduced cerebral perfusion, syncope and unexplained falls following periods of bed rest, impaired mobility or hospitalization.

Orthostatic Tolerance

Orthostatic intolerance ~5 h after first being allowed upright following 14 days of bed rest was confirmed by eight (7 women) of 20 participants being unable to complete 8 min standing post-bed rest, plus two additional women were unable to attempt the stand test due to pre-existing symptoms of intolerance. The inability of the high-intensity exercise program to maintain orthostatic tolerance after bed rest is consistent with studies identifying that exercise must be combined with either a gravity-simulating stimulus (Watenpaugh *et al.*, 2007) or fluid loading procedure (Shibata *et al.*, 2010; Hastings *et al.*, 2012) to protect orthostatic tolerance. Although 10 of 11 men within this study were able to complete 8 min of standing post-bed rest, these results should not be misconstrued as orthostatic intolerance post-bed rest only being an issue for women. For the men who completed 8 min of standing after bed rest, heart rate was markedly elevated ($+35 \pm 12 \text{ beats} \cdot \text{min}^{-1}$), while MCAv ($-5.9 \pm 5.7 \text{ cm} \cdot \text{s}^{-1}$) and P_{ETCO_2} ($-6.0 \pm 4.0 \text{ mmHg}$) were greatly reduced in the eighth minute of standing compared to pre-bed rest, suggesting that men were also under considerable stress. If the standing portion of the test continued beyond 8 min, reduced orthostatic tolerance times post-bed rest likely would have been observed in many more of the male participants in the present study, as these changes are consistent with changes that are expected to occur in the progression towards syncope (Zuj *et al.*, 2013). Nevertheless, the finding of lower orthostatic tolerance of post-menopausal women compared to similar age men following bed rest is consistent with reports of increased susceptibility of younger women to orthostatic intolerance before (Fu *et al.*, 2004; Grenon *et al.*, 2006) and after bed rest (Grenon *et al.*, 2006), and for female astronauts following spaceflight (Fritsch-Yelle *et al.*, 1996a; Waters *et al.*, 2002; Blaber *et al.*, 2011). These data confirm the elevated risk of syncope several hours after resuming upright posture and normal ambulation.

In this study, we had the opportunity to compare responses to standing ~5 h after the end of bed rest with the responses of the same participants to an 80° head-up tilt test, which was completed as the first instance of upright posture (Mastrandrea *et al.*, 2023). Orthostatic tolerance seemed to improve between the initial tilt test and the supine-to-stand test, as 5 of 22 participants were unable to complete at least 3 min of 80° head-up tilt whereas all participants, except the 2 women who could not attempt the stand test, completed at least 3 min standing on the first day out of bed. The longer duration tolerated in the upright posture by many of the participants during the stand test likely reflected an adaptation to upright posture and the importance of the muscle pump for venous return post-bed rest. Interestingly, very short tilt tolerance times were observed within this same cohort for both men and women immediately after bed rest (i.e., no sex difference in orthostatic tolerance) (Mastrandrea *et al.*, 2023). The finding of reduced tolerance of women compared to men during the 8-min stand test after bed rest, but not during the post-bed rest tilt test (Mastrandrea *et al.*, 2023), raises questions about potential differences in how quickly men and women in this age group readapt to upright posture or respond to passive versus active orthostatic stresses.

Initial Response to Standing

Following two weeks of bed rest, post-menopausal women and similar age men presented with higher supine heart rates to compensate for reduced stroke volumes in order to maintain cardiac output, an observation consistent with younger adult responses to bed rest (Edgell *et al.*, 2007; Hastings *et al.*, 2012). Unexpectedly, both men and women were mildly hypocapnic while supine post-bed rest, in contrast with observations of unchanged $P_{ET}CO_2$ while seated at the end of bed rest in young men (Katayama *et al.*, 2004). Women also had lower blood pressure while

supine post-bed rest, which was compensated for by reduced CVRi to maintain MCAv via static autoregulation, resulting in lower supine CVRi for women than men after bed rest.

Upon transitioning from supine to standing posture, a hydrostatic gradient reduces arterial pressure at the level of the brain by ~20-30 mmHg in addition to an initial transient drop in MAP, causing a reduction in cerebral blood flow (Harms *et al.*, 2021). In the present study, post-menopausal women and similar age men had comparable initial cerebrovascular responses to standing after bed rest. The absolute SBP, DBP, and MAP nadirs of men and women were not different after bed rest. Systemic arterial pressure was protected during this transient phase by increased TPR. Interestingly, the MCAv nadir for both sexes during the transient phase of standing up reached lower values post-bed rest, suggesting that reduced cerebral perfusion immediately upon standing after bed rest was independent of blood pressure for many of the participants in the present study. At the timing of the MAP nadir, P_{ETCO_2} was reduced across groups and sexes after bed rest, with the magnitude of reduction being most prominent in the exercise group. However, it is difficult to discern whether the lower P_{ETCO_2} at the nadir directly influenced the reduction in MCAv nadir during the initial ~5s of upright posture due to the lung-to-brain transit time and the latency of cerebrovascular responses to changes in P_{ETCO_2} (Harms *et al.*, 2021). It is also noteworthy that the magnitude of drop in blood pressure and MCAv from supine to nadir was exacerbated for participants within the exercise group, especially for the men. We know of no prior reports of bed rest effects on the initial blood pressure responses to standing; however, it was unexpected that the exercise group would experience larger initial drops in blood pressure than control participants following bed rest. Whether pure inactivity versus exercise training during bed rest impacted initial blood pressure responses to standing in the present study remains to be determined, but this could align with paradoxical effects of

aerobic exercise training on orthostatic tolerance (van Lieshout, 2003) and Shibata *et al.*'s (Shibata *et al.*, 2010) bed rest study results with younger adults, where the cycling exercise group had exaggerated reductions in orthostatic tolerance following bed rest compared to the inactive control group (-43% vs. -25%).

Regardless of the precise mechanism provoking the larger initial reductions in MCAv upon standing or lower absolute MCAv nadirs, bed rest could have important implications for fall risk upon standing. Larger reductions in cerebral perfusion experienced immediately after standing have been linked to postural instability in both younger (Fitzgibbon-Collins *et al.*, 2019; Amelard *et al.*, 2020) and older adults (Fitzgibbon-Collins *et al.*, 2021), potentially translating into an increased risk of unexplained falls following periods of hospitalization or severe immobility (Mahoney *et al.*, 2000). Given that lower MCAv nadirs were observed in many participants without having simultaneously lower MAP nadirs, this suggests that factors beyond simply orthostatic hypotension, such as $P_{ET}CO_2$ and other cerebrovascular regulation mechanisms, should be considered as risk factors for syncope and unexplained falls following bed rest hospitalization for illness or surgery.

Prolonged Standing

After bed rest, both men and women were hypocapnic, with reduced MCAv. The lower $P_{ET}CO_2$ measured after bed rest was likely a result of increased alveolar ventilation; however, ventilation was not measured in the present study. Lower $P_{ET}CO_2$ and cerebral perfusion while upright have previously been identified in individuals who experience orthostatic intolerance compared to those who do not (Novak *et al.*, 1998). Interestingly, the average standing $P_{ET}CO_2$

in the present study was 5.8 mmHg lower post-bed rest, which is similar in magnitude to the difference in upright $P_{ET}CO_2$ observed between clinical patients with orthostatic intolerance and healthy control participants (-6.9 mmHg) (Novak *et al.*, 1998). In the present study, the contribution of mechanisms to reductions in MCAv appear to differ based on sex. The men in the cohort presented with increased CVRi while standing after bed rest, which is consistent with the vasoconstrictor effect of lower PCO_2 mediating the reduction in MCAv (Aaslid *et al.*, 1989). These reductions in male MCAv post-bed rest occurred while MAP was maintained at pre-bed rest levels. Conversely, CVRi of female participants was unchanged (if not slightly reduced) from pre- to post-bed despite being hypocapnic ($\Delta P_{ET}CO_2 = -6.4$ mmHg). It is noteworthy that cerebrovascular reactivity is reduced in women following menopause, with a step reduction in reactivity occurring around the 5th decade of life, with no similar change occurring in men (Kastrup *et al.*, 1998). The blunted cerebrovascular reactivity to CO_2 after menopause may contribute to the lack of increase in CVRi during hypocapnia post-bed rest. In interpreting the absence of an increase in CVRi in women during hypocapnia while standing, it is also imperative to consider that women also had reduced MAP, with cerebral autoregulation having opposite effects compared to lower $P_{ET}CO_2$ on vascular tone. The autoregulatory response to a reduction in MAP will reduce CVRi and maintain MCAv to compensate for the reduction in perfusion pressure, whereas low $P_{ET}CO_2$ will act to increase CVRi. In essence, the competing effects of autoregulation and $P_{ET}CO_2$ on CVRi in these women appeared to offset each other, resulting in no net change in CVRi from pre- to post-bed rest while standing. Without a difference in CVRi, MCAv of women decreased with the fall in MAP, according to Ohm's Law.

Our finding of reduced MAP during prolonged standing post-bed rest in only post-menopausal women is interesting, as women appear to regulate their blood pressure more

similarly to men after menopause (Hart *et al.*, 2011; Barnes *et al.*, 2014). Following menopause, the β -adrenergic vasodilator response characteristic of younger women disappears, and TPR and MAP become associated with the level of muscle sympathetic nerve activity (MSNA) (Hart *et al.*, 2011). This is thought to predispose post-menopausal women to hypertension, as MSNA also increases with age (Hart *et al.*, 2011; Barnes *et al.*, 2014). In pre-menopausal astronauts, low TPR was proposed to be the mechanism underlying impaired orthostatic intolerance following spaceflight; however, that is inconsistent with our findings in post-menopausal women following bed rest, as the women had similar or higher TPR values than men while standing yet could not maintain MAP. This suggests that pre- and post-menopausal women likely have different mechanisms contributing to their impaired orthostatic tolerance following bed rest or spaceflight. Previous bed rest studies identified a reduction in cardiac baroreflex gain in proportion to the increase in resting heart rate (Hughson *et al.*, 1994), as occurred in this study. Post-menopausal women have been reported to be highly dependent on the cardiac baroreflex and heart rate in order to maintain arterial pressure during hypotensive stimulation (Barnes *et al.*, 2012). The importance of the cardiac baroreflex for maintaining MAP in post-menopausal women while upright after bed rest may even be accentuated by higher resting MSNA that limits the potential for sympatho-excitatory responses (Akins *et al.*, 2023). Accordingly, the impairment in cardiac baroreflex sensitivity post-bed rest may affect MAP and orthostatic tolerance of post-menopausal women more than similar age men, which is consistent with our finding of shorter orthostatic tolerance times of post-menopausal women than men after bed rest. It is also possible that the post-menopausal women in the present study were more susceptible to the previously reported ~10 % reduction in blood volume observed during bed rest (Mastrandrea *et al.*, 2023) than the men, as acute hypovolemia has been reported to have exaggerated effects on orthostatic tolerance

of young women compared to men (Fu *et al.*, 2005). Future bed rest studies that include post-menopausal women should include measurements of MSNA during orthostatic stress in order to better characterize the sex-dependent effects of bed rest on the arterial vs. cardiac arms of the baroreflex.

Limitations

Transcranial Doppler ultrasound was used to measure MCAv as an index of global cerebral blood flow, as it provides high temporal resolution and is able to provide stable signals during postural transitions. In the interpretation of MCAv data, it was assumed that MCA diameter was constant such that reduced blood velocity was proportional to the reduction in flow. It is unknown if MCA diameter increased upon standing, which could reduce MCAv. Small changes in MCA diameter have been reported following large changes in P_{ETCO_2} (Coverdale *et al.*, 2014; Verbree *et al.*, 2014). The mean reduction in P_{ETCO_2} observed from pre- to post-bed rest while standing in the present study (-5.8 mmHg) was within the range of hypocapnia (-7.5 mmHg) where MCA diameter has been reported to be stable (Verbree *et al.*, 2014). However, it is possible that the observed post-bed rest hypocapnic state caused a small reduction in MCA diameter that would further exaggerate the reduction in cerebral blood flow reported while standing from pre- to post-bed rest. Additionally, this study relied on pulse contour analysis of the finger arterial pressure waveform with the ModelFlow® algorithm to estimate SVi and Qi. The ModelFlow® algorithm has been previously shown to underestimate changes in stroke volume when arterial pressure rapidly falls, such as during the initial dynamic phase of posture transitions, but tracks well during constant posture (Gibbons *et al.*, 2019).

Our study was also limited by its relatively small sample size and that it did not include a non-bed rest control group. The present study was likely under-powered to detect smaller main effects or interactions between bed rest, sex, and exercise, meaning that some effects may have gone undetected. However, this is one of the first bed rest studies to include both men and women of this age group and investigate sex-based differences, which will inform the design of future investigations.

Lastly, the 8-min maximum for the supine-to-stand test produced a ceiling effect, with 36 of 44 tests reaching the maximum duration. Accordingly, it remains unknown how much longer participants who completed the test could tolerate being upright beyond 8 min. This ceiling effect may confound the output of the linear mixed model used to assess the effects of bed rest, exercise, and sex on standing tolerance times. Despite the statistical modeling limitations imposed by this ceiling effect (Šimkovic & Träuble, 2019), it is remarkable that 9 of 11 women were unable to complete 8 min of standing on the first day after bed rest (7 early termination and 2 participants had tests postponed until recovery day 3; see Methods), whereas only 1 of 11 men was unable to complete the protocol.

Conclusion

Fourteen days of head-down bed rest induced sex-specific effects on orthostatic tolerance and cerebrovascular responses to upright posture. Post-menopausal women had lower standing tolerance times than similar age men after bed rest, and daily high-intensity exercise countermeasures provided no protective effect on orthostatic tolerance. We identified lower MCAv nadirs post-bed rest in both men and women without a simultaneous reduction in MAP

nadirs, and sex-specific effects of $P_{ET}CO_2$, CVRi, and MAP on cerebral perfusion while standing following bed rest. Men had lower MCAv while standing after bed rest linked to hypocapnia and increased CVRi while MAP was maintained, whereas the lower MCAv while standing in women after bed rest was linked to lower MAP. These data suggest that orthostatic hypotension was not obligatory for reduced cerebral perfusion while standing after bed rest. Therefore, $P_{ET}CO_2$ and cerebrovascular regulation should be assessed in addition to MAP in order to fully understand the mechanisms underlying increased risk for reduced cerebral perfusion and syncope following bed rest. These results have implications for increased risk for unexplained falls following periods of bed rest, impaired mobility or hospitalization.

Chapter 5: Vertebral artery blood flow and respiratory interactions during graded lower-body negative pressure to pre-syncope

Abstract

The interactions between cerebral blood flow and respiration during orthostatic stress, as well as the contribution of postural hypocapnia to the development of orthostatic intolerance remain unclear. Vertebral artery blood flow may be an important mediator of respiratory-cerebrovascular interactions, as it supplies the chemosensitive area of the brain. This study investigated how reductions in vertebral artery blood flow interact with minute ventilation ($\dot{V}_E$) and end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) during orthostatic stress. Fourteen young healthy adults (6 women; age: 28 ± 4 years) completed graded lower-body negative pressure (LBNP) tests to pre-syncope while vertebral artery blood flow, mean arterial pressure (MAP), $\dot{V}_E$, and $P_{ET}CO_2$ were simultaneously measured. A wide range of vertebral artery blood flow and $\dot{V}_E$ responses were observed despite MAP being consistently maintained until pre-syncope. The reduction in vertebral artery blood flow was highly correlated with the increase in $\dot{V}_E$ from rest to pre-syncope ($\rho = -0.808$, $p < 0.001$). $P_{ET}CO_2$ also fell alongside increased $\dot{V}_E$, and was associated with reductions in vertebral artery blood flow ($r_{rm} = 0.675$, $p < 0.001$). These data provide evidence of important interactions between respiration and cerebral blood flow during the progression towards syncope in healthy adults. However, the direction of causality between changes in vertebral artery blood flow and $\dot{V}_E$ in the present study could not be determined, and remains a novel area of investigation.

Introduction

Blood flow to the brain can become compromised during upright postures, leading to symptoms of orthostatic intolerance and eventually syncope (Van Lieshout *et al.*, 2003). The mechanisms underlying postural reductions in cerebral blood flow are complex and multifactorial (Van Lieshout *et al.*, 2003), and can be simulated in a controlled manner using lower-body negative pressure (Levine *et al.*, 1994; Ogoh *et al.*, 2015). Systemic hypotension and hemodynamic collapse are obvious factors immediately prior to syncope (Van Dijk & Wieling, 2013), but cerebrovascular-specific mechanisms that are independent of a drop in mean arterial pressure (MAP) may also play an important role in precipitating the decline of cerebral perfusion leading up to intolerance (Grubb *et al.*, 1991; Carey *et al.*, 2001; Zuj *et al.*, 2013).

Multiple factors that affect cerebral blood flow change during orthostatic stress, even when mean arterial pressure (MAP) is maintained. Central blood volume and cardiac output ($\dot{Q}$) are reduced (Ogoh *et al.*, 2005a) and arterial partial pressure of CO₂ (P_aCO₂) decreases (Bjurstedt *et al.*, 1962; van Lieshout *et al.*, 2001; Serrador *et al.*, 2006). Proposed mechanisms underlying postural hypocapnia include venous pooling and reduced $\dot{Q}$ (Serrador *et al.*, 2006), as well as increased chemosensitivity or a lower PCO₂ set point while in the upright posture (Jordan *et al.*, 2000; Wang *et al.*, 2004). However, the contribution of postural hypocapnia to the reduction in cerebral blood flow and development of syncope continues to be debated (Carey *et al.*, 2001; Serrador *et al.*, 2006; Zuj *et al.*, 2013; Immink *et al.*, 2014).

A potential key mediator of cerebrovascular and respiratory interactions during orthostatic stress is vertebral artery blood flow. The vertebral arteries deliver blood to the brainstem (Tatu *et al.*, 1996), which includes the cardiovascular and respiratory control centers and areas responsible for the central chemoreflex (Ainslie & Duffin, 2009; Nattie & Li, 2012).

Cerebrovascular reactivity acts as an important buffering mechanism to reduce the influence of changes in $P_a\text{CO}_2$ on cerebral tissue PCO_2 and pH, with changes in cerebral blood flow stabilizing central chemoreceptor stimulation (Ainslie & Duffin, 2009; Nattie & Li, 2012). However, a reduction in vertebral artery blood flow independent of $P_a\text{CO}_2$ during orthostatic stress may reduce CO_2 clearance from the chemosensitive areas of the brain, leading to reduced central pH and a compensatory increase in alveolar ventilation ($\dot{V}_A$) that lowers $P_a\text{CO}_2$. Indeed, reduced middle cerebral artery blood velocity (MCAv) during a constant level of lower-body negative pressure (LBNP) was associated with increased minute ventilation ($\dot{V}_E$) and a leftward shift in the $\dot{V}_E$ -end-tidal PCO_2 (P_{ETCO_2}) relationship (Ogoh *et al.*, 2013). This work provided preliminary evidence that decreased cerebral blood flow during orthostatic stress may stimulate breathing and cause P_{ETCO_2} to fall, but was limited by the assumption that changes in MCAv were directly proportional to changes in vertebral artery blood flow (Ogoh *et al.*, 2013), as regional differences in perfusion may be present during orthostatic stress (Sato *et al.*, 2012; Ogoh *et al.*, 2015). Nevertheless, the effect of reduced cerebral blood flow on ventilatory control during orthostatic stress may be an important factor contributing to postural hypocapnia and further reductions in cerebral blood flow in the pathway to syncope.

Therefore, the purpose of this study was to investigate how reductions in vertebral artery blood flow interact with $\dot{V}_E$ and P_{ETCO_2} during graded LBNP to pre-syncope. We hypothesized that simulated orthostatic stress will reduce vertebral artery blood flow independent of MAP, which will stimulate the central chemoreceptor via reduced CO_2 clearance, prompting a compensatory increase in $\dot{V}_E$ and subsequent reduction in P_{ETCO_2} . In addition, we explored a potential modifying effect of central chemosensitivity on the interaction between the cerebrovascular and respiratory systems during simulated orthostatic stress.

Methods

Participants

Fourteen young, healthy adults (8 men, 6 women; age: 28 ± 4 years, height: 1.73 ± 0.07 m, mass: 72 ± 12 kg; mean $\pm$ SD) volunteered to participate in the study. Based on previous work that identified within-participant relationships between changes in mean MCAv and $\dot{V}_E$ during LBNP (Ogoh *et al.*, 2013), we anticipated observing a large effect size for the relationship between changes in ventilation and cerebral blood flow during graded LBNP and calculated a required sample size of 13 ($r = 0.65$, $\alpha = 0.05$, power = 0.80). To account for potential data loss, we recruited an additional participant. Participants were instructed to: i) arrive for testing at least 2 h post-prandial, ii) not consume alcohol nor perform vigorous exercise in the preceding 24 h, and iii) avoid caffeine consumption on the day of the tests. The study was conducted in accordance with the Declaration of Helsinki, except for registration in a trial database, and all procedures were approved by University of Waterloo's Clinical Research Ethics Board (ORE 45763). All participants signed an informed consent before participating, and were made aware of their right to withdraw at any time.

Experimental Protocol

During a single experimental visit, participants performed: i) a 5-min resting assessment of cerebral autoregulation followed immediately by a two-breath hypercapnic cerebrovascular reactivity test (5 sets of two breaths of 10% CO₂ gas at minute intervals), ii) a hypocapnic cerebrovascular reactivity test with controlled hyperventilation and inspired hypercapnic gas (~5

mmHg step changes in $P_{ET}CO_2$ from ~20 mmHg to normocapnia), iii) a modified hyperoxic-hypercapnic rebreathing test to assess central chemosensitivity, and iv) a graded LBNP test to pre-syncope. Following familiarization with equipment, participants were instrumented in the supine position, and remained supine at rest for ~10 min prior to commencing data collection. All tests were performed in the supine position, with ~10-15 min breaks provided between tests for $\dot{V}_E$, $P_{ET}CO_2$ and cerebral blood flow to return to baseline levels. Only data from the modified rebreathing and graded LBNP tests were used in the present manuscript, with detailed procedures for these tests provided below.

Modified Rebreathing Test. Baseline data were collected for ~90 s, and then participants were coached to hyperventilate to reduce their $P_{ET}CO_2$ to ~20 mmHg for ~3 min (Boulet *et al.*, 2016). At the end of the hyperventilation period, participants were asked to hold their breath at the end of expiration while a three-way valve was turned to switch participants from breathing room air to a 6-L rebreathing bag filled with 93% O_2 and 7% CO_2 gas. Participants were asked to immediately take three deep breaths, to equilibrate with the rebreathing circuit, followed by spontaneous breathing until they reached a $P_{ET}CO_2$ of 60 mmHg. All participants reached a $P_{ET}CO_2$ of 60 mmHg, except for 3 individuals who self-terminated the test at a mean $P_{ET}CO_2$ of 55.4 mmHg. Participants then rested for ~20 min prior to commencing the graded LBNP test.

Graded LBNP Test. Participants were sealed at the level of the iliac crest to a custom-made decompression chamber. Baseline data were collected for 2 min, followed by 2 min at -20 mmHg and subsequent step-wise chamber decompressions of -5 mmHg every 2 min until pre-syncope or reaching a chamber decompression of -100 mmHg. Criteria for pre-syncope and test termination were: i) participant's request to stop the test due to symptoms of orthostatic intolerance (e.g., dizzy, nauseous, light-headed, sweaty, etc.), ii) a sudden drop in heart rate

greater than 15 beats per minute, iii) absolute systolic brachial blood pressure below 80 mmHg, or iv) a decrease in systolic blood pressure greater than 25mmHg for 10 consecutive heart beats.

Data Collection

During all tests, participants breathed through a mask (7450 SeriesV2™ Mask, Hans Rudolph, Inc., Shawnee, USA). Inspired and expired flow were measured using a bi-directional turbine (Universal Ventilation Meter, VacuMed, Ventura, USA) and used to determine breathing rate, tidal volume, and $\dot{V}_E$. O₂ and CO₂ were continuously sampled at the mouth (ML206 gas analyzer, AD Instruments, Colorado Springs, USA), and converted to partial pressures based on the ambient atmospheric conditions. The flow sensor and the gas analyzer were calibrated prior to each testing session using a 3-L syringe and precision analyzed gas mixtures, respectively.

Lead II electrocardiogram was recorded (ECG module, Finapres Medical Systems, Enschede, Netherlands). Finger arterial blood pressure was measured continuously using the volume clamp method from which brachial artery pressure waveforms were reconstructed, height corrected to the level of the heart, and calibrated using two brachial pressure measures obtained using an automated arm cuff (Finapres NOVA, Finapres Medical Systems). MCAv and aortic outflow velocity were measured continuously using 2-MHz pulsed Doppler ultrasound probes held in place by a headset over the right temporal sonication window (WAKIe 2TC, Atys Medical, Soucieu-en-Jarrest, France) and by hand at the suprasternal notch (Multigon, New York, USA), respectively. All cardiovascular and respiratory signals were recorded simultaneously at 1000 Hz (PowerLab and Labchart, version 7.3.7; AD Instruments). Separately, left vertebral artery blood flow was measured using duplex ultrasound (9-3 MHz linear array

probe with iE33 xMatrix, Philips Healthcare, Andover, USA). Video output from the ultrasound unit was time-synchronized with the other acquired signals and stored on a computer for post-processing. After completion of the LBNP test, transthoracic echocardiography in the parasternal long axis view (5-1 MHz sector array probe, Philips Healthcare) was performed to measure aortic root diameter with on-screen calipers from at least 3 different cardiac cycles during diastole.

Data Analysis

Modified Rebreathing Test. Baseline values for $P_{ET}CO_2$ and $\dot{V}_E$ were calculated as 1-min averages from the breath-by-breath data prior to initiating hyperventilation. Segmented linear regression was utilized to identify the inflection point of the $\dot{V}_E - P_{ET}CO_2$ relationship, with the point of intersection between the two regression lines representing the ventilatory recruitment threshold (VRT) and the slope of the linear segment after the inflection point representing the sensitivity of the central chemoreflex. Basal ventilation was also calculated as the average ventilation during the time period between stabilization of $\dot{V}_E$ following the equilibration breaths and the VRT.

Graded LBNP Test. Heart rate, brachial artery blood pressure, stroke volume, MCAv, and vertebral artery blood flow were extracted beat-by-beat. Stroke volume was calculated as the product of the area under the curve of the outer envelope of the aortic Doppler spectrum and the aortic root cross sectional area. $\dot{Q}$ was subsequently calculated as the product of heart rate and stroke volume, and total peripheral resistance was calculated as the quotient of $MAP/\dot{Q}$. Mean MCAv was calculated as the time-averaged outer envelope of the transcranial Doppler spectrum

for each cardiac cycle. Two cerebrovascular indices describing the instantaneous relationship between cerebral blood velocity and arterial pressure – resistance-area product and critical closing pressure – were calculated beat-by-beat using the two-point mean/diastolic method (Panerai *et al.*, 2011). Tidal volume, breathing rate, and $\dot{V}_E$, CO₂ output ($\dot{V}CO_2$), and $P_{ET}CO_2$ were determined for each breath.

Intensity-weighted mean blood velocity of the recorded vertebral artery duplex ultrasound videos was extracted using automated software (MAUI, Hedgehog Medical, Waterloo, Canada). Vertebral artery diameter was measured in triplicate at end-diastole during rest and the end of each LBNP stage with calipers, and used to calculate vessel cross-sectional area. Fluctuations in diameter across stages within each participant were smoothed using first-degree polynomial local regression. Vertebral blood flow was calculated as the product of vessel cross-sectional area and the intensity-weighted mean blood velocity.

One-min averages were used to quantify the baseline state of cardiovascular and respiratory variables prior to starting LBNP, and at the end of each subsequent completed stage of LBNP. The value of each variable was also noted at pre-syncope, using a 15-s average immediately preceding test termination. The relationship between the percent vertebral blood flow normalized to supine rest at 0 mmHg of LBNP and $\dot{V}_E$ was curve fit using a rational function for each individual:

$$\dot{V}_E = \frac{p}{\dot{Q}_{VA} + q}, \quad \text{Eq. 3}$$

where $\dot{V}_E$ is minute ventilation, $\dot{Q}_{VA}$ is vertebral artery blood flow, and p and q are the constants for the numerator and denominator, respectively. A rational function was selected based on the following assumptions: i) brain CO₂ production is relatively constant during the LBNP test, ii)

clearance of CO₂ from the brain is dependent on both blood flow and the tissue-to-arterial PCO₂ gradient, and iii) an increase in $\dot{V}_E$ reduces P_aCO₂, which increases the tissue-to-arterial PCO₂ gradient.

Statistical Analysis

Statistical analyses were conducted in R (v. 4.0.3). Due to wide variation in LBNP tolerance times, individual time-series data are presented and statistical comparisons for variables of interest were only made between supine rest and pre-syncope using Wilcoxon signed rank tests. Spearman correlation was used to examine the relationship between the percent change in vertebral artery blood flow and the change in $\dot{V}_E$ from rest to pre-syncope. Repeated measures correlations (Bakdash & Marusich, 2017) were also used to assess the relationships between P_{ET}CO₂ and vertebral artery blood flow or mean MCAv throughout the LBNP test, as well as the relationship between mean MCAv and vertebral blood flow. Statistical significance was set to $p \leq 0.05$, and data are reported as medians (first, third quartiles).

Results

The median time to pre-syncope was 18.05 min (16.55, 22.40 min), with test termination occurring at median LBNP of -62.5 mmHg (-75, -60 mmHg). Ten tests were terminated due to falling blood pressure, and 4 tests were terminated by participant request due to symptoms of orthostatic intolerance prior to reaching the systolic blood pressure threshold for termination. Of the 10 tests that were terminated due to falling blood pressure, only 2 participants reported feeling no symptoms of intolerance at pre-syncope. One female participant's vertebral artery

blood flow and another male participant's stroke volume and $\dot{Q}$ were unable to be measured to pre-syncope due to poor imaging quality or loss of the aortic Doppler signal in the later stages of LBNP.

Due to considerable variation in LBNP tolerance, individual cardiovascular, respiratory and cerebrovascular time-series responses are presented in Figure 14, with group responses compared between baseline and pre-syncope in Table 8. As expected, heart rate increased, and stroke volume and $\dot{Q}$ decreased during progressive LBNP. MAP, however, was well-maintained by increases in TPR until the point of pre-syncope. $\dot{V}_E$ and $\dot{V}_{CO_2}$ both increased from baseline to pre-syncope, and $P_{ET}CO_2$ decreased. Vertebral artery blood flow and mean MCAv also decreased from baseline to pre-syncope, with 9 of 13 participants exhibiting reductions in vertebral artery diameter >4% (Figure 15). The changes in vertebral artery blood flow ($r_{rm} = 0.675$, $p < 0.001$; Figure 16A) and mean MCAv ($r_{rm} = 0.901$, $p < 0.001$; Figure 16B) throughout LBNP were correlated with the changes in $P_{ET}CO_2$ (measures at pre-syncope were not included to avoid confounding effects of falling MAP). The common regression slopes from the repeated measures correlation analysis were $3.14\% \cdot mmHg^{-1}$ for vertebral artery blood flow and $3.01\% \cdot mmHg^{-1}$ for mean MCAv. Repeated measures correlation also revealed a strong association between the measured vertebral artery blood flow and MCAv throughout LBNP to pre-syncope ($r_{rm} = 0.797$, $p < 0.001$; Figure 17). Progressive increases in critical closing pressure as an indicator of cerebrovascular tone were observed beyond mild simulated orthostatic stress, but the resistance-area product remained relatively constant until MAP fell at pre-syncope (Figure 18).

The relationship between $\dot{V}_E$ and vertebral artery blood flow during LBNP for each individual is presented in Figure 19. Reductions in vertebral artery blood flow and associated changes in $\dot{V}_E$ were observed in participants of both sexes. The relationship was non-linear, with

a predictable pattern of increased $\dot{V}_E$ at lower vertebral artery blood flow for only participants who had a substantial reduction in vertebral artery blood flow from baseline. This is supported by the strong negative relationship between the change in $\dot{V}_E$ and the change in vertebral artery blood flow from pre-LBNP to pre-syncope ($\rho = -0.808$, $p < 0.001$; Figure 20). A summary of responses from the modified hyperoxic-hypercapnic rebreathing test for chemosensitivity is presented in Table 9. Differences in central chemosensitivity did not appear to explain between-person differences for the magnitude of change in $\dot{V}_E$ during LBNP. Participants with the largest increases in $\dot{V}_E$ were not the participants with the highest $\dot{V}_E$ - $P_{ET}CO_2$ slope during the rebreathing test, nor were participants with smallest changes in $\dot{V}_E$ the participants with the lowest $\dot{V}_E$ - $P_{ET}CO_2$ slope (see color of dots in Figure 20).

Table 8. Summary of cardiovascular and respiratory responses at rest and pre-syncope.

Variable	Baseline	Pre-Syncope	p-value
LBNP, mmHg	0	-62.5 (-75, -60)	NA
Heart Rate, beats·min ⁻¹	63 (59, 73)	126 (96, 141)	<0.001
Stroke Volume, mL (<i>n</i> = 13)	82 (71, 108)	23 (16, 39)	<0.001
$\dot{Q}$, L·min ⁻¹ (<i>n</i> = 13)	5.4 (4.2, 6.4)	2.7 (2.2, 3.8)	<0.001
MAP, mmHg	101 (91, 106)	86 (78, 93)	0.002
SBP, mmHg	125 (121, 138)	98 (93, 107)	<0.001
DBP, mmHg	80 (71, 84)	79 (70, 85)	0.952
PP, mmHg	52 (41, 59)	23 (16, 28)	<0.001
TPR, mmHg·min·L ⁻¹ (<i>n</i> = 13)	18.7 (15.4, 22.5)	35.2 (26.1, 44.8)	0.002
Breathing Rate, breaths·min ⁻¹	15 (10, 18)	21 (17, 30)	0.003
Tidal Volume, L	0.51 (0.45, 0.68)	0.62 (0.52, 0.94)	0.173
$\dot{V}_E$, L·min ⁻¹	7.4 (6.4, 10.4)	12.6 (10.6, 17.1)	<0.001
$\dot{V}CO_2$, L·min ⁻¹	0.27 (0.23, 0.32)	0.35 (0.28, 0.41)	0.035
P _{ET} CO ₂ , mmHg	39.5 (36.2, 41.6)	30.7 (27.8, 33.5)	<0.001
$\dot{V}_E/\dot{Q}$, ratio (<i>n</i> = 13)	1.43 (1.14, 1.70)	4.69 (2.60, 7.15)	<0.001
VA Blood Flow, mL·min ⁻¹ (<i>n</i> = 13)	137.9 (113.1, 204.2)	90.0 (71.4, 155.9)	0.006
Mean VAv, cm·s ⁻¹ (<i>n</i> = 13)	21.9 (21.1, 23.6)	17.0 (13.3, 22.5)	0.057
VA Diameter, cm (<i>n</i> = 13)	0.34 (0.33, 0.43)	0.34 (0.30, 0.39)	0.005
Mean MCAv, cm·s ⁻¹	65.9 (59.8, 75.0)	48.8 (41.0, 51.8)	<0.001
MCA CrCP, mmHg	30 (19, 36)	52 (38, 64)	<0.001
MCA RAP, mmHg·s·cm ⁻¹	1.06 (0.92, 1.21)	0.80 (0.53, 1.00)	<0.001

Data are median (first – third quartile). All variables are *n* = 14, unless noted otherwise. LBNP, lower-body negative pressure; $\dot{Q}$, cardiac output; MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; TPR, total peripheral resistance; $\dot{V}_E$, minute ventilation; $\dot{V}CO_2$, carbon dioxide output; P_{ET}CO₂, end-tidal partial pressure of carbon dioxide; VA, vertebral artery; VAv, vertebral artery velocity; MCAv, middle cerebral artery blood velocity; MCA CrCP and RAP, critical closing pressure and resistance-area product estimated using measures of velocity from the middle cerebral artery.

Table 9. Modified rebreathing test results ($n = 14$).

Variable	Value
Resting $P_{ET}CO_2$, mmHg	38.2 (36.5, 41.9)
Resting $\dot{V}_E$, $L \cdot min^{-1}$	7.8 (6.6, 9.4)
Basal $\dot{V}_E$, $L \cdot min^{-1}$	6.7 (5.2, 12.4)
VRT, mmHg	47.0 (44.0, 49.0)
Slope, $L \cdot min^{-1} \cdot mmHg^{-1}$	3.18 (2.08, 3.99)

Data are median (first, third quartile). $P_{ET}CO_2$, end-tidal partial pressure of carbon dioxide; $\dot{V}_E$, minute ventilation; VRT, ventilatory recruitment threshold.

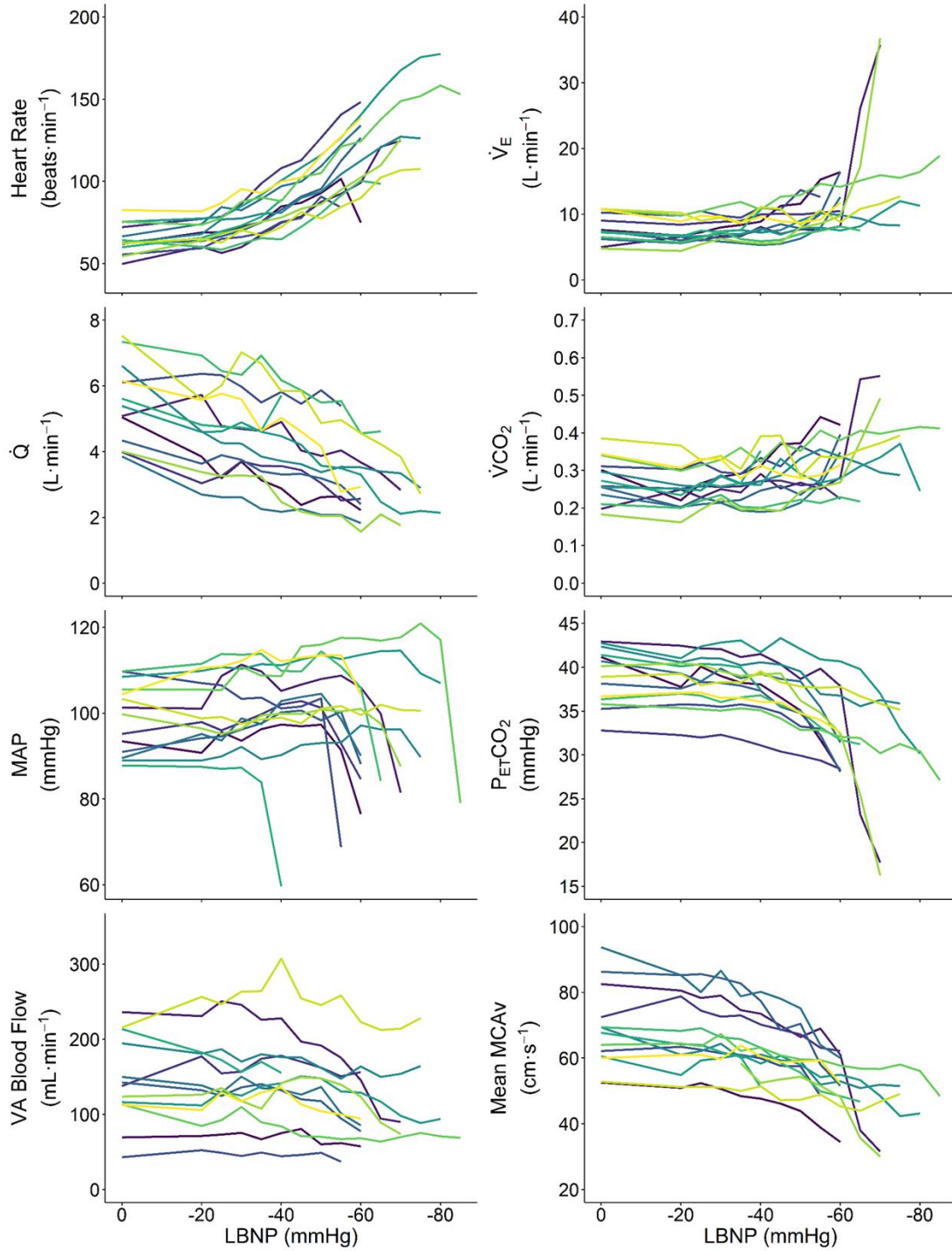


Figure 14. Individual time-series responses to progressive lower-body negative pressure (LBNP). Each line represents a unique individual, with the line ending at the level of LBNP when the test was terminated. $n = 14$, except $n = 13$ for vertebral artery blood flow and cardiac output. $\dot{Q}$, cardiac output, MAP, mean arterial pressure; VA, vertebral artery; $\dot{V}_E$, minute ventilation; $\dot{V}_{CO_2}$, rate of carbon dioxide output; P_{ETCO_2} , end-tidal partial pressure of carbon dioxide; MCAv, middle cerebral artery blood velocity.

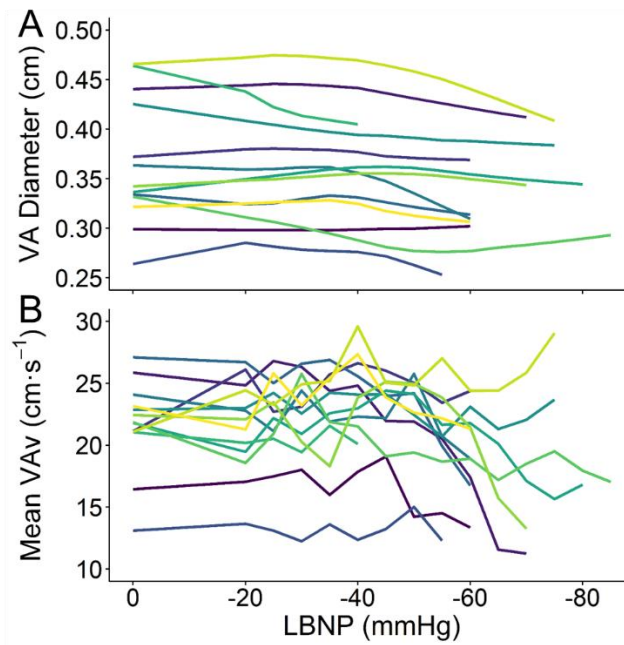


Figure 15. Individual ($n = 13$) vertebral artery (VA) diameter (A) and mean velocity (VAv; B) responses to progressive lower-body negative pressure (LBNP). Vertebral artery diameters were smoothed using first-degree polynomial local regression. Each line represents a unique individual, with the line ending at the level of LBPN when the test was terminated.

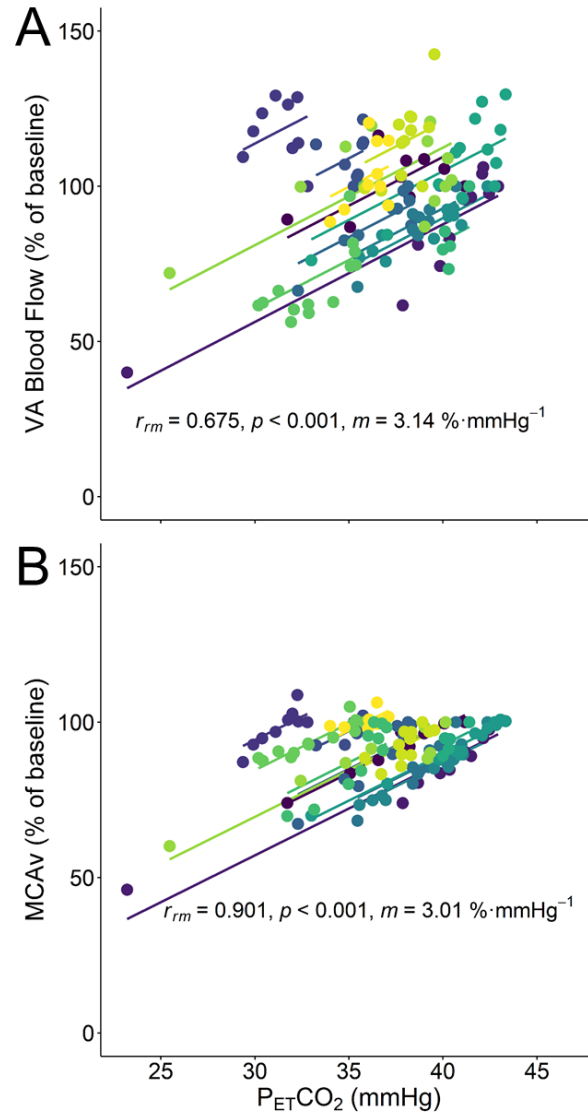


Figure 16. Repeated measures correlations between vertebral artery (VA) blood flow (A; $n = 13$) or middle cerebral artery blood velocity (MCAv; B; $n = 14$) and the end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$) throughout the lower-body negative pressure protocol. Measurements at pre-syncope were excluded from these analyses to prevent a confounding influence of the large and rapid reduction in mean arterial pressure on measures of vertebral artery blood flow or MCAv. A different color represents each participant. Parallel lines represent the best fit regression line slope across conditions between participants, and the length of each parallel line is constrained to the bounds of each participant's response range.

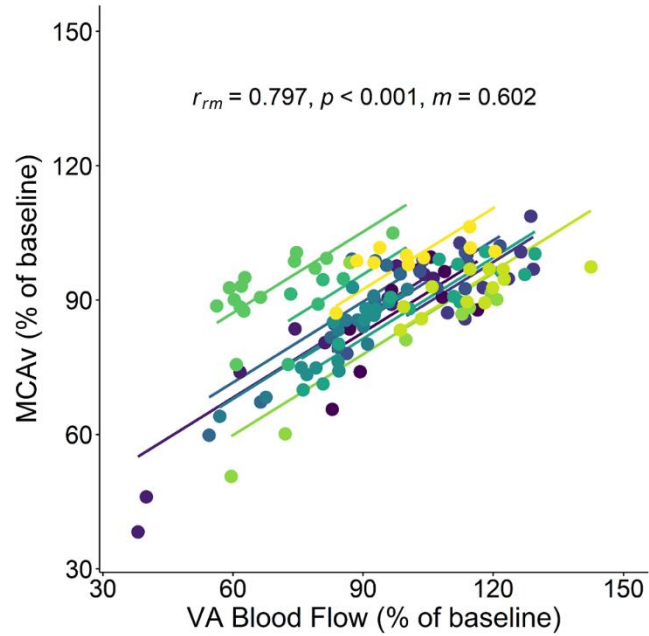


Figure 17. Repeated measures correlations ($n = 13$) between vertebral artery (VA) blood flow and middle cerebral artery blood velocity (MCAv). A different color represents each participant. Parallel lines represent the best fit regression line slope across conditions between participants, and the length of each parallel line is constrained to the bounds of each participant's response range.

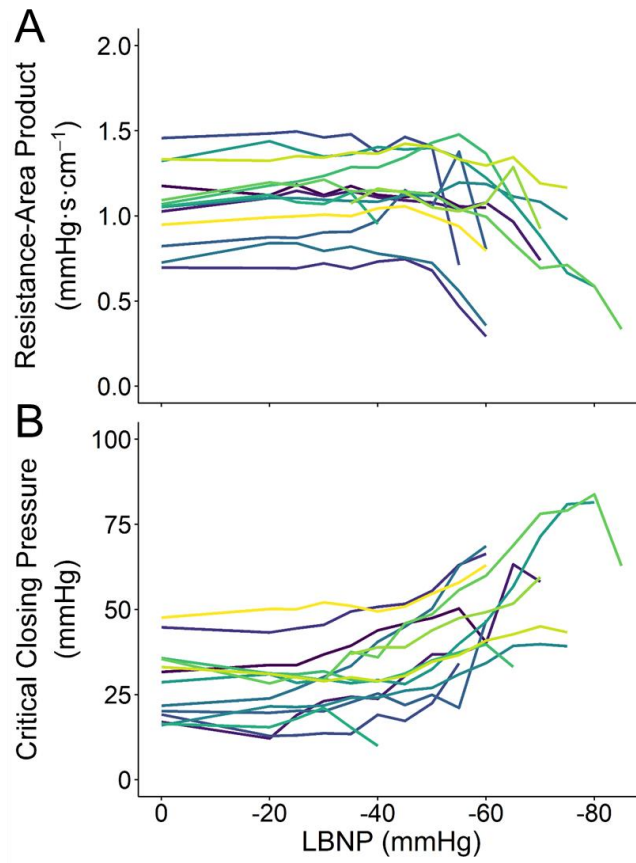


Figure 18. Individual time-series responses ($n = 14$) to lower-body negative pressure (LBNP) for resistance-area product (A), and critical closing pressure (B). Each line represents a unique individual, with the line ending at the level of LBNP when the test was terminated.

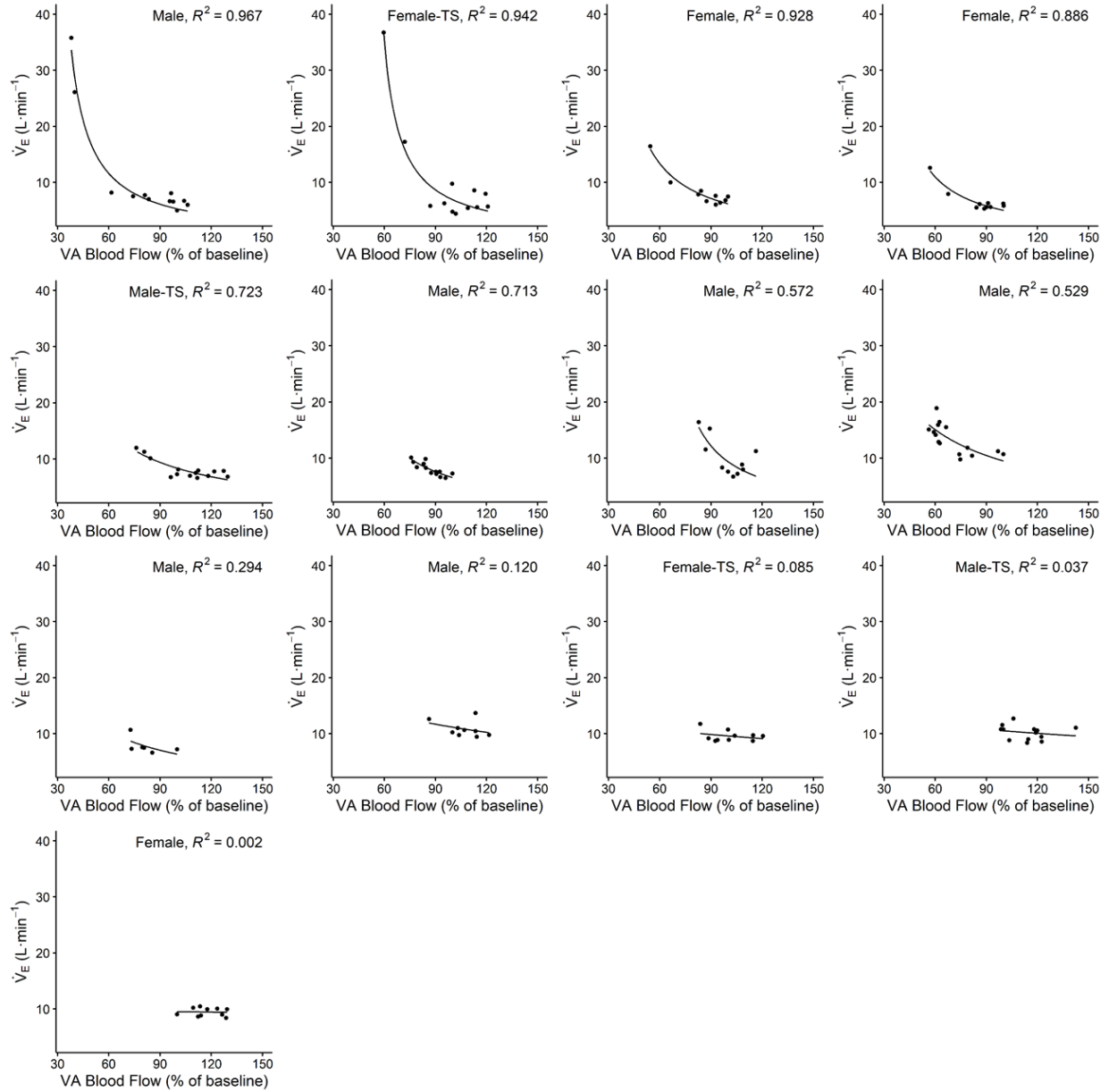


Figure 19. Individual plots of the minute ventilation ($\dot{V}_E$) – vertebral artery (VA) blood flow relationship during lower-body negative pressure. The sex of each participant is displayed on each panel, with “TS” signifying that the test was terminated by the participant due to symptoms of intolerance. Data were fit using a rational function to illustrate the non-linear relationship between changes in vertebral artery blood flow and ventilation during simulated orthostatic stress. Notably, participants without a substantial reduction in vertebral artery blood flow from baseline, do not appear to exhibit a pattern of increased ventilation.

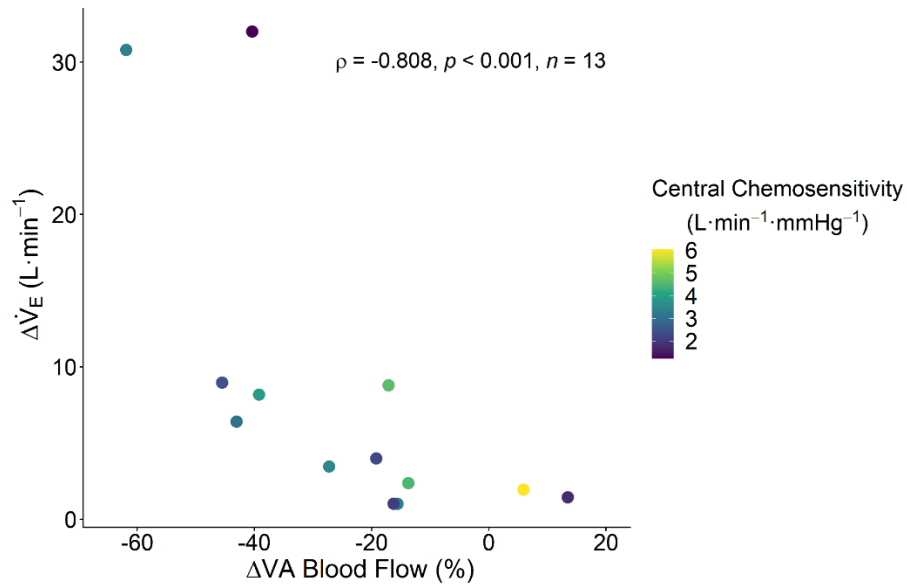


Figure 20. Relationship between the change in vertebral artery (VA) blood flow and the change in minute ventilation ($\dot{V}_E$) from before lower-body negative pressure to pre-syncope ($n = 13$). Spearman correlation was used to assess the strength of the relationship. The color of the individual data points represents the $\dot{V}_E$ -end-tidal partial pressure of carbon dioxide slope measured during the modified rebreathing protocol as an indicator of central chemosensitivity.

Discussion

This study investigated cerebrovascular and respiratory interactions during simulated orthostatic stress, focusing on the changes in vertebral artery blood flow and $\dot{V}_E$ in the progression to pre-syncope. We observed a wide range of vertebral artery blood flow and $\dot{V}_E$ responses to graded LBNP, with the magnitude of reduction in vertebral artery blood flow being highly correlated with the increase in $\dot{V}_E$ from rest to pre-syncope. $P_{ET}CO_2$ fell alongside increased $\dot{V}_E$, and was tightly associated with reductions in vertebral artery blood flow and mean MCAv. Measures of central chemosensitivity did not provide additional insight into why some individuals had stronger ventilatory responses to simulated orthostatic stress than others. Despite not being able to determine the direction of causality between changes in vertebral artery blood flow and $\dot{V}_E$ in the present study, these results provide strong evidence that interactions between respiratory and cerebrovascular control are in play during orthostatic stress.

We observed a wide range of responses within our cohort of young healthy adults. This was clearly illustrated by the range of heart rate responses to graded LBNP, where multiple individuals experienced rapid reductions in blood pressure at heart rates only approaching 100 beats·min⁻¹, while others tolerated LBNP very well, reaching -80 and -85 mmHg of LBNP with heart rates approaching 180 and 160 beats·min⁻¹, respectively. Two of the individuals with lower heart rate responses had strong vasovagal responses at lower LBNP pressures (-40 and -55 mmHg) despite not having substantial reductions in $\dot{Q}$ (5.6 to 5.7 L·min⁻¹, and 6.1 to 5.4 L·min⁻¹, respectively). Variation in respiratory responses to LBNP were also observed, with some individuals having large increases in $\dot{V}_E$ leading up to pre-syncope, whereas others had small or negligible changes. The observed variability for tolerance, cardiovascular and respiratory responses to LBNP reinforces that the mechanisms leading to syncope can vary considerably

from person-to-person (Fritsch-Yelle *et al.*, 1996b; Fu *et al.*, 2005, 2012), and that cerebrovascular-respiratory interactions may be an important factor to consider in the pathway to orthostatic intolerance in some individuals, but not others.

Maintenance of vertebral artery blood flow is essential for respiratory stability due to its effects on CO₂ clearance and the central chemoreflex (Ainslie & Duffin, 2009), but also because it supplies blood to the sites of cardiovascular and respiratory control within the brain stem. Accordingly, it has been suggested that vertebral artery blood flow is preferentially protected compared to the anterior cerebral circulation during orthostatic stress, and that reductions in posterior rather than anterior cerebral blood flow are responsible for the development of orthostatic intolerance (Sato *et al.*, 2012; Ogoh *et al.*, 2015). In support of this, it was demonstrated that vertebral artery blood flow was unchanged during the first 2 min of 60° head-up tilt (Sato *et al.*, 2012), and during submaximal levels of LBNP (Ogoh *et al.*, 2015), but internal carotid artery blood flow was reduced in proportion to the magnitude of orthostatic stress. We measured vertebral artery blood flow to the point of pre-syncope. In agreement with previous work, our results demonstrate that vertebral artery blood flow was relatively well maintained at low levels of LBNP (e.g., -20 mmHg), but did decrease relative to baseline in 11 of our 13 participants at the point of pre-syncope. One participant without a decrease in vertebral blood flow relative to baseline self-terminated the test due to the onset of symptoms prior to a fall in blood pressure. Interestingly though, the other participant without reduced vertebral artery blood flow did have falling blood pressure at test termination, which occurred simultaneously with a request to stop the test due to symptoms (systolic blood pressure at termination was 84 mmHg). Similar reductions in vertebral artery blood flow have been reported at pre-syncope during exposure to a combination of orthostatic and heat stresses (Shibasaki *et al.*, 2020).

The magnitude of reduction in vertebral artery blood flow was negatively associated with the change in $\dot{V}_E$, suggesting that participants with reduced brain blood flow during simulated orthostatic stress had a greater drive to breathe. The observed association provides correlative evidence to support the hypothesized mechanism of reduced vertebral artery blood flow leading to reduced CO_2 clearance from the chemosensitive areas of the brain triggering an increase in ventilation via the central chemoreflex (Ogoh *et al.*, 2013). Mechanistically, reduced $\dot{Q}$ and central hypovolemia could independently reduce cerebral blood flow during orthostatic stress (Ogoh *et al.*, 2005a), which could potentially initiate the hypothesized cerebrovascular-respiratory cascade of events. However, the possibility of another factor causing the increase in $\dot{V}_E$, thereby reducing $P_a\text{CO}_2$ and subsequently vertebral artery blood flow, cannot be ruled out. Baroreflex unloading during LBNP could also potentially initiate a ventilatory response in our participants (Stewart *et al.*, 2011). Accordingly, additional work is needed in order to determine the direction of causality between the changes in vertebral artery blood flow and $\dot{V}_E$.

Work with postural orthostatic tachycardia syndrome (POTS) patients supports the hypothesis that reduced cerebral blood flow precedes ventilatory responses to upright posture (Del Pozzi *et al.*, 2014; Baker *et al.*, 2024). While hyperventilation when upright may be mediated by a hypoxic peripheral chemoreflex response (Del Pozzi *et al.*, 2014), recent evidence challenges this, instead pointing to important roles for stroke volume and brain perfusion (Baker *et al.*, 2024). An elegant experiment using decerebrate rat preparations determined that spontaneous phrenic nerve activity in response to reduced brain perfusion was independent of both peripheral chemoreceptor and baroreceptor inputs, implying that the respiratory response to reduced cerebral perfusion is mediated centrally (Baker *et al.*, 2024). Although the participants in

the present study do not have POTS, our observations of reduced cerebral blood flow, stroke volume and $\dot{Q}$ in parallel with increased $\dot{V}_E$ during LBNP is consistent with their results.

The effect of postural hypocapnia on cerebral blood flow measures remains debated (Serrador *et al.*, 2006; Zuj *et al.*, 2013; Immink *et al.*, 2014). Investigations using non-invasive measures of $P_{ET}CO_2$ as an index of P_aCO_2 during posture transitions have to account for changes in physiological deadspace that exaggerate the reductions in $P_{ET}CO_2$ compared to P_aCO_2 upon standing (Bjurstedt *et al.*, 1962; Immink *et al.*, 2006; Serrador *et al.*, 2006). In the present study, participants remained in the supine position during LBNP, which eliminated the effect of posture change on the distribution of lung blood flow and the $P_{ET}CO_2$ - P_aCO_2 gradient. Strong repeated measures correlations were observed between $P_{ET}CO_2$ and vertebral artery blood flow or mean MCAv throughout the LBNP test, suggesting that hypocapnia did contribute to the reductions in cerebral blood flow. Increased critical closing pressure as an indicator of cerebrovascular tone, which is sensitive to changes in P_aCO_2 (Panerai *et al.*, 1999, 2020), also supports the effect of reduced $P_{ET}CO_2$ on cerebral blood flow. The progressive increase in critical closing pressure during LBNP is in agreement with observations of increasing critical closing pressure in the lead up to syncope during head-up tilt testing (Carey *et al.*, 2001; Zuj *et al.*, 2013). Despite strong repeated measures correlations between $P_{ET}CO_2$ and vertebral artery blood flow or MCAv during LBNP, changes in $P_{ET}CO_2$ did not explain all of the variation in our measures of blood flow or velocity, so other factors, such as $\dot{Q}$ (Ogoh *et al.*, 2005a), must be contributing to reductions in cerebral perfusion as well.

The effects of $P_{ET}CO_2$ on vertebral artery blood flow and mean MCAv were most apparent during the later stages of LBNP leading up to pre-syncope. This observation is consistent with the findings of Zuj *et al.* (Zuj *et al.*, 2013), where reductions in $P_{ET}CO_2$ and mean

MCAv during head-up tilt testing appeared to accelerate in the ~5 min prior to syncope without a change in MAP. Interestingly, studies identifying small or negligible effects of changes in P_aCO_2 or $P_{ET}CO_2$ on cerebral blood flow during orthostatic stress merely observed minor degrees of hypocapnia ($\Delta \sim 2\text{-}3$ mmHg) and did not test all participants to pre-syncope (Immink *et al.*, 2006; Serrador *et al.*, 2006). The degree and duration of orthostatic stress may be important determinants of whether P_aCO_2 changes substantially to affect cerebral blood flow.

Limitations

Large reductions in vertebral artery blood flow leading up to pre-syncope and associated increases in $\dot{V}_E$ (or the absence of these responses) were not unique to a specific sex (Figure 19), suggesting that the relationship between vertebral artery blood flow and $\dot{V}_E$ exists in both men and women. However, sex differences have been reported for LBNP tolerance of young adults, with men typically having higher tolerance than women (Fu *et al.*, 2004, 2005; Jarrard *et al.*, 2024). Resting $\dot{V}_E$ and $P_{ET}CO_2$ of women also fluctuates slightly across the menstrual cycle (MacNutt *et al.*, 2012). Accordingly, it is possible that participants' sex and menstrual cycle phase could influence the magnitude of change in vertebral artery blood flow or $\dot{V}_E$ during simulated orthostatic stress.

We used $\dot{V}_E$ and $P_{ET}CO_2$ as indices of $\dot{V}_A$ and P_aCO_2 , respectively. With posture transitions, changes in the distribution of lung perfusion alters physiological deadspace and gas exchange efficiency, which reduces the $\dot{V}_A/\dot{V}_E$ ratio and increases the $P_{ET}CO_2$ - P_aCO_2 gradient (Riley *et al.*, 1959; Bjurstedt *et al.*, 1962). We used supine LBNP as our model of orthostatic

stress to eliminate these effects of posture transition on our measures, but cannot account for potential changes in physiological deadspace as a consequence of reduced $\dot{Q}$ during LBNP.

We collected MCAv as a complimentary index of cerebral blood flow. We acknowledge that interpreting changes in velocity as an index of flow requires assuming that vessel diameter does not change. It is unknown whether the MCA diameter changed during the graded LBNP test to pre-syncope, but importantly MCAv data in the present study were only provided to supplement conclusions based on volumetric flow through the vertebral artery. Additionally, we only measured blood flow through the left vertebral artery. Volumetric flow between the right and left vertebral arteries may differ, with one artery having higher flow rate; however, side-to-side differences likely vary from person-to-person (Seidel *et al.*, 1999).

Lastly, multiple physiological tests that manipulated $P_{ET}CO_2$ were performed during a single experimental visit. The hypocapnic cerebrovascular reactivity test included hyperventilating to reduce $P_{ET}CO_2$ to ~20 mmHg followed by 5 mmHg step-wise increases in $P_{ET}CO_2$ back to normocapnic levels, with each level of hypocapnia lasting for 2-3 min and the whole test typically lasting ~15 min. The modified rebreathing test included ~3 min of hyperventilation to drive $P_{ET}CO_2$ down to ~20 mmHg, followed by a gradual rise in $P_{ET}CO_2$ to a maximum of 60 mmHg. All the tests were performed during the same visit in order to eliminate day-to-day variability in measurements; however, it is unknown whether one test influenced the outcome of a subsequent test, as there is evidence of hysteresis in the $P_{ET}CO_2$ – cerebral blood flow relationship (Ide *et al.*, 2003). It is also possible that reductions in $P_{ET}CO_2$ with hyperventilation during the hypocapnic cerebrovascular reactivity test, or increase in $P_{ET}CO_2$ during the rebreathing test could have affected CO_2 stores in the body for the subsequent test. Importantly though, participants were given a 10-15 min break between each test to allow

$P_{ET}CO_2$, $\dot{V}_E$, and cerebral blood flow to recover back to baseline levels, and it is anticipated that the respiratory manipulations only affected fast response, extracellular CO_2 stores (Giosa *et al.*, 2021).

Conclusion

The present study examined cerebrovascular and respiratory responses during graded LBNP to pre-syncope. We identified considerable between-person variation in the changes in vertebral artery blood flow, $P_{ET}CO_2$, and $\dot{V}_E$ in the progression to pre-syncope in a cohort of young healthy adults. The magnitude of reduction in vertebral artery blood flow was strongly associated with the increase in $\dot{V}_E$ from pre-LBNP to pre-syncope, potentially implicating vertebral artery blood flow as an important mediator of respiratory responses to orthostatic stress; however, this remains speculative as direction of causality between altered vertebral artery blood flow and $\dot{V}_E$ remained elusive in the present study. Nevertheless, these data provide strong support for important interactions between respiratory and cerebrovascular control during orthostatic stress, and that not all healthy adults follow the same pathway to syncope.

Chapter 6: General Discussion

Cerebrovascular regulation of blood flow is complex, with multiple integrative and redundant mechanisms acting together to ensure that the brain receives adequate blood flow (Claassen *et al.*, 2021). It is widely accepted that habitual exercise and associated increases in cerebral blood flow are beneficial for cerebrovascular and cognitive function (Barnes, 2015; Calverley *et al.*, 2020; Bliss *et al.*, 2021), while excessive reductions in cerebral blood flow during orthostatic stress are problematic leading to syncope (Van Lieshout *et al.*, 2003). Despite the clear benefits or consequences of increased or reduced blood flow, the contribution of the different cerebrovascular control mechanisms to the increase in cerebral blood flow with exercise (Ogoh & Ainslie, 2009; Smith & Ainslie, 2017) and the reduction in flow during orthostatic stress (Serrador *et al.*, 2006; Zuj *et al.*, 2013; Immink *et al.*, 2014) remains debated. Therefore, the overall aim of this thesis was to identify regulatory factors that play prominent roles in modulating cerebral blood flow during exercise and orthostatic stress. A series of 4 experiments were conducted, which included moderate- and high-intensity interval cycling to investigate cerebrovascular dynamics during exercise, as well as a bed rest intervention and graded orthostatic stress to pre-syncope to identify factors contributing to the development of orthostatic intolerance.

Summary of Findings

Dynamic changes in exercise intensity provided strong stimuli to study the contribution of different mechanisms to the cerebral hyperemic response to exercise. Through time-series modeling, it was identified that changes in MAP, eP_aCO_2 and cerebral autoregulation largely explained the dynamic changes in MCAv during both moderate- (chapter 2) and high-intensity

exercise (chapter 3). Initial transient responses within the first ~10 s of changes in work rate were linked to rapid changes in MAP that occurred too quickly for autoregulation to buffer. Following this initial dynamic phase, changes in eP_aCO_2 drove the dynamic changes in MCAv following a short time delay. The intensity of exercise also appeared to have a modifying effect on cerebrovascular control. Initial ARMA analysis of the entire high-intensity interval exercise protocol resulted in a pattern in the residuals for the predicted MCAv response that was not evident during moderate-intensity interval exercise. By splitting the protocol in half, with the first half having higher eP_aCO_2 than the second half, we identified that the simulated ARMA MAP step response was elevated in the first half of the test. Given that hypercapnia impairs dynamic cerebral autoregulation whilst hypocapnia improves autoregulation (Aaslid *et al.*, 1989; Edwards *et al.*, 2004), it is plausible that the changes in eP_aCO_2 that are observed during high-intensity interval exercise have an effect on the ability of autoregulation to buffer changes in MAP. Overall, the results from chapters 2 and 3 support hypothesis #1 and #2, as P_aCO_2 was identified as a causal mediator of dynamic MCAv responses to both moderate and high-intensity exercise transitions; however, contrary to hypothesis #2, dynamic autoregulation changed over the course of a bout of high-intensity interval exercise, suggesting that the contribution of different mechanisms to changes in MCAv during exercise may change with intensity.

Bed rest deconditioning coupled with supine-to-stand transitions, or graded LBNP tests were used as stimuli to increase orthostatic stress to the point of pre-syncope. Changes in cerebrovascular responses to supine-to-stand tests from pre- to post-bed rest of late-middle-aged adults (chapter 4) and the observed variation in the pathway to syncope in young healthy adults during graded LBNP (chapter 5) suggested that there are important interactions between cerebral blood flow and $P_{ET}CO_2$ during severe orthostatic stress leading to syncope. In support of

hypothesis #3, we observed sex-specific effects of bed rest on orthostatic tolerance, MAP, and CVRi while standing. Post-menopausal women had reduced orthostatic tolerance while standing after bed rest compared to similar-aged men. Despite orthostatic tolerance being reduced in the female participants, both men and women were hypocapnic with reduced mean MCAv while standing post-bed rest. Interestingly, only the men had increased CVRi associated with hypocapnia, while the women had unchanged CVRi and were hypotensive.

Graded LBNP tests to pre-syncope in young healthy men and women identified a relationship between changes in vertebral artery blood flow and $\dot{V}_E$ in the progression towards syncope. Participants with the largest reductions in vertebral artery blood flow had the greatest increases in $\dot{V}_E$ as they reached orthostatic intolerance, potentially implicating vertebral artery blood flow and its interaction with the central chemoreceptor as an important mediator of respiratory responses to orthostatic stress. This relationship between the changes in $\dot{V}_E$ and vertebral artery blood flow during simulated orthostatic stress supports hypothesis #4; however, this correlative relationship cannot definitively confirm the mechanism of action, as the direction of causality between altered vertebral artery blood flow and $\dot{V}_E$ remained elusive.

In summary, the findings of these 4 studies indicate important cerebrovascular-respiratory interactions, with competing effects of arterial blood pressure and P_aCO_2 on cerebral blood flow during both exercise and orthostatic stress.

Significance

The findings outlined in this thesis enhance our fundamental understanding of cerebrovascular control during exercise and the integrative cerebrovascular cascade leading to

syncope. Our identification of a critical role of eP_aCO_2 in driving cerebrovascular dynamics and increased MCAv during exercise challenges recent works that have suggested that changes in P_aCO_2 do not influence the dynamic MCAv response at the onset of exercise (Billinger *et al.*, 2017; Witte *et al.*, 2019). Instead, our results indicate that changes in mean MCAv follow the changes in eP_aCO_2 after step transitions in work rate, which do not necessarily follow an exponential rise to a new steady-state after a time delay (Billinger *et al.*, 2017). Our tight temporal associations between eP_aCO_2 dynamics and changes in MCAv during transitions in exercise intensity also contrast with the report of similar increases in cerebral blood flow during incremental levels of steady-state isocapnic and poikilocapnic exercise (Smith *et al.*, 2016). This discrepancy between an important role for $P_{ET}CO_2$ for dynamic changes in MCAv, but not for steady-state changes raises insightful questions for future work. Given that there are multiple integrative and redundant mechanisms responsible for ensuring that the brain is adequately perfused, it may be possible that different factors mediate the exercise-induced increase in cerebral blood flow in the absence of a change in another factor. Investigation of the time-course of change in cerebral blood flow during step transitions in work rate where $P_{ET}CO_2$ is clamped in comparison to a poikilocapnic condition may reveal important differences in blood flow dynamics and suggest an altered primary control mechanism leading to cerebral hyperemia.

The results from the bed rest study and graded LBNP tests reinforce the idea that there are a variety of mechanisms that can contribute to the development of orthostatic intolerance and syncope (Fritsch-Yelle *et al.*, 1996b; Fu *et al.*, 2005, 2012). Our identification of sex-differences in orthostatic tolerance, MAP, and CVRi while standing following 2 weeks of bed rest in late-middle-aged adults is of clinical relevance, as this identifies late-middle-aged women as being at potentially higher risk for syncope and falls than men after a period of severe inactivity or

hospitalization. Increased susceptibility of post-menopausal women to orthostatic intolerance after bed rest is consistent with reports of older women being more likely to present to the emergency department due to syncope (Grossman *et al.*, 2005). It was notable that men and women had similar reductions in MCAv and $P_{ET}CO_2$ while standing, but that only women were hypotensive by the third minute of standing, suggesting that reduced MAP contributed to lower MCAv while standing in women post-bed rest but not in men. Increased sensitivity of post-menopausal women to either reduced cardiac baroreflex sensitivity (Barnes *et al.*, 2014) or hypovolemia (Fu *et al.*, 2005) following bed rest may contribute to the observed sex differences.

Vertebral artery blood flow may be an important mediator of both cardiovascular and respiratory responses to orthostatic stress, as the vertebral arteries supply the cardiovascular and respiratory control centres, as well as the chemosensitive areas of the brain (Tatu *et al.*, 1996). Previous work has identified that vertebral artery blood flow is protected during low-to-moderate orthostatic stress compared to internal carotid artery flow (Sato *et al.*, 2012; Ogoh *et al.*, 2015), likely because maintenance of blood flow to this brainstem region is critical for basic life function. Accordingly, it has been suggested that reductions in vertebral artery blood flow may be more linked to orthostatic intolerance and syncope than the anterior circulation fed by the internal carotid arteries (Ogoh *et al.*, 2015). It has also been suggested that reductions in cerebral blood flow (estimated by MCAv) during orthostatic stress may initiate a ventilatory response via reduced CO_2 clearance from the chemosensitive area of the brain and subsequent stimulation of the central chemoreflex (Ogoh *et al.*, 2013). Our results build on these findings by demonstrating a strong relationship between reduced vertebral artery blood flow and increased $\dot{V}_E$, providing correlative evidence that reductions in posterior blood supply to the brain affect respiratory control during simulated orthostatic stress. Although hyperventilation on its own is not typically

enough of a stimulus to induce syncope (Levine *et al.*, 1994), how reductions in vertebral blood flow, increased $\dot{V}_E$, and reduced $P_a\text{CO}_2$ interact with systemic cardiovascular regulation could be an important factor in the progression towards syncope. Within our study, we did not observe large reductions in vertebral artery blood flow in all participants, nor did all participants substantially increase their $\dot{V}_E$ leading up to test termination. These differences between participants emphasise that the progression to syncope is not uniform for all individuals. Larger effects of simulated orthostatic stress on vertebral artery blood flow and $\dot{V}_E$ may have been observed in some participants if tests were not stopped at the onset of symptoms prior to a fall in blood pressure, or if blood pressure was able to be further reduced prior to test termination. It is noteworthy to state that large reductions in vertebral blood flow and increased $\dot{V}_E$ at test termination, or lack thereof, was not unique to a specific sex, despite sex differences in orthostatic tolerance existing between men and women (Convertino, 1998; Fu *et al.*, 2004, 2005).

Limitations

In this thesis, all studies were conducted on cohorts of healthy men and women. Three studies used young adults and one study used late-middle-aged adults. Accordingly, our results are only generalizable to healthy populations of those age groups for each respective study. Additionally, our exercise studies were conducted using an upright cycle ergometer, so the exercise-based results may not be generalizable to all modes of exercise.

Multiple factors affect the regulation of cerebral blood flow (Smith & Ainslie, 2017; Claassen *et al.*, 2021). During our exercise investigations, it was not possible to measure all potential factors that could affect blood flow, and as a result our ARMA analysis included only 2

inputs to model the MCAv response to exercise. We acknowledge that other factors that are known to change with exercise (e.g., $\dot{Q}$, cerebral metabolism, etc.) may have some influence on the cerebrovascular response to exercise; however, within the scope of our experimental conditions, it appears that P_aCO_2 , MAP and autoregulation largely explain dynamic changes in MCAv during exercise-to-exercise transitions.

As stated within the limitations/methodological considerations sections of chapters 2, 3, 4, and 5, the investigations of the present thesis relied heavily on non-invasive estimates of multiple cardiovascular, respiratory and cerebrovascular variables. Blood pressure was measured via the finger volume clamp method in all studies. Stroke volume was estimated from the non-invasive pressure waveform using the ModelFlow® algorithm, and subsequently used to calculate $\dot{Q}$ and TPR during exercise tests and the supine-to-stand tests. $ePaCO_2$ and $P_{ET}CO_2$ were used as estimates of P_aCO_2 during exercise and orthostatic stress tests, respectively, and $\dot{V}_E$ was also used as an indicator of $\dot{V}_A$ during LBNP. MCAv was measured as an index of cerebral blood flow in all studies, which requires assuming that MCA diameter is not changing during exercise or orthostatic stress in order to interpret the observed changes in velocity. In future work, if experimentally feasible, more direct measures of variables of interest should be utilized. The use of invasive arterial blood pressure, P_aCO_2 and volumetric blood flow measures in future exercise and orthostatic stress test investigations would be beneficial.

Future Directions

There are many potential future directions stemming from these research projects. As previously discussed, assessing dynamic MCAv or volumetric blood flow responses during

isocapnic versus poikilocapnic conditions will be very important in understanding how different mechanisms may contribute to changes in cerebral blood flow at the onset of exercise and during rapid transitions in exercise intensity. Isocapnic exercise transitions may provide critical insights into the interaction between MAP and $P_a\text{CO}_2$ in the regulation of RAP and CrCP during exercise, which appears to differ from rest. Additionally, given that $P_a\text{CO}_2$ appears to be the main driver of MCAv dynamics during exercise transitions and that there is an interest in modelling dynamic cerebrovascular responses to step changes in exercise as an indicator of cerebrovascular health and function (Billinger *et al.*, 2017, 2021; Witte *et al.*, 2019; Weston *et al.*, 2022), future work examining the how ventilatory control at the onset of exercise interacts with cerebral blood flow would be beneficial. Time-varying models (Dineen *et al.*, 2010; Liu *et al.*, 2010; Panerai *et al.*, 2010; Kostoglou *et al.*, 2019) may also be useful in the future for assessing potentially dynamic changes in cerebrovascular control mechanisms during high-intensity exercise.

To build on the results of our orthostatic stress investigations, future work should focus on the dynamic coupling between changes in vertebral blood flow and $\dot{V}_E$ during severe intermittent orthostatic stress in order to potentially resolve the direction of causality between changes in vertebral artery blood flow and $\dot{V}_E$. Extending these investigations to older adults will also be important, as the risk of syncope increases with advancing age (Soteriades *et al.*, 2002; O'Brien & Kenny, 2014).

Thesis Conclusion

This thesis identified important cerebrovascular-respiratory interactions, with important effects of arterial blood pressure and $P_a\text{CO}_2$ on cerebral blood flow observed during both

exercise and orthostatic stress. The magnitude of reduction in vertebral artery blood flow also appeared to be an important factor during orthostatic stress, potentially mediating increases in $\dot{V}_E$ in the progression to orthostatic intolerance. Overall, these findings enhance our understanding of cerebrovascular control during exercise and the cerebrovascular cascade leading to syncope.

References

- Aaslid R (1987). Visually evoked dynamic blood flow response of the human cerebral circulation. *Stroke* **18**, 771–775.
- Aaslid R, Lindegaard K-F, Sorteberg W & Nornes H (1989). Cerebral autoregulation dynamics in humans. *Stroke* **20**, 45–52.
- Aaslid R, Markwalder T-M & Nornes H (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* **57**, 769–774.
- Ainslie PN & Duffin J (2009). 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–R1495.
- Ainslie PN & Ogoh S (2010). Regulation of cerebral blood flow in mammals during chronic hypoxia: a matter of balance. *Exp Physiol* **95**, 251–262.
- Ainslie PN & Poulin MJ (2004). Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *J Appl Physiol* **97**, 149–159.
- Akins JD, Okada Y, Hendrix JM, Vongpatanasin W & Fu Q (2023). Greater resting muscle sympathetic nerve activity reduces cold pressor autonomic reactivity in older women but not older men. *Am J Physiol Regul Integr Comp Physiol* **324**, R656–R665.
- Alborch E, Gomez B, Dieguez G, Marin J & Lluch S (1977). Cerebral blood flow and vascular reactivity after removal of the superior cervical sympathetic ganglion in the goat. *Circ Res* **41**, 278–282.
- Amelard R, Murray KR, Hedge ET, Cleworth TW, Noguchi M, Laing AC & Hughson RL

- (2020). Monocular 3D sway tracking for assessing postural instability in cerebral hypoperfusion during quiet standing. *IEEE Trans Neural Syst Rehabil Eng* **28**, 720–729.
- Angerer O, Larina I & Cromwell R (2014). *Guidelines for standardization of bed rest studies in the spaceflight contexted*. Sundblad P & Orlov O. International Academy of Astronautics, Paris, France.
- Arbeille P, Kerbeci P, Mattar L, Shoemaker JK & Hughson R (2008). Insufficient flow reduction during LBNP in both splanchnic and lower limb areas is associated with orthostatic intolerance after bedrest. *Am J Physiol Heart Circ Physiol* **295**, H1846–H1854.
- Attwell D, Buchan AM, Charpak S, Lauritzen M, MacVicar BA & Newman EA (2010). Glial and neuronal control of brain blood flow. *Nature* **468**, 232–243.
- Bailey DM, Evans KA, McEneny J, Young IS, Hullin DA, James PE, Ogoh S, Ainslie PN, Lucchesi C, Rockenbauer A, Culcasi M & Pietri S (2011). Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. *Exp Physiol* **96**, 1196–1207.
- Bakdash JZ & Marusich LR (2017). Repeated measures correlation. *Front Psychol* **8**, 1–13.
- Baker JR, Incognito A V., Ranada SI, Sheldon RS, Sharkey KA, Phillips AA, Wilson RJA & Raj SR (2024). Reduced stroke volume and brain perfusion drive postural hyperventilation in postural orthostatic tachycardia syndrome. *JACC Basic to Transl Sci*; DOI: 10.1016/j.jacbts.2024.04.011.
- Barnes JN (2015). Exercise, cognitive function, and aging. *Adv Physiol Educ* **39**, 55–62.
- Barnes JN, Hart EC, Curry TB, Nicholson WT, Eisenach JH, Wallin BG, Charkoudian N &

- Joyner MJ (2014). Aging enhances autonomic support of blood pressure in women. *Hypertension* **63**, 303–308.
- Barnes JN, Matzek LJ, Charkoudian N, Joyner MJ, Curry TB & Hart EC (2012). Association of cardiac baroreflex sensitivity with blood pressure transients: influence of sex and menopausal status. *Front Physiol* **3**, 187.
- Baumbach GL & Heistad DD (1983). Effects of sympathetic stimulation and changes in arterial pressure on segmental resistance of cerebral vessels in rabbits and cats. *Circ Res* **52**, 527–533.
- Beaver WL, Wasserman K & Whipp BJ (1986). A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* **60**, 2020–2027.
- Beltrame T & Hughson RL (2017). Linear and non-linear contributions to oxygen transport and utilization during moderate random exercise in humans. *Exp Physiol* **102**, 563–577.
- Bennett FM, Reischl P, Grodins FS, Yamashiro SM & Fordyce WE (1981). Dynamics of ventilatory response to exercise in humans. *J Appl Physiol Respir Environ Exerc Physiol* **51**, 194–203.
- Billinger SA, Craig JC, Kwapiszeski SJ, Sisante J-F V., Vidoni ED, Maletsky R & Poole DC (2017). Dynamics of middle cerebral artery blood flow velocity during moderate-intensity exercise. *J Appl Physiol* **122**, 1125–1133.
- Billinger SA, Whitaker AA, Morton A, Kaufman CS, Perdomo SJ, Ward JL, Eickmeyer SM, Bai SX, Ledbetter L & Abraham MG (2021). Pilot study to characterize middle cerebral artery dynamic response to an acute bout of moderate intensity exercise at 3- and 6-months

- poststroke. *J Am Heart Assoc* **10**, e017821.
- Bjurstedt H, Hesser CM, Liljestrand G & Matell G (1962). Effects of posture on alveolar-arterial CO₂ and O₂ differences and on alveolar dead space in man. *Acta Physiol Scand* **54**, 65–82.
- Blaber AP, Goswami N, Bondar RL & Kassam MS (2011). Impairment of cerebral blood flow regulation in astronauts with orthostatic intolerance after flight. *Stroke* **42**, 1844–1850.
- Bliss ES, Wong RHX, Howe PRC & Mills DE (2021). Benefits of exercise training on cerebrovascular and cognitive function in ageing. *J Cereb Blood Flow Metab* **41**, 447–470.
- Du Bois D & Du Bois EF (1916). A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med* **17**, 863–871.
- Bos WJW, van Goudoever J, van Montfrans GA, van den Meiracker AH & Wesseling KH (1996). Reconstruction of brachial artery pressure from noninvasive finger pressure measurements. *Circulation* **94**, 1870–1875.
- Boulet LM, Tymko MM, Jamieson AN, Ainslie PN, Skow RJ & Day TA (2016). Influence of prior hyperventilation duration on respiratory chemosensitivity and cerebrovascular reactivity during modified hyperoxic rebreathing. *Exp Physiol* **101**, 821–835.
- Brassard P, Ferland-Dutil H, Smirl JD, Paquette M, Le Blanc O, Malenfant S & Ainslie PN (2017). Evidence for hysteresis in the cerebral pressure-flow relationship in healthy men. *Am J Physiol Heart Circ Physiol* **312**, H701–H704.
- Brown AM & Ransom BR (2007). Astrocyte glycogen and brain energy metabolism. *Glia* **55**, 1263–1271.
- Brys M, Brown CM, Marthol H, Franta R & Hilz MJ (2003). Dynamic cerebral autoregulation

- remains stable during physical challenge in healthy persons. *Am J Physiol Heart Circ Physiol* **285**, H1048–H1054.
- Burma JS, Macaulay A, Copeland P, Khatra O, Bouliane KJ & Smirl JD (2020). Comparison of cerebrovascular reactivity recovery following high-intensity interval training and moderate-intensity continuous training. *Physiol Rep* **8**, e14467.
- Burton AC (1951). On the physical equilibrium of small blood vessels. *Am J Physiol* **164**, 319–329.
- Calverley TA, Ogoh S, Marley CJ, Steggall M, Marchi N, Brassard P, Lucas SJE, Cotter JD, Roig M, Ainslie PN, Wisløff U & Bailey DM (2020). HIITing the brain with exercise: mechanisms, consequences and practical recommendations. *J Physiol* **598**, 2513–2530.
- Carey BJ, Eames PJ, Panerai RB & Potter JF (2001). Carbon dioxide, critical closing pressure and cerebral haemodynamics prior to vasovagal syncope in humans. *Clin Sci* **101**, 351–358.
- Casaburi R, Stremel RW, Whipp BJ, Beaver WL & Wasserman K (1980). Alteration by hyperoxia of ventilatory dynamics during sinusoidal work. *J Appl Physiol Respir Environ Exerc Physiol* **48**, 1083–1091.
- Casaburi R, Whipp BJ, Wasserman K, Beaver WL & Koyal SN (1977). Ventilatory and gas exchange dynamics in response to sinusoidal work. *J Appl Physiol Respir Environ Exerc Physiol* **42**, 300–311.
- Cassaglia PA, Griffiths RI & Walker AM (2008). Sympathetic nerve activity in the superior cervical ganglia increases in response to imposed increases in arterial pressure. *Am J Physiol Regul Integr Comp Physiol* **294**, R1255–R1261.

- Cencetti S, Bandinelli G & Lagi A (1997). Effect of Pco₂ changes induced by head-upright tilt on transcranial Doppler recordings. *Stroke* **28**, 1195–1197.
- Claassen JAHR, Thijssen DHJ, Panerai RB & Faraci FM (2021). Regulation of cerebral blood flow in humans: physiology and clinical implications of autoregulation. *Physiol Rev* **101**, 1487–1559.
- Convertino VA (1998). Gender differences in autonomic functions associated with blood pressure regulation. *Am J Physiol Regul Integr Comp Physiol* **275**, R1909–R1920.
- Coverdale NS, Gati JS, Opalevych O, Perrotta A & Shoemaker JK (2014). Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *J Appl Physiol* **117**, 1090–1096.
- D’Antona L, Craven CL, Bremner F, Matharu MS, Thorne L, Watkins LD & Toma AK (2022). Effect of position on intracranial pressure and compliance: a cross-sectional study including 101 patients. *J Neurosurg* **136**, 1781–1789.
- Deegan BM, Devine ER, Geraghty MC, Jones E, ÓLaighin G & Serrador JM (2010). The relationship between cardiac output and dynamic cerebral autoregulation in humans. *J Appl Physiol* **109**, 1424–1431.
- Diaz-Canestro C, Pentz B, Sehgal A & Montero D (2022). Sex differences in orthostatic tolerance are mainly explained by blood volume and oxygen carrying capacity. *Crit Care Explor* **4**, E0608.
- Diedrich A, Paranjape SY & Robertson D (2007). Plasma and blood volume in space. *Am J Med Sci* **334**, 80–86.

- Van Dijk JG & Wieling W (2013). Pathophysiological basis of syncope and neurological conditions that mimic syncope. *Prog Cardiovasc Dis* **55**, 345–356.
- Dineen NE, Brodie FG, Robinson TG & Panerai RB (2010). Continuous estimates of dynamic cerebral autoregulation during transient hypocapnia and hypercapnia. *J Appl Physiol* **108**, 604–613.
- van Dinther M, Hooghiemstra AM, Bron EE, Versteeg A, Leeuwis AE, Kalay T, Moonen JE, Kuipers S, Backes WH, Jansen JFA, van Osch MJP, Biessels GJ, Staals J & van Oostenbrugge RJ (2024). Lower cerebral blood flow predicts cognitive decline in patients with vascular cognitive impairment. *Alzheimer's Dement* **20**, 136–144.
- Edgell H, Zuj KA, Greaves DK, Shoemaker JK, Custaud M-A, Kerbeci P, Arbeille P & Hughson RL (2007). WISE-2005: adrenergic responses of women following 56-days, 6° head-down bed rest with or without exercise countermeasures. *Am J Physiol Regul Integr Comp Physiol* **293**, R2343–R2352.
- Edwards MR, Devitt DL & Hughson RL (2004). Two-breath CO₂ test detects altered dynamic cerebrovascular autoregulation and CO₂ responsiveness with changes in arterial PCO₂. *Am J Physiol Regul Integr Comp Physiol* **287**, R627–R632.
- Edwards MR, Topor ZL & Hughson RL (2003). A new two-breath technique for extracting the cerebrovascular response to arterial carbon dioxide. *Am J Physiol Regul Integr Comp Physiol* **284**, R853–R859.
- Faisal A, Beavers KR, Robertson AD & Hughson RL (2009). Prior moderate and heavy exercise accelerate oxygen uptake and cardiac output kinetics in endurance athletes. *J Appl Physiol* **106**, 1553–1563.

- Fitzgibbon-Collins LK, Coombs GB, Noguchi M, Parihar S, Hughson RL, Borrie M, Peters S, Shoemaker JK & Bhangu J (2024). Standing middle cerebral artery velocity predicts cognitive function and gait speed in older adults with cognitive impairment, and is impacted by sex differences. *Cereb Circ - Cogn Behav* **6**, 100198.
- Fitzgibbon-Collins LK, Heckman GA, Bains I, Noguchi M, McIlroy WE & Hughson RL (2021). Older adults' drop in cerebral oxygenation on standing correlates with postural instability and may improve with sitting prior to standing. *J Gerontol Ser A, Biol Sci Med Sci* **76**, 1124–1133.
- Fitzgibbon-Collins LK, Noguchi M, Heckman GA, Hughson RL & Robertson AD (2019). Acute reduction in cerebral blood velocity on supine-to-stand transition increases postural instability in young adults. *Am J Physiol Heart Circ Physiol* **317**, H1342–H1353.
- Fritsch-Yelle JM, Charles JB, Jones MM & Wood ML (1996a). Microgravity decreases heart rate and arterial pressure in humans. *J Appl Physiol* **80**, 910–914.
- Fritsch-Yelle JM, Whitson PA, Bondar RL & Brown TE (1996b). Subnormal norepinephrine release relates to presyncope in astronauts after spaceflight. *J Appl Physiol* **81**, 2134–2141.
- Fu Q, Arbab-Zadeh A, Perhonen MA, Zhang R, Zuckerman JH & Levine BD (2004). Hemodynamics of orthostatic intolerance: implications for gender differences. *Am J Physiol Heart Circ Physiol* **286**, H449–H457.
- Fu Q, Verheyden B, Wieling W & Levine BD (2012). Cardiac output and sympathetic vasoconstrictor responses during upright tilt to presyncope in healthy humans. *J Physiol* **590**, 1839–1848.

- Fu Q, Witkowski S, Okazaki K & Levine BD (2005). Effects of gender and hypovolemia on sympathetic neural responses to orthostatic stress. *Am J Physiol Regul Integr Comp Physiol* **289**, R109–R116.
- Gibbons TD, Zuj KA, Peterson SD & Hughson RL (2019). Comparison of pulse contour, aortic Doppler ultrasound and bioelectrical impedance estimates of stroke volume during rapid changes in blood pressure. *Exp Physiol* **104**, 368–378.
- Giller CA, Bowman G, Dyer H, Mootz L & Krippner W (1993). Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery* **32**, 737–742.
- Giosa L, Busana M, Bonifazi M, Romitti F, Vassalli F, Pasticci I, Macrì MM, D’Albo R, Collino F, Gatta A, Palumbo MM, Herrmann P, Moerer O, Iapichino G, Meissner K, Quintel M & Gattinoni L (2021). Mobilizing carbon dioxide stores: an experimental study. *Am J Respir Crit Care Med* **203**, 318–327.
- Gordon GRJ, Choi HB, Rungta RL, Ellis-Davies GCR & MacVicar BA (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature* **456**, 745–750.
- Grenon SM, Xiao X, Hurwitz S, Sheynberg N, Kim C, Seely EW, Cohen RJ & Williams GH (2006). Why is orthostatic tolerance lower in women than in men? Renal and cardiovascular responses to simulated microgravity and the role of midodrine. *J Invest Med* **54**, 180–190.
- Gross PM, Marcus ML & Heistad DD (1980). Regional distribution of cerebral blood flow during exercise in dogs. *J Appl Physiol Respir Environ Exerc Physiol* **48**, 213–217.
- Grossman SA, Shapiro NI, Van Epp S, Konen R, Arnold R, Moore R, Lee L, Wolfe RE &

- Lipsitz LA (2005). Sex differences in the emergency department evaluation of elderly patients with syncope. *J Gerontol A Biol Sci Med Sci* **60A**, 1202–1205.
- Grubb BP, Gerard G, Roush K, Temesy-Armos P, Montford P, Elliott L, Hahn H & Brewster P (1991). Cerebral vasoconstriction during head-upright tilt-induced vasovagal syncope: a paradoxical and unexpected response. *Circulation* **84**, 1157–1164.
- Guelen I, Westerhof BE, Van Der Sar GL, Van Montfrans GA, Kiemeneij F, Wesseling KH & Bos WJW (2008). Validation of brachial artery pressure reconstruction from finger arterial pressure. *J Hypertens* **26**, 1321–1327.
- Guinet P, Schneider SM, Macias BR, Watenpaugh DE, Hughson RL, Pavy Le Traon A, Bansard JY & Hargens AR (2009). WISE-2005: effect of aerobic and resistive exercises on orthostatic tolerance during 60 days bed rest in women. *Eur J Appl Physiol* **106**, 217–227.
- Hafkenschiel JH, Crumpton CW, Shenkin HA, Moyer JH, Zintel HA, Wendel H & Jeffers WA (1951). The effects of twenty degree head-up tilt upon the cerebral circulation of patients with arterial hypertension before and after sympathectomy. *J Clin Invest* **30**, 793–798.
- Hajj-Boutros G, Sonjak V, Faust A, Balram S, Lagacé J-C, St-Martin P, Divsalar DN, Sadeghian F, Liu-Ambrose T, Blaber AP, Dionne IJ, Duchesne S, Kontulainen S, Theou O & Morais JA (2024). Myths and methodologies: understanding the health impact of head down bedrest for the benefit of older adults and astronauts. Study protocol of the Canadian Bedrest Study. *Exp Physiol* **109**, 812–827.
- Hajj-Boutros G, Sonjak V, Hedge ET, Mastrandrea C, Lagacé J-C, St-Martin P, Divsalar DN, Sadeghian F, Chevalier S, Liu-Ambrose T, Blaber AP, Dionne IJ, Duchesne S, Hughson R, Kontulainen S, Theou O & Morais JA (2023). Impact of 14 days of bed rest in older adults

- and an exercise countermeasure on body composition, muscle strength, and cardiovascular function: Canadian Space Agency standard measures. *Gerontology* **69**, 1284–1294.
- Hargens AR & Vico L (2016). Long-duration bed rest as an analog to microgravity. *J Appl Physiol* **120**, 891–903.
- Harms MPM, Finucane C, Pérez-Denia L, Jurachek S, van Wijnen VK, Lipsitz LA, van Lieshout JJ & Wieling W (2021). Systemic and cerebral circulatory adjustment within the first 60 s after active standing: an integrative physiological view. *Auton Neurosci Basic Clin* **231**, 102756.
- Harms MPM, Wesseling KH, Pott F, Jenstrup M, van Goudoever J, Secher NH & van Lieshout JJ (1999). Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci* **97**, 291–301.
- Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach J & Joyner MJ (2011). Sex and ageing differences in resting arterial pressure regulation: the role of the β -adrenergic receptors. *J Physiol* **589**, 5285–5297.
- Hastings JL, Krainski F, Snell PG, Pacini EL, Jain M, Bhella PS, Shibata S, Fu Q, Palmer MD & Levine BD (2012). Effect of rowing ergometry and oral volume loading on cardiovascular structure and function during bed rest. *J Appl Physiol* **112**, 1735–1743.
- Hedge ET & Hughson RL (2020). Frequency domain analysis to extract dynamic response characteristics for oxygen uptake during transitions to moderate- and heavy-intensity exercises. *J Appl Physiol* **129**, 1422–1430.
- Hedge ET, Hughson RL & Dominelli PB (2022a). Repeatability and reproducibility of changes

- in thoracoabdominal compartmental volumes and breathing pattern during low-, moderate- and heavy-intensity exercise. *Eur J Appl Physiol* **122**, 1217–1229.
- Hedge ET, Mastrandrea CJ & Hughson RL (2023). Loss of cardiorespiratory fitness and its recovery following two weeks of head-down bed rest and the protective effects of exercise in 55- to 65-yr-old adults. *J Appl Physiol* **134**, 1022–1031.
- Hedge ET, Patterson CA, Mastrandrea CJ, Sonjak V, Hajj-Boutros G, Faust A, Morais JA & Hughson RL (2022b). Implementation of exercise countermeasures during spaceflight and microgravity analogue studies: developing countermeasure protocols for bedrest in older adults (BROA). *Front Physiol* **13**, 928313.
- Hughson RL (2009). Oxygen uptake kinetics: historical perspective and future directions. *Appl Physiol Nutr Metab* **34**, 840–850.
- Hughson RL & Inman MD (1986). Oxygen uptake kinetics from ramp work tests: variability of single test values. *J Appl Physiol* **61**, 373–376.
- Hughson RL, Maillet A, Gharib C, Fortrat J, Yamamoto Y, Pavy-Le Traon A, Riviere D & Guell A (1994). Reduced spontaneous baroreflex response slope during lower body negative pressure after 28 days of head-down bed rest. *J Appl Physiol* **77**, 69–77.
- Hughson RL & Morrissey M (1982). Delayed kinetics of respiratory gas exchange in the transition from prior exercise. *J Appl Physiol Respir Environ Exerc Physiol* **52**, 921–929.
- Hughson RL, Winter DA, Patla AE, Swanson GD & Cuervo LA (1990). Investigation of VO₂ kinetics in humans with pseudorandom binary sequence work rate change. *J Appl Physiol* **68**, 796–801.

Huntsman LL, Stewart DK, Barnes SR, Franklin SB, Colocousis JS & Hessel EA (1983).

Noninvasive Doppler determination of cardiac output in man: clinical validation.

Circulation **67**, 593–602.

Huttmann SE, Windisch W & Storre JH (2014). Techniques for the measurement and monitoring of carbon dioxide in the blood. *Ann Am Thorac Soc* **11**, 645–652.

Iadecola C (2017). The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. *Neuron* **96**, 17–42.

Ide K, Eliasziw M & Poulin MJ (2003). Relationship between middle cerebral artery blood velocity and end-tidal PCO₂ in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* **95**, 129–137.

Ihlen H, Amlie JP, Dale J, Forfang K, Nitter-Hauge S, Otterstad JE, Simonsen S & Myhre E (1984). Determination of cardiac output by Doppler echocardiography. *Br Heart J* **51**, 54–60.

Immink R V., Pott FC, Secher NH & Van Lieshout JJ (2014). Hyperventilation, cerebral perfusion, and syncope. *J Appl Physiol* **116**, 844–851.

Immink R V., Secher NH, Roos CM, Pott F, Madsen PL & van Lieshout JJ (2006). The postural reduction in middle cerebral artery blood velocity is not explained by PaCO₂. *Eur J Appl Physiol* **96**, 609–614.

Ito S (2019). High-intensity interval training for health benefits and care of cardiac diseases - the key to an efficient exercise protocol. *World J Cardiol* **11**, 171–188.

Jans Ø, Bundgaard-Nielsen M, Solgaard S, Johansson PI & Kehlet H (2012). Orthostatic

- intolerance during early mobilization after fast-track hip arthroplasty. *Br J Anaesth* **108**, 436–443.
- Jarrard CP, Watso JC, Atkins WC, Mckenna ZJ, Foster J, Huang M, Belval LN & Crandall CG (2024). Sex differences in sympathetic responses to lower-body negative pressure. *Med Sci Sports Exerc* **56**, 1056–1065.
- Jarrett CL, Shields KL, Broxterman RM, Hydren JR, Park SH, Gifford JR & Richardson RS (2020). Imaging transcranial Doppler ultrasound to measure middle cerebral artery blood flow: the importance of measuring vessel diameter. *Am J Physiol Regul Integr Comp Physiol* **319**, R33–R42.
- Jeng JS, Yip PK, Huang SJ & Kao MC (1999). Changes in hemodynamics of the carotid and middle cerebral arteries before and after endoscopic sympathectomy in patients with palmar hyperhidrosis: preliminary results. *J Neurosurg* **90**, 463–467.
- Jones NL, Robertson DG & Kane JW (1979). Difference between end-tidal and arterial PCO₂ in exercise. *J Appl Physiol Respir Environ Exerc Physiol* **47**, 954–960.
- Jordan J, Shannon JR, Diedrich A, Black B, Costa F, Robertson D & Biaggioni I (2000). Interaction of carbon dioxide and sympathetic nervous system activity in the regulation of cerebral perfusion in humans. *Hypertension* **36**, 383–388.
- Jorgensen LG, Perko G & Secher NH (1992a). Regional cerebral artery mean flow velocity and blood flow during dynamic exercise in humans. *J Appl Physiol* **73**, 1825–1830.
- Jorgensen LG, Perko M, Hanel B, Schroeder T V. & Secher NH (1992b). Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia in humans. *J Appl*

Physiol **72**, 1123–1132.

Kamiya A, Michikami D, Fu Q, Iwase S, Hayano J, Kawada T, Mano T & Sunagawa K (2003).

Pathophysiology of orthostatic hypotension after bed rest: paradoxical sympathetic withdrawal. *Am J Physiol Heart Circ Physiol* **285**, H1158–H1167.

Kastrup A, Dichgans J, Niemeier M & Schabet M (1998). Changes of cerebrovascular CO₂ reactivity during normal aging. *Stroke* **29**, 1311–1314.

Kastrup A, Thomas C, Hartmann C & Schabet M (1997). Sex dependency of cerebrovascular CO₂ reactivity in normal subjects. *Stroke* **28**, 2353–2356.

Katayama K, Sato K, Akima H, Ishida K, Yanagiya T, Kanehisa H, Fukuoka H, Fukunaga T & Miyamura M (2004). Ventilatory and cardiovascular responses to hypercapnia after 20 days of head-down bed rest. *Aviat Space Environ Med* **75**, 312–316.

Kaufman CS, Bai SX, Ward JL, Eickmeyer SM & Billinger SA (2019). Middle cerebral artery velocity dynamic response profile during exercise is attenuated following multiple ischemic strokes: a case report. *Physiol Rep* **7**, e14268.

Kavaliuskas M, Aspe RR & Babraj J (2015). High-intensity cycling training: the effect of work-to-rest intervals on running performance measures. *J Strength Cond Res* **29**, 2229–2236.

Kempf KS, Whitaker AA, Lui Y, Witte E, Perdomo SJ, Ward JL, Eickmeyer S, Ledbetter L, Abraham M & Billinger SA (2019). The effect of stroke on middle cerebral artery blood flow velocity dynamics during exercise. *J Neurol Phys Ther* **43**, 212–219.

Kety SS & Schmidt CF (1945). The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. *Am J Physiol* **143**, 53–66.

- Kety SS & Schmidt CF (1948). The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. *J Clin Invest* **27**, 476–483.
- Kontos HA, Raper AJ & Patterson JL (1977). Analysis of vasoactivity of local pH, PCO₂ and bicarbonate on pial vessels. *Stroke* **8**, 358–360.
- Kostoglou K, Robertson AD, Macintosh BJ & Mitsis GD (2019). A novel framework for estimating time-varying multivariate autoregressive models and application to cardiovascular responses to acute exercise. *IEEE Trans Biomed Eng* **66**, 3257–3266.
- Kovacs GTA & Shadden M (2017). Analysis of age as a factor in NASA astronaut selection and career landmarks. *PLoS One* **12**, e0181381.
- Kulkarni N, Mody P & Levine BD (2020). Abolish the tilt table test for the workup of syncope! *Circulation* **141**, 335–337.
- Kuznetsova A, Brockhoff PB & Christensen RHB (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *J Stat Softw* **82**, 1–26.
- ter Laan M, Van Dijk JMC, Elting JWJ, Staal MJ & Absalom AR (2013). Sympathetic regulation of cerebral blood flow in humans: A review. *Br J Anaesth* **111**, 361–367.
- Labrecque L, Drapeau A, Rahimaly K, Imhoff S, Billaut F & Brassard P (2020). Comparable blood velocity changes in middle and posterior cerebral arteries during and following acute high-intensity exercise in young fit women. *Physiol Rep* **8**, e14430.
- Lassen NA (1959). Cerebral blood flow and oxygen consumption in man. *Physiol Rev* **39**, 183–238.

- Lenth R V., Buerkner P, Herve M, Love J, Miguez F, Riebl H & Singmann H (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. Available at: <https://cran.r-project.org/package=emmeans>.
- Levine BD, Giller CA, Lane LD, Buckey JC & Blomqvist CG (1994). Cerebral versus systemic hemodynamics during graded orthostatic stress in humans. *Circulation* **90**, 298–306.
- Levine BD, Zuckerman JH & Pawelczyk JA (1997). Cardiac atrophy after bed-rest deconditioning: a non-neural mechanism for orthostatic intolerance. *Circulation* **96**, 517–525.
- van Lieshout JJ (2003). Exercise training and orthostatic intolerance: a paradox? *J Physiol* **551**, 401.
- van Lieshout JJ, Pott F, Madsen PL, van Goudoever J & Secher NH (2001). Muscle tensing during standing: effects on cerebral tissue oxygenation and cerebral artery blood velocity. *Stroke* **32**, 1546–1551.
- Van Lieshout JJ, Wieling W, Karemaker JM & Secher NH (2003). Syncope, cerebral perfusion, and oxygenation. *J Appl Physiol* **94**, 833–848.
- Lin A-L, Fox PT, Hardies J, Duong TQ & Gao J-H (2010). Nonlinear coupling between cerebral blood flow, oxygen consumption, and ATP production in human visual cortex. *Proc Natl Acad Sci U S A* **107**, 8446–8451.
- Linkis P, Jorgensen LG, Olesen HL, Madsen PL, Lassen NA & Secher NH (1995). Dynamic exercise enhances regional cerebral artery mean flow velocity. *J Appl Physiol* **78**, 12–16.
- Liu J, David Simpson M, Yan J & Allen R (2010). Tracking time-varying cerebral

- autoregulation in response to changes in respiratory PaCO₂. *Physiol Meas* **31**, 1291–1307.
- Lucas SJE, Cotter JD, Brassard P & Bailey DM (2015). High-intensity interval exercise and cerebrovascular health: curiosity, cause, and consequence. *J Cereb Blood Flow Metab* **35**, 902–911.
- MacNutt MJ, De Souza MJ, Tomczak SE, Homer JL & Sheel AW (2012). Resting and exercise ventilatory chemosensitivity across the menstrual cycle. *J Appl Physiol* **112**, 737–747.
- Mahoney JE, Palta M, Johnson J, Jalaluddin M, Gray S, Park S & Sager M (2000). Temporal association between hospitalization and rate of falls after discharge. *Arch Intern Med* **160**, 2788–2795.
- Markwalder T-M, Grolimund P, Seiler RW & Roth F (1984). Dependency of blood flow velocity in the middle cerebral artery on end-tidal carbon dioxide partial pressure - a transcranial ultrasound Doppler study. *J Cereb Blood Flow Metab* **4**, 368–372.
- Mastrandrea CJ, Hedge ET, Robertson AD, Heckman GA, Ho J, Granados Unger F & Hughson RL (2023). High-intensity exercise does not protect against orthostatic intolerance following bedrest in 55- to 65-yr-old men and women. *Am J Physiol Regul Integr Comp Physiol* **325**, R107–R119.
- Matteis M, Troisi E, Monaldo BC, Caltagirone C & Silvestrini M (1998). Age and sex differences in cerebral hemodynamics: a transcranial Doppler study. *Stroke* **29**, 963–967.
- Meng L, Hou W, Chui J, Han R & Gelb AW (2015). Cardiac Output and Cerebral Blood Flow. *Anesthesiology* **123**, 1198–1208.
- Murrell CJ, Cotter JD, Thomas KN, Lucas SJE, Williams MJA & Ainslie PN (2013). Cerebral

- blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: effect of age and 12-week exercise training. *Age (Omaha)* **35**, 905–920.
- Nattie E & Li A (2012). Central chemoreceptors: locations and functions. *Compr Physiol* **2**, 221–254.
- Novak V, Spies JM, Novak P, McPhee BR, Rummans TA & Low PA (1998). Hypocapnia and cerebral hypoperfusion in orthostatic intolerance. *Stroke* **29**, 1876–1881.
- Nybo L, Møller K, Pedersen BK, Nielsen B & Secher NH (2003). Association between fatigue and failure to preserve cerebral energy turnover during prolonged exercise. *Acta Physiol Scand* **179**, 67–74.
- O'Brien H & Kenny RA (2014). Syncope in the elderly. *Eur Cardiol Rev* **9**, 28–36.
- Ogoh S & Ainslie PN (2009). Cerebral blood flow during exercise: mechanisms of regulation. *J Appl Physiol* **107**, 1370–1380.
- Ogoh S, Brothers M, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, O-Yurvati A & Raven PB (2005a). The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol* **569**, 697–704.
- Ogoh S, Brothers RM, Jeschke M, Secher NH & Raven PB (2010). Estimation of cerebral vascular tone during exercise; evaluation by critical closing pressure in humans. *Exp Physiol* **95**, 678–685.
- Ogoh S, Dalsgaard MK, Yoshiga CC, Dawson EA, Keller DM, Raven PB & Secher NH (2005b). Dynamic cerebral autoregulation during exhaustive exercise in humans. *Am J Physiol - Hear Circ Physiol* **288**, 1461–1467.

- Ogoh S, Fisher JP, Young CN & Fadel PJ (2011). Impact of age on critical closing pressure of the cerebral circulation during dynamic exercise in humans. *Exp Physiol* **96**, 417–425.
- Ogoh S, Hayashi N, Inagaki M, Ainslie PN & Miyamoto T (2008). Interaction between the ventilatory and cerebrovascular responses to hypo- and hypercapnia at rest and during exercise. *J Physiol* **586**, 4327–4338.
- Ogoh S, Nakahara H, Okazaki K, Bailey DM & Miyamoto T (2013). Cerebral hypoperfusion modifies the respiratory chemoreflex during orthostatic stress. *Clin Sci* **125**, 37–44.
- Ogoh S, Sato K, Okazaki K, Miyamoto T, Hirasawa A, Sadamoto T & Shibasaki M (2015). Blood flow in internal carotid and vertebral arteries during graded lower body negative pressure in humans. *Exp Physiol* **100**, 259–266.
- Olin JT, Dimmen AC, Subudhi AW & Roach RC (2011). Cerebral blood flow and oxygenation at maximal exercise: the effect of clamping carbon dioxide. *Respir Physiol Neurobiol* **175**, 176–180.
- Panerai RB (2003). The critical closing pressure of the cerebral circulation. *Med Eng Phys* **25**, 621–632.
- Panerai RB, Deverson ST, Mahony P, Hayes P & Evans DH (1999). Effect of CO₂ on dynamic cerebral autoregulation measurement. *Physiol Meas* **20**, 265–275.
- Panerai RB, Dineen NE, Brodie FG & Robinson TG (2010). Spontaneous fluctuations in cerebral blood flow regulation: contribution of PaCO₂. *J Appl Physiol* **109**, 1860–1868.
- Panerai RB, Haunton VJ, Llwyd O, Minhas JS, Katsogridakis E, Salinet ASM, Maggio P & Robinson TG (2021). Cerebral critical closing pressure and resistance-area product: the

- influence of dynamic cerebral autoregulation, age and sex. *J Cereb Blood Flow Metab* **41**, 2456–2469.
- Panerai RB, Minhas JS, Llwyd O, Salinet ASM, Katsogridakis E, Maggio P & Robinson TG (2020). The critical closing pressure contribution to dynamic cerebral autoregulation in humans: influence of arterial partial pressure of CO₂. *J Physiol* **598**, 5673–5685.
- Panerai RB, Salinet ASM, Brodie FG & Robinson TG (2011). The influence of calculation method on estimates of cerebral critical closing pressure. *Physiol Meas* **32**, 467–482.
- Panerai RB, Simpson DM, Deverson ST, Mahony P, Hayes P & Evans DH (2000). Multivariate dynamic analysis of cerebral blood flow regulation in humans. *IEEE Trans Biomed Eng* **47**, 419–423.
- Penaz J (1973). Photo-electric measurement of blood pressure, volume and flow in the finger. In *Digest of the 10th International Conference on Medical and Biological Engineering*, p. 104. Dresden.
- Perrott MH & Cohen RJ (1996). An efficient approach to ARMA modeling of biological systems with multiple inputs and delays. *IEEE Trans Biomed Eng* **43**, 1–14.
- Poole DC & Jones AM (2012). Oxygen uptake kinetics. *Compr Physiol* **2**, 933–996.
- Poulin MJ, Liang P-J & Robbins PA (1996). Dynamics of the cerebral blood flow response to step changes in end-tidal PCO₂ and PO₂ in humans. *J Appl Physiol* **81**, 1084–1095.
- Poulin MJ, Liang P-J & Robbins PA (1998). Fast and slow components of cerebral blood flow response to step decreases in end-tidal PCO₂ in humans. *J Appl Physiol* **85**, 388–397.
- Del Pozzi AT, Schwartz CE, Tewari D, Medow MS & Stewart JM (2014). Reduced cerebral

- blood flow with orthostasis precedes hypocapnic hyperpnea, sympathetic activation, and postural tachycardia syndrome. *Hypertension* **63**, 1302–1308.
- Rasmussen P, Stie H, Nielsen B & Nybo L (2006). Enhanced cerebral CO₂ reactivity during strenuous exercise in man. *Eur J Appl Physiol* **96**, 299–304.
- Riley RL, Permutt S, Said S, Godfrey M, Cheng TO, Howell JB & Shepard RH (1959). Effect of posture on pulmonary dead space in man. *J Appl Physiol* **14**, 339–344.
- Rolfe DFS & Brown GC (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev* **77**, 731–758.
- Romero-Ortuno R, Cogan L, Foran T, Fan CW & Kenny RA (2010). Using the Finometer to examine sex differences in hemodynamic responses to orthostasis in older people. *Blood Press Monit* **15**, 8–17.
- Sabbahi A, Arena R, Kaminsky LA, Myers J & Phillips SA (2018). Peak blood pressure responses during maximum cardiopulmonary exercise testing: reference standards from FRIEND (Fitness Registry and the Importance of Exercise: A National Database). *Hypertension* **71**, 229–236.
- Saito S, Washio T, Watanabe H, Katayama K & Ogoh S (2022). Influence of cardiac output response to the onset of exercise on cerebral blood flow. *Eur J Appl Physiol* **122**, 1939–1948.
- Sato K, Fisher JP, Seifert T, Overgaard M, Secher NH & Ogoh S (2012). Blood flow in internal carotid and vertebral arteries during orthostatic stress. *Exp Physiol* **97**, 1272–1280.
- Seidel E, Eicke BM, Tettenborn B & Krummenauer F (1999). Reference values for vertebral

- artery flow volume by duplex sonography in young and elderly adults. *Stroke* **30**, 2692–2696.
- Serrador JM, Hughson RL, Kowalchuk JM, Bondar RL & Gelb AW (2006). Cerebral blood flow during orthostasis: Role of arterial CO₂. *Am J Physiol Regul Integr Comp Physiol* **290**, 1087–1093.
- Shenkin HA, Cabieses F & Van Den Noordt G (1951). The effect of bilateral stellectomy upon the cerebral circulation of. *J Clin Invest* **30**, 90–93.
- Shibao C, Lipsitz LA & Biaggioni I (2013). Evaluation and treatment of orthostatic hypotension. *J Am Soc Hypertens* **7**, 317–324.
- Shibasaki M, Sato K, Hirasawa A, Sadamoto T, Crandall CG & Ogoh S (2020). An assessment of hypercapnia-induced elevations in regional cerebral perfusion during combined orthostatic and heat stresses. *J Physiol Sci* **70**, 25.
- Shibata S, Perhonen M & Levine BD (2010). Supine cycling plus volume loading prevent cardiovascular deconditioning during bed rest. *J Appl Physiol* **108**, 1177–1186.
- Šimkovic M & Träuble B (2019). *Robustness of statistical methods when measure is affected by ceiling and/or floor effect.*
- Smirl JD, Haykowsky MJ, Nelson MD, Altamirano-Diaz LA & Ainslie PN (2012). Resting and exercise cerebral blood flow in long-term heart transplant recipients. *J Hear Lung Transplant* **31**, 906–908.
- Smith KJ & Ainslie PN (2017). Regulation of cerebral blood flow and metabolism during exercise. *Exp Physiol* **102**, 1356–1371.

- Smith KJ, Wildfong KW, Hoiland RL, Harper M, Lewis NC, Pool A, Smith SL, Kuca T, Foster GE & Ainslie PN (2016). Role of CO₂ in the cerebral hyperemic response to incremental normoxic and hyperoxic exercise. *J Appl Physiol* **120**, 843–854.
- Sorond FA, Kiely DK, Galica A, Moscufo N, Serrador JM, Iloputaife I, Egorova S, Dell'Oglio E, Meier DS, Newton E, Milberg WP, Guttmann CRG & Lipsitz LA (2011). Neurovascular coupling is impaired in slow walkers: The MOBILIZE Boston Study. *Ann Neurol* **70**, 213–220.
- Soteriades ES, Evans JC, Larson MG, Chen MH, Chen L, Benjamin EJ & Levy D (2002). Incidence and prognosis of syncope. *N Engl J Med* **347**, 878–885.
- Stewart JM, Rivera E, Clarke DA, Baugham IL, Ocon AJ, Taneja I, Terilli C & Medow MS (2011). Ventilatory baroreflex sensitivity in humans is not modulated by chemoreflex activation. *Am J Physiol Heart Circ Physiol* **300**, H1492–H1500.
- Tari B, Vanhie JJ, Belfry GR, Shoemaker JK & Heath M (2020). Increased cerebral blood flow supports a single-bout postexercise benefit to executive function: evidence from hypercapnia. *J Neurophysiol* **124**, 930–940.
- Tatu L, Moulin T, Bogousslavsky J & Duvernoy H (1996). Arterial territories of human brain: brainstem and cerebellum. *Neurology* **47**, 1125–1135.
- Thomas KN, Lewis NCS, Hill BG & Ainslie PN (2015). Technical recommendations for the use of carotid duplex ultrasound for the assessment of extracranial blood flow. *Am J Physiol Regul Integr Comp Physiol* **309**, R707–R720.
- Thomas SN, Schroeder T, Secher NH & Mitchell JH (1989). Cerebral blood flow during

- submaximal and maximal dynamic exercise in humans. *J Appl Physiol* **67**, 744–748.
- Tsukamoto H, Hashimoto T, Olesen ND, Petersen LG, Sorensen H, Nielsen HB, Secher NH & Ogoh S (2019). Dynamic cerebral autoregulation is maintained during high-intensity interval exercise. *Med Sci Sports Exerc* **51**, 372–378.
- Tymko MM, Ainslie PN & Smith KJ (2018). Evaluating the methods used for measuring cerebral blood flow at rest and during exercise in humans. *Eur J Appl Physiol* **118**, 1527–1538.
- Tymko MM, Drapeau A, Vieira-Coelho MA, Labrecque L, Imhoff S, Coombs GB, Langevin S, Fortin M, Châteauvert N, Ainslie PN & Brassard P (2024). Acute isometric and dynamic exercise do not alter cerebral sympathetic nerve activity in healthy humans. *J Cereb Blood Flow Metab*; DOI: 10.1177/0271678X241248228.
- Tzeng Y-C, Willie CK, Atkinson G, Lucas SJE, Wong A & Ainslie PN (2010). Cerebrovascular regulation during transient hypotension and hypertension in humans. *Hypertension* **56**, 268–273.
- Verbree J, Bronzwaer AGT, van Buchem MA, Daemen MJAP, van Lieshout JJ & van Osch MJP (2017). Middle cerebral artery diameter changes during rhythmic handgrip exercise in humans. *J Cereb Blood Flow Metab* **37**, 2921–2927.
- Verbree J, Bronzwaer ASGT, Ghariq E, Versluis MJ, Daemen MJAP, van Buchem MA, Dahan A, van Lieshout JJ & van Osch MJP (2014). Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *J Appl Physiol* **117**, 1084–1089.

- Wang X, Richardson L, Krishnamurthy S, Pennington K, Evans J, Bruce E, Abraham W, Bhakta D & Patwardhan A (2004). Orthostatic modification of ventilatory dynamic response to carbon dioxide perturbations. *Auton Neurosci* **116**, 76–83.
- Ward JL, Craig JC, Liu Y, Vidoni ED, Maletsky R, Poole DC & Billinger SA (2018). Effect of healthy aging and sex on middle cerebral artery blood velocity dynamics during moderate-intensity exercise. *Am J Physiol Heart Circ Physiol* **315**, H492–H501.
- Ward SA, Whipp BJ, Koyal S & Wasserman K (1983). Influence of body CO₂ stores on ventilatory dynamics during exercise. *J Appl Physiol Respir Environ Exerc Physiol* **55**, 742–749.
- Watenpaugh DE, O’Leary DD, Schneider SM, Lee SMC, Macias BR, Tanaka K, Hughson RL & Hargens AR (2007). Lower body negative pressure exercise plus brief postexercise lower body negative pressure improve post-bed rest orthostatic tolerance. *J Appl Physiol* **103**, 1964–1972.
- Waters WW, Ziegler MG & Meck J V. (2002). Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance. *J Appl Physiol* **92**, 586–594.
- Wesseling KH, Jansen JRC, Settels JJ & Schreuder JJ (1993). Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol* **74**, 2566–2573.
- Weston ME, Barker AR, Tomlinson OW, Coombes JS, Bailey TG & Bond B (2022). The effect of exercise intensity and cardiorespiratory fitness on the kinetic response of middle cerebral artery blood velocity during exercise in healthy adults. *J Appl Physiol* **133**, 214–222.
- Whipp BJ, Lamarra N, Ward SA, Davis JA & Wasserman K (1989). Estimating arterial PCO₂

- from flow weighted and time-average alveolar PCO₂ during exercise. In *Respiratory Control*, ed. Swanson GD, Grodins FS & Hughson RL, pp. 91–99. Springer, Boston, MA.
- Whipp BJ, Ward SA, Lamarra N, Davis JA & Wasserman K (1982). Parameters of ventilatory and gas exchange dynamics during exercise. *J Appl Physiol Respir Environ Exerc Physiol* **52**, 1506–1513.
- Whitaker AA, Aaron SE, Kaufman CS, Kurtz BK, Bai SX, Vidoni ED, Montgomery RN & Billinger SA (2022). Cerebrovascular response to an acute bout of low-volume high-intensity interval exercise and recovery in young healthy adults. *J Appl Physiol* **132**, 236–246.
- Williams JS & Babb TG (1997). Differences between estimates and measured PaCO₂ during rest and exercise in older subjects. *J Appl Physiol* **83**, 312–316.
- Williams LR & Leggett RW (1989). Reference values for resting blood flow to organs of man. *Clin Phys Physiol Meas* **10**, 187–217.
- Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA & Ainslie PN (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol* **590**, 3261–3275.
- Willie CK, Tzeng YC, Fisher JA & Ainslie PN (2014). Integrative regulation of human brain blood flow. *J Physiol* **592**, 841–859.
- Witte E, Liu Y, Ward JL, Kempf KS, Whitaker A, Vidoni ED, Craig JC, Poole DC & Billinger SA (2019). Exercise intensity and middle cerebral artery dynamics in humans. *Respir Physiol Neurobiol* **262**, 32–39.

- Wolters FJ, Zonneveld HI, Hofman A, van der Lugt A, Koudstaal PJ, Vernooij MW & Ikram MA (2017). Cerebral perfusion and the risk of dementia: a population-based study. *Circulation* **136**, 719–728.
- Wright RL, Swain DP & Branch JD (1999). Blood pressure responses to acute static and dynamic exercise in three racial groups. *Med Sci Sports Exerc* **31**, 1973–1978.
- Xing CY, Tarumi T, Liu J, Zhang Y, Turner M, Riley J, Tinajero CD, Yuan L-J & Zhang R (2017). Distribution of cardiac output to the brain across the adult lifespan. *J Cereb Blood Flow Metab* **37**, 2848–2856.
- Zago S, Ferrucci R, Marceglia S & Priori A (2009). The Mosso method for recording brain pulsation: the forerunner of functional neuroimaging. *Neuroimage* **48**, 652–656.
- Zarrinkoob L, Ambarki K, Wåhlin A, Birgander R, Eklund A & Malm J (2015). Blood flow distribution in cerebral arteries. *J Cereb Blood Flow Metab* **35**, 648–654.
- Zhang R, Crandall CG & Levine BD (2004). Cerebral hemodynamics during the Valsalva maneuver: insights from ganglionic blockade. *Stroke* **35**, 843–847.
- Zhang R, Zuckerman JH, Giller CA & Levine BD (1998). Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol Heart Circ Physiol* **274**, H233–H241.
- Zhang R, Zuckerman JH, Pawelczyk JA & Levine BD (1997). Effects of head-down-tilt bed rest on cerebral hemodynamics during orthostatic stress. *J Appl Physiol* **83**, 2139–2145.
- Zuj KA, Arbeille P, Shoemaker JK & Hughson RL (2013). Cerebral critical closing pressure and CO₂ responses during the progression toward syncope. *J Appl Physiol* **114**, 801–807.