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An Exploration into the Effects of High Altitude Exposure on the Neurovascular Coupling Response

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents of this thesis. I have read and understood the regulations of University College Cork concerning plagiarism.

Signed: 

Date: 18/12/2022

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List of Abbreviations

Listed according to appearance in thesis (excluding main abstract).

Abbreviation	Word
NVC	Neurovascular coupling
CA	Cerebral autoregulation
CVR	Cerebrovascular reactivity
HA	High altitude
rCBF	Regional cerebral blood flow
BBB	Blood brain barrier
ATP	Adenosine triphosphate
CMR_{GLU}	Cerebral metabolic rate of glucose consumption
CMR_{O2}	Cerebral metabolic rate of oxygen consumption
PPP	Pentose phosphate pathway
CNS	Central nervous system
ANLS	Astrocyte to neuron lactate shuttle
LDH5	Lactate dehydrogenase 5
MCTs	Monocarboxylic acid transporters
LDH1	Lactate dehydrogenase 1
TCA cycle	Tricarboxylic acid cycle
GS	Glycogen synthase
GP	Glycogen phosphorylase
TIA	Transient ischemic attack
CBF	Cerebral blood flow
VSMCs	Vascular smooth muscle cells
NVU	Neurovascular unit

BOLD	Blood oxygen level dependent
fMRI	Functional magnetic resonance imaging
$PaCO_2$	Partial pressure of arterial carbon dioxide concentration
N_20	Nitrous oxide
γ-camera	Gamma-camera
Kr^{85}	$Krypton^{85}$
Xr^{133}	$Xenon^{133}$
EEG	Electroencephalography
PET	Positron emission tomography
TCD	Transcranial Doppler ultrasound
NIRS	Near-infrared spectroscopy
DVA	Dynamic vessel analysis
CO_2	Carbon dioxide
H^+	Hydrogen ion concentration
NO	Nitric oxide
K^+	Potassium ions
NMDAr	N-methyl-D-aspartate receptors
AMPAr	α-amino-3-hydroxy-5-methyl-4-isoxazol-epropionic acid receptors
iCa^{2+}	Intracellular cytosolic calcium concentrations
nNOS	Neuronal nitric oxide synthase
COX2	Cyclooxygenase 2
PGE_2	Prostaglandin E_2
sGC	Soluble guanylate cyclase
cGMP	Cyclic guanosine monophosphate
A_{2A}	Adenosine A2A receptor

ACh	Acetylcholine
5-HT	Serotonin
$K_{IR}4.1$	Inward rectifying potassium channels
mGLUR5	Metabotropic glutamate receptors
IP_3R	Inositol 1,4,5-triphosphate receptor
AA	Arachidonic acid
EET	Epoxyeicosatrienoic acid
CYP2C11	Cytochrome P450-2C11-epoxygenase
COX1	Cyclooxygenase 1
20-HETE	20-hydroxyeicosatetraenoic acid
PO_2	Partial pressure of ambient oxygen
AMS	Acute mountain sickness
HVR	Hypoxic ventilatory response
VAH	Ventilatory acclimatisation to hypoxia
PaO_2	Partial pressure of arterial oxygen
SNS	Sympathetic nervous system
BP	Blood pressure
HCO_3^-	Bicarbonate
EPO	Erythropoietin
Hb	Haemoglobin
CDO_2	Cerebral O_2 delivery
CaO_2	Cerebral arterial oxygen content
RBCs	Red blood cells
SNO-Hb	S-nitroso haemoglobin
PCAv	Posterior cerebral artery velocity
tAUC	Total area under the curve
MCA	Middle cerebral artery

ACA	Anterior cerebral artery
PCA	Posterior cerebral artery
UCC	University college cork
MRU	Mount Royal University
ECG	Electrocardiogram
HR	Heart rate
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
R_R	Respiratory rate
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
SpO_2	Peripheral oxygen saturation
VS	Visual stimulation
Δmean	Change in mean cerebral blood velocity during visual stimulation
Δpeak	Peak change in mean cerebral blood velocity during visual stimulation
ΔtAUC	Change in total area under the curve during visual stimulation
T2p	Time to peak haemodynamic response during visual stimulation
RMR	Resting metabolic rate
ABGs	Arterial blood gases
Hct	Haematocrit
P_{ATM}	Total atmospheric pressure
P_IO_2	Partial pressure of inspired oxygen
HIF	Hypoxia-inducible factors
HARS	High altitude renal syndrome

HAPE	High altitude pulmonary oedema
HACE	High altitude cerebral oedema
$\dot{V}_I$	Inspiratory minute ventilation
$\dot{V}_{TI}$	Inspiratory tidal volume
R_R	Respiratory rate
rCO_2	Regional cerebral oxygen saturation
OCT	Optical coherence tomography
Iso-Hx	Isocapnic hypoxia
CBv	Cerebral blood velocity

Thesis Abstract

Introduction

The neural infrastructure within cerebral tissue is both elaborate and metabolically volatile. Owing to limitations in energy substrate storage capacity, the neural nexus relies upon continuous perfusion for the delivery of essential nutrients. Vital substrates which are transported within the blood help to support energy-dependent metabolic processes throughout the central nervous system at a global and regional level. Despite constituting ≈2% of total body mass, the brain receives a disproportionate ≈20% of total cardiac output. The physiological phenomenon which describes the intimate link between local neuronal activity and regional cerebral blood flow is termed neurovascular coupling (NVC). This phenomenon has been observed across all cerebral structures wherein dynamic changes in local metabolic activity are met with a concomitant increase in local perfusion of the active tissue. A vast body of work has assessed the translational implications of NVC for neurophysiological function and behavioural outcomes across several pathologies (aging, neurodegenerative disease, autoimmune disorders, trauma and metabolic disorders).

Thesis Aims

Herein, we sought to characterise the effects of high altitude (HA) exposure on NVC in healthy human volunteers encompassing different ascent paradigms (chapters 3 and 4). HA exposure is a pernicious multimodal stressor among habitual lowlanders, which necessitates an integrative physiological acclimatisation to sustain homeostasis and overall health and function. This environmental stressor has pronounced effects on other components of cerebral blood flow control. Prior to this

thesis there was a dearth of literature regarding the effects of HA exposure on NVC. In addition, we conducted a complementary laboratory-based investigation which examined the degree of variance within the NVC response attributable to either age and/or sex (chapter 2). This comprehensive study was essential with respect to appreciating the interaction between participant demographics (age, sex) and NVC. Demographic heterogeneity is an inherent component of many HA research expedition. We therefore determined that it was of paramount importance to ascertain the magnitude to which heterogeneity influences our primary physiological measures.

Methods

Transcranial Doppler ultrasound (TCD) was employed to provide non-invasive measures of cerebral blood velocity. NVC was indexed as the change in posterior cerebral artery velocity (PCAv) during intermittent visual stimulation (30s on/off; 6Hz) in all experiments. This technique is widely used within the literature, as visual stimulation mediates an increase in metabolic activity of the visual cortex, a neural territory perfused by the PCA. Healthy human volunteers were recruited for studies described in chapter 2 (n=125, 41 male), 3 (n=10, 4 male) and 4 (n=12, 7 male). Arterial blood draws were sampled from the radial artery during HA expeditions (chapter 3 and 4), providing and index of ventilatory and acid-base acclimatisation status within each participant. Ascent profiles differed between chapters 3 and 4 relevant to the experimental question.

Key results and conclusions

Collectively, our results demonstrate that approximately 0-6.1% of the variance across several NVC metrics are attributable to either age and/or sex (chapter 2).

Moreover, the magnitude of the haemodynamic response across several NVC metrics was remarkably intact following incremental and acute ascent to 4240m and 3800m respectively (chapters 3 and 4 respectively). Taken in conjunction, this thesis reveals that: (1) Neither age nor sex greatly affect the magnitude of the NVC response in healthy human volunteers (21-66yrs old) and (2) NVC response magnitude remains remarkably intact across multiple HA exposure paradigms in habitual lowlanders.

Chapter 1. Introduction and Background Physiology

1.1 Opening Remarks

1.1.1 Introduction to Cerebral Paradox

Notwithstanding unparalleled sophistication, the brain's cytoarchitecture is the catalyst for its own vulnerability. Despite constituting ≈2% of total body mass, it receives ≈20% of total cardiac output and is responsible for ≈15-25% of total body energy consumption at rest (Beishon et al., 2021; Camandola & Mattson, 2017; Deitmer et al., 2019; Fedorovich & Waseem, 2018; Howarth et al., 2021; Jha & Morrison, 2018; Lourenço et al., 2016; Nippert et al., 2018; Niven, 2016; Oyarzabal & Marin-Valencia, 2019; Phillips et al., 2015; Stackhouse & Mishra, 2021; Watts et al., 2018). Unlike systemic tissue and other organ systems, the brain is ill-equipped for the energy storage required to support its own metabolic demand (Attwell & Laughlin, 2001; Brown & Ransom, 2007). Consequently, cerebral tissue relies upon continuous perfusion for the delivery of substrates required to maintain neurophysiological function.

1.1.2 Introduction Outline

Appropriately, the brain has robust regulatory mechanisms, which ensure global and local perfusion is maintained within optimal limits; (1) neurovascular coupling (NVC), (2) cerebral autoregulation (CA) and (3) cerebrovascular reactivity (CVR) (Phillips et al., 2015; Willie, Tzeng, et al., 2014). The homeostatic limits of these regulatory mechanisms have been examined across a milieu of stimuli, stressors and pathologies. This thesis explores the effects of high altitude (HA) exposure on NVC

response dynamics in a healthy human model. Prior to experimental assessment of NVC it is important to develop a foundational understanding of the key components, which underlie and facilitate the NVC response. This thesis begins with a comprehensive appraisal of the major components underlying cerebral metabolism, neuroanatomy and NVC signalling pathways, which allow sophisticated and robust regulation of regional cerebral blood flow (rCBF). The introduction concludes with a summary of HA physiology across multiple physiological systems.

1.2 Cerebral Metabolism and Neuroanatomy

1.2.1 Basic Cellular Composition of the Brain

At a rudimentary level, the adult brain is a composite of neural, vascular and glial cells. Subtypes of both neural and glial cells have been observed within specific regions and depths of the cerebral tissue. Neurons coalesce to form neural pathways and intricate neural circuits. These circuits facilitate communication within, and between brain regions at rest and during domain specific tasks (Pulvermüller et al., 2014). Neural communication is achieved through neurotransmitter (excitatory, inhibitory) release at the synaptic cleft, a process termed neurotransmission in chemical synapses (Patel & Feucht, 2011; Shulman et al., 2004). Glial cells serve important functions including, but not limited to neuroimmune signalling, facilitation of neurotransmission, vasoactive signalling to intraparenchymal vessels and providing structural integrity to the blood brain barrier (BBB) (Abbott et al., 2006; Allaman et al., 2011; Alvarez et al., 2013; Tanigami et al., 2012).

1.2.2 Basic Components of Cerebral Metabolism

Cerebral metabolism is the total bioenergetic cost within the brain and is the net product of a coalition between several energy-dependent mechanisms underlying neurotransmission processes (e.g., neurotransmitter shuttling, packaging docking and release). The largest proportion of adenosine triphosphate (ATP) is consumed at the synapse, which gives rise to regional differences in metabolism between cortical grey and white matter (Attwell & Laughlin, 2001; Barros & Deitmer, 2010; Harris et al., 2013; Magistretti & Allaman, 2008; Oyarzabal & Marin-Valencia, 2019). Major synaptic energy-dependent processes include: (1) generation and propagation of an action potential, (2) maintenance and restoration of resting membrane potentials, (3) recycling and replenishment of pre-synaptic vesicles and (4) the release and reuptake of neurotransmitters (Attwell & Laughlin, 2001; Barros & Deitmer, 2010; Bélanger et al., 2011; Harris et al., 2013; Magistretti & Allaman, 2008; Oyarzabal & Marin-Valencia, 2019). Neuronal signalling accounts for approximately 70-80% of the total ATP used during neurotransmission, with non-signalling components contributing to the remaining 20-30% (Bélanger et al., 2011; Dienel, 2019; Magistretti & Allaman, 2015; Watts et al., 2018). Collectively, these processes allow for resting membrane potential, as well as the functional depolarization and repolarisation of neurons during neurotransmission, and fuel astrocytic energy dependent-processes involved in neurotransmitter recycling (i.e., glutamate-glutamine cycle) and glial cell metabolism (Falkowska et al., 2015; Harris et al., 2013; Oyarzabal & Marin-Valencia, 2019).

1.2.3 Substrate Utilisation Within Cerebral Metabolism

The catabolism of glucose within neural and glial cells is the primary metabolic substrate used for ATP generation (Chi & Roberts, 2003; Deitmer et al., 2019; Dienel, 2019; Magistretti & Allaman, 2015; Oyarzabal & Marin-Valencia, 2019). Excitatory (glutamatergic) neurons are responsible for ≈80-85% of ATP consumption within the brain, the remaining ≈15-20% is attributed to inhibitory (GABAergic) neuron and glial cell function (Dienel, 2019). At rest, the normal adult brain has a cerebral metabolic rate of glucose (CMR_{GLU}) of ≈0.2-0.3 µmol/g/min, which reflects an average total glucose consumption of ≈91g of glucose/day (Dienel, 2019). Cerebral metabolic rate of oxygen (CMR_{O2}) is ≈3.3-4.2 ml/100g/min within the normal resting adult brain, reflecting an average total O_2 consumption of ~68-86 litres of O_2/day (Dienel, 2019). Along with glucose, the brain can metabolize ketones, amino acids, and lactate under certain conditions (fasting, exercise, specific diets, across various stages of neurodevelopment) (Chi & Roberts, 2003; Dienel, 2019; Falkowska et al., 2015; Jha & Morrison, 2018; Magistretti & Allaman, 2008, 2015). The metabolism of glucose has four distinct pathways within the brain; (1) glycolysis; (2) oxidative phosphorylation; (3) glycogen synthesis (astrocytes); and the (4) pentose phosphate pathway (PPP) (Bélanger et al., 2011; Camandola & Mattson, 2017; Dienel, 2019; Falkowska et al., 2015; Jha & Morrison, 2018; Magistretti & Allaman, 2015). Although the primacy of glucose as a main fuel source within the CNS is well-recognised, the metabolic profile of how this monosaccharide is utilised across each cell within the central nervous system (CNS) is currently under debate.

1.2.4 Metabolic Profiles in Neural & Glial Cells

While glucose is the preferred metabolic substrate within the adult brain, its utility among neurons and astrocytes is often contested. The astrocyte to neuron lactate shuttle (ANLS) hypothesis was first developed by Magistretti and colleagues (1994). This phenomenon describes the process by which glucose is metabolised to lactate through pyruvate via lactate dehydrogenase-5 (LDH5) in astrocytes (Bélanger et al., 2011; Falkowska et al., 2015; Magistretti & Allaman, 2015). Astrocytic lactate is then transported to neurons through monocarboxylic acid transporters (MCTs) found on the astrocytic and neural membranes (Bélanger et al., 2011; Falkowska et al., 2015; Jha & Morrison, 2018; Prebil et al., 2011). Once inside the neuron, lactate is synthesised into pyruvate, through the action of lactate dehydrogenase-1 (LDH1). Pyruvate is subsequently taken into the mitochondria where it enters the tricarboxylic acid (TCA) cycle, once inside the TCA cycle it can be fully metabolised along the electron transport chain (Bélanger et al., 2011; Jha & Morrison, 2018; Watts et al., 2018). The basis of this hypothesis proposes that neurons derive most of the ATP they require from the metabolism of astrocyte-derived lactate. Glucose is primarily metabolised within neurons to support the pentose phosphate pathway (PPP), which maintains the necessary intracellular neural antioxidant capacity helping to offset superoxide formation from mitochondrial respiration. The differences in preferred metabolic pathways of glucose are due largely to differences within intracellular enzyme expression levels involved in the metabolism of glucose. This hypothesis is still debated with the exact metabolic profiles of both neural and glial cells undetermined.

1.2.5 Energy Storage Within Cerebral Tissue

The brain is under constant bombardment with afferent signalling and subsequent central processing, which induces an insatiable and volatile metabolic demand. The brain's resting metabolic rate is third to only the heart and renal cortex (Brown & Ransom, 2007; McKenna, 2007). Glycogen is the preferred substance for stored glucose and is present in most mammalian tissues (e.g., liver, skeletal muscle) providing an energy reserve during instances of hypoglycaemia and hypoperfusion, in a process termed glycogenolysis (Falkowska et al., 2015). Within the adult CNS, astrocytes are the sole location for glycogen storage due to preferable intracellular enzyme expression of glycogen synthase (GS) and glycogen phosphorylase (GP), relative to neurons (Brown & Ransom, 2007; Camandola & Mattson, 2017). Owing to limited space within the cranium, only a negligible concentration of glycogen can be stored within the astrocytes (Bak & Walls, 2018; Brown & Ransom, 2007; Falkowska et al., 2015). The glycogen reserve of the brain is approximately 6-12µmol, compared with 100-500 and 300-350µmol of the liver and skeletal muscle respectively (Brown & Ransom, 2007).

1.2.6 Final Remarks on Cerebral Metabolism & Cerebral Tissue Vulnerability

Taken in conjunction, these considerations of energetic capacity exemplify the importance of glucose and O_2 as key metabolic substrates for normal astrocyte and neuronal metabolism. Aberrant cerebral and glucose metabolism has been observed across several pathologies such as hypoglycaemia, starvation, neurological (e.g., epilepsy, Parkinson's disease) and neurodegenerative disease (e.g., Alzheimer's disease, dementia) (Oyarzabal & Marin-Valencia, 2019; Watts et al., 2018).

Moreover, genetic pathologies affecting the capacity of glucose metabolism have been shown to have profound neurological and behavioural effects throughout neurodevelopment. These observations stress the importance of adequate perfusion across all cerebral territories during rest and instances of increased neural activity, as neurometabolic coupling is essential for acute and chronic cerebral health. Consequently, cerebral tissue relies upon continuous perfusion for the direct supply of essential nutrients which facilitate and sustain energy-dependent processes of neurophysiological function. This renders the brain uniquely vulnerable to anoxic and/or ischaemic injury during instances of transient and/or chronic cerebral hypoperfusion. Cessation of cerebral perfusion can have debilitating effects in a 'dose'-dependent manner as observed during stroke (ischaemic and/or haemorrhage), transient ischemic attack (TIA), orthostatic hypotension and cardiac arrest (Drake & Iadecola, 2007a). Cerebral hypoperfusion and aberrant cerebrovascular control have now been implicated in many age-related neurodegenerative conditions such as Alzheimer's disease and dementia (Beishon et al., 2021; Beishon & Panerai, 2021; de la Torre, 2018; Ozturk & Tan, 2018; Parkes et al., 2018; Shabir et al., 2018; Sweeney et al., 2018; Toth et al., 2017).

1.2.7 The Role of Cerebral Blood Flow

Cerebral blood flow (CBF) facilitates the transport of essential nutrients to all regions of the brain, allowing for continued metabolic homeostasis and support of neurophysiological function (Ainslie et al., 2016; Camandola & Mattson, 2017; Muoio et al., 2014; Quaegebeur et al., 2011; Willie, Tzeng, et al., 2014). The major nutrient transported within CBF and used by neurons and glial cells to facilitate

metabolic function is glucose, however other substrates can also be used under certain conditions (*see section 1.2.3 Substrate Utilisation Within Cerebral Metabolism)* (Barros & Deitmer, 2010; Bélanger et al., 2011; Chi & Roberts, 2003; Lam et al., 2009; Shulman et al., 2004; Simpson et al., 2007). Through the process of glycolysis, the cellular components within the CNS can develop sufficient ATP to support energy-dependent metabolic functions (Barros & Deitmer, 2010; Bélanger et al., 2011; Harris et al., 2013; Oyarzabal & Marin-Valencia, 2019). Additionally, transport of sufficient O_2 within the CBF is essential in supporting oxidative phosphorylation within neural and glial cells (Watts et al., 2018). Moreover, CBF allows the removal of neurotoxic metabolites from within the interstitial fluid wherein neural and glial structures reside. Several potentially neurotoxic metabolites are developed and released within the interstitial fluid throughout neural and glial oxidative phosphorylation. CBF allows the washout of these metabolites ensuring the homeostatic environment of the cerebral tissue is maintained within narrow limits. Owing to the gravity and relative importance of CBF for neurophysiological homeostasis, it is unsurprising that all regions of the brain are densely vascularised to accommodate this reliance.

1.2.8 Anatomy and Structure of the Intracranial Cerebrovascular Tree

Three distinct hierarchical levels of the cerebrovascular tree have been recognised: (1) the pial vessels; (2) pial arterioles; and (3) parenchymal arterioles/capillaries (Iadecola, 2017b). The structural components of the cerebral vessels change at each level of the cerebrovascular tree (McConnell et al., 2017). The pial arteries, which run along the surface of the cerebral tissue within the

subarachnoid space are composed of a single endothelial layer, internal elastic lamina and multiple layers of vascular smooth muscle cells (VSMCs) (Drake & Iadecola, 2007a; Iadecola, 2017b; McConnell et al., 2017). Pial arteries are innervated by nerve fibres of cranial autonomic and sensory ganglia origin (i.e., sympathetic, parasympathetic and trigeminal ganglia) (Drake & Iadecola, 2006; Hamel, 2006; Iadecola, 2017b; McConnell et al., 2017). Upon initial penetration of the parenchyma, penetrating arterioles are surrounded by the Virchow of Robin space endowed with perivascular macrophages. With continued penetration of the parenchyma, the perivascular space diminishes, and arterioles become enveloped by the terminal processes of surrounding astrocytes, commonly referred to as astrocytic end-feet. Intraparenchymal arterioles are composed of a single layer of VSMC, endothelial layer and basement membrane (Iadecola, 2017b). At the capillary level the vessel wall is completely enveloped by astrocytic end-feet and composed of a single endothelial layer and pericytes. Both intraparenchymal arterioles and capillaries are innervated by nerve fibres of local interneurons and projections from sub cortical nuclei (i.e., locus coeruleus, raphe nuclei, ventral tegmental area and nucleus basalis) (Drake & Iadecola, 2006; Hamel, 2006). Approximately 60% of total cerebrovascular resistance is attributed to large cranial and pial arteries. The remaining 40% is provided by the subcortical vasculature (Iadecola, 2017b). The cellular components of the cerebrovascular system amalgamate to form the neurovascular unit (NVU).

1.2.9 Basic Structure and Function of the NVU

The NVU refers to the aggregate of structural components at all levels of the cerebrovascular tree. The major components of the NVU includes neurons, astrocytes, endothelial cells, connexins, basal lamina, vascular smooth muscle cells and pericytes (capillary level) (Bhalerao et al., 2020; Caffrey et al., 2021; Hawkins & Davis, 2005; Lecrux & Hamel, 2011; McConnell et al., 2017; Muoio et al., 2014; Netto et al., 2018; Sweeney et al., 2016; Weiss et al., 2009; Yu et al., 2020; Zhao et al., 2015).

The structural integrity of the BBB makes it difficult to pharmacologically treat neurological disorders within the CNS. This is primarily due to difficulty in pharmacological agents penetrating the BBB to exert their influence (Bhalerao et al., 2020; McConnell et al., 2017). The NVU serves several important functions within the CNS such as: (1) removal of metabolic toxins from within the CNS, (2) formation of the BBB, ensuring separation from the CNS environment and the systemic circulation, (3) transport of glucose and other metabolic substrates across the BBB to facilitate energy-dependent mechanisms of cerebral metabolism and (4) signalling and regulation of rCBF to active neural territories, a process termed neurovascular coupling (NVC), which is most relevant with respect to this thesis (Bhalerao et al., 2020; Hawkins & Davis, 2005; Mizee & de Vries, 2013; Netto et al., 2018; Persidsky et al., 2006; Stanimirovic & Friedman, 2012; Sweeney et al., 2016; Weiss et al., 2009; Yu et al., 2020).

1.3 History and Mechanisms of NVC

1.3.1 Basic NVC overview

NVC is an innate physiological phenomenon, which describes the functional link between local neuronal activity and rCBF (Howarth, 2014; Iadecola, 2004; McConnell et al., 2017; Nippert et al., 2018; Petzold & Murthy, 2011a). NVC is achieved through inter- and intra-cellular signalling between and within the components of the neurovascular unit (NVU) (Iadecola, 2004; McConnell et al., 2017; Nippert et al., 2018). The necessity of NVC arises as a consequence of (1) the lack of energy reserves within the CNS, (2) the metabolic dynamism of cerebral tissue, (3) volume restraints within the cranial cavity and (4) the requirement for efficient energy use within the CNS (Hosford & Gourine, 2019a). Changes in rCBF following focal neuronal activity form the foundational basis for blood oxygen level dependent (BOLD) and functional magnetic resonance imaging (fMRI) techniques (Phillips et al., 2015) The precise temporal sequence and proportional involvement of each signalling pathway encompassing NVC have yet to be fully elucidated. The difficulty in delineating the mechanistic sequence is consequent to morphological and innervation differences within the NVU across each layer of the cerebrovascular tree. The likelihood is that the mechanistic sequalae following localised neuronal activity is region-specific and involves a combination of primary and auxiliary pathways (Iadecola, 2004).

1.3.2 Historical overview of NVC - Origin

NVC has been researched across several *in vivo* experimental models (human and animal) using a plethora of neuroimaging techniques. Herein, the following paragraph(s) discuss the early historical landscape of NVC-related research, from point of origin to current techniques, and conclude with a recognition of the current theorized mechanistic model which regulates NVC. Employing this method, we gain an appreciation for the key literature and researchers which have catalysed this field. The birth of NVC is often credited with the pioneering work of Roy & Sherrington (1890) who investigated continuous recordings of cerebral volume and arterial blood pressure equipped with an oncograph and kymograph, respectively. These primary investigations in the anaesthetized dog, cat and rabbit demonstrated the influence of afferent nerve stimulation, asphyxia and chemical administration on hemispheric volume properties and systemic blood pressure. The authors concluded their work by positing the idea of an intrinsic cerebral mechanism by which the brain could regulate its own blood supply in accordance with functional activity:

"We conclude then, that the chemical products of cerebral metabolism contained in the lymph which bathes the walls of the arterioles of the brain can cause variations of the calibre of the cerebral vessels: that in this re-action the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations of functional activity" (Roy & Sherrington, 1890).

1.3.3 Initial Scepticism of an NVC Response

Although this study lacked the experimental sophistication to draw precise mechanistic conclusions, its prescient deduction, made ≈132 years ago, would become synonymous with our interpretation of the NVC response. This rhetoric was met with much contempt and disregard. Leonard Hill, a renowned British physiologist, was a principal sceptic of Roy and Sherrington's (1890) proposition. His disbelief of an intrinsic cerebral vasomotor mechanism led him to publish *The Physiology and Pathology of the Cerebral Circulation: An Experimental Research (1896)* (S. Kety, 1964). This review provided a narrative which challenged the possibility of an NVC response. Its damning, but erroneous, conclusion was that CBF is passively controlled by changes in systemic blood pressure and indirectly through vasomotor centres within the splanchnic region. Hill's appraisal of the literature proclaimed that the brain did not possess the capacity to regulate its own blood supply. This doctrine would govern the field for subsequent generations until further advancements in neuroimaging techniques allowed increasingly accurate temporal and spatial measurements of cerebral metabolism and perfusion to be determined in parallel.

1.3.4 Developments in Neuroanatomical Research Supporting NVC

Attempts at challenging Hill's axiom were revived by the 1920's. Developments in neuroanatomical research were the likely catalyst for the revival. Several investigations, armed with an ocular microscope and square-ruled disc micrometer demonstrated regional and cortical differences in cerebral capillary density of the albino rat (Craigie, 1920, 1921, 1925) and rabbit (Cobb, 1927) within cortical and

sub-cortical structures. Significant differences in cerebral vascularisation were observed between gray and white matter structures as well as sensory vs. motor function areas. The authors proposed that the differentiation of capillary vascularity was likely relative to neural density and consequent metabolic demand of the tissue. This interpretation suggests an organisational link between cerebral metabolic demand and vascularisation, providing structural support for the earlier observation made by Roy & Sherrington (1890).

1.3.5 First Clinical Reference to NVC

The first clinical inference of an NVC response was observed at Peter Bent Brigham Hospital, Boston, Massachusetts (Fulton, 1928). A patient presented to clinic with an angioma and secondary auscultatory bruit within the occipital lobe territory. Patient symptoms included persistent headache and progressive loss of visual fields. During treatment, it was found that intermittent visual stimulation exacerbated patient symptoms and caused a detectable increase in auscultatory bruit signals which were alleviated following removal of the visual stimulus. Moreover, other sensory stimuli (auditory, olfactory) did not elicit an exacerbation of symptoms. The authors proposed that the exacerbation was the result of a localised increase in cerebral blood flow toward the occipital territory following visually induced increases in regional functional activity. Although unsubstantiated, the authors interpretation of the underlying physiology would prove correct.

1.3.6 Growing Appreciation for Inherent NVC Response

By the 1930s there was a growing appreciation for the dynamic relationship between functional activity of the brain and cerebral blood flow. An updated review on the intricacies of cerebral blood flow regulatory mechanisms was published (Wolff, 1936). While acknowledging a lack of empirical evidence, this review supported the concept of an intrinsic cerebral vasomotor mechanism and dismissed the supposition of cerebral tissue being passively perfused by systemic pressures. Wolf (1936) made important references to developments in neuroanatomy of the brain, regional differences in vascularisation and even metabolic profiling of the cerebral tissue (oxidative metabolism, glycolysis). While technical development limited researchers to gross observations at the time, this review would encourage the possibility of a functional link between cerebral blood flow and metabolic demand.

1.3.7 Application of Thermoelectric Techniques

Throughout the 1930s we saw the introduction of thermoelectric methods as conduits for inferring changes in cerebral blood flow (Schmidt, 1936; Serota & Gerard, 1938). Thermojunctions were inserted approximately 1-3mm below the surface of the cortex using a Horsley Clark instrument. The underlying principle requires that the thermode is either continually warmed or cooled relative to blood temperature. Ergo, any increase in blood flow to that region is reflected by a cooling or warming of the thermode, respectively. This method was initially used to determine innervation properties of cerebral vessels. Application of faradic current stimulation to the cervical sympathetic nerve was shown to induce gross

vasoconstriction of the parietal cortex within the anaesthetized cat (Schmidt, 1936). These experiments would demonstrate that neural activity modulates cerebral vessel calibre through an intrinsic mechanism that was yet unknown.

Serota and Gerard (1938) built upon this technique and recorded thermal changes within the visual cortex of the anaesthetized cat and dog in response to somatosensory and visual stimulation. The results demonstrated that intermittent visual stimulation induced a rise in temperature within the optic pathway (e.g., lateral geniculate, visual cortex) which returns to baseline once the visual stimulus is removed. Moreover, infusion of ammonia gas into the nostrils of the anaesthetized animal led to a temperature increase in Ammon's horn of the hippocampus, a key structure within the olfactory pathway (Serota & Gerard, 1938). Importantly, these observations were made in the absence of any systemic arterial blood pressure changes. The results demonstrate region-specific perfusion increases during cortex-specific stimuli, as indicated by temperature changes.

1.3.8 Acceptance of an NVC Response

The exponential growth of experimental evidence rendered the passive brain ideology proposed by Hill (1896) as untenable. An updated summary of the available evidence was published by Forbes & Cobb, (1938), ≈42 years following the publication of Hill and colleagues' (1896) thesis. The updated review supported the existence of both intra- and extracranial regulators of CBF. Major intrinsic regulators were: (1) chemical changes in arterial carbon dioxide ($PaCO_2$), (2) local neuron activity (regional metabolism), and (3) neurogenic signalling (vasoconstriction and vasodilation). Systemic arterial pressure was noted as a major extracranial regulator

of CBF. By the mid-20th century, we gained an appreciation for all tenets of CBF regulatory mechanisms, encompassing NVC as well as cerebrovascular reactivity and cerebral autoregulation.

1.3.9 Developments of Global CBF measurement techniques

The next major progression in the context of CBF measurement was developed by (S. S. Kety & Schmidt, 1945). Their adaptation of the *Fick* principle allowed the quantitative determination of global CBF *in vivo* without the confounding effects of anaesthesia. Their method followed the basic principle that the rate at which the arterial-venous (a-v) difference of an inert gas approaches equilibrium is a function of the volume of blood which perfuses the organ/tissue (S. S. Kety & Schmidt, 1945, 1947). Kety and Schmidt (1947) employed the utility of nitrous oxide (N_2O), a common prophylactic in pain relief, however other inert gases have also been employed (Gibbs et al., 1947). This method was used to quantify total cerebral blood flow in the unanaesthetised man (Gibbs et al., 1947; S. S. Kety & Schmidt, 1947; Sokoloff et al., 1955). The same principle was used to determine total cerebral metabolic rates of oxygen ($CMRO_2$) and glucose (CMR_{GLU}) in humans (S. S. Kety & Schmidt, 1947; Sokoloff et al., 1955) and monkeys (Schmidt et al., 1944). This method consistently demonstrated a correlative relationship between $CMRO_2/CMR_{GLU}$ and total CBF in support of an intrinsic link between functional activity of the cerebral tissue and its perfusion (S. S. Kety, 1950). Unfortunately, this technique lacked the spatial and temporal resolution to examine changes in metabolic rate and perfusion at the local level.

1.3.10 Developments in rCBF Measurement Techniques

Building on the principles developed by Kety and Schmidt (1944), the utility of autoradiographic techniques and inert radioactive tracers (vs. N_2O-method) were proposed as an alternative method which would allow assessment of localised changes in metabolic rates and perfusion at rest and during activity (Freygang & Sokoloff, 1958; Landau et al., 1955). Employing this method requires administration of inert radioactive isotopes and external detection by a γ-camera. Weak beta-emitting radioisotopes such as I^{131}-tagged trifluoro iodomethane, CF_3I^{131}, Krypton^{85} (Kr^{85}) and Xenon^{133} (Xr^{133}) were routinely used for assessment of rCBF changes (Cronqvist et al., 1966; Ingvar, 1975; Ingvar, Baldy-Moulinier, et al., 1965; Ingvar, Cronqvist, et al., 1965; Ingvar & Lassen, 1962; Ingvar & Risberg, 1967; Landau et al., 1955; Obrist et al., 1963; Olesen, 1971). Localised changes in glucose and oxygen metabolism were captured following administration of 2-deoxy-D-[^{14}C]glucose-6-phosphate (Altenau & Agranoff, 1979; Kennedy et al., 1976) and ^{15}O-labeled oxyhaemoglobin (Raichle et al., 1976), respectively. The method relies upon the rapid uptake and slow clearance rates of diffusible tracers from within the cerebral tissue. Recirculation of radioisotopes is negated due to rapid and efficient washout by the respiratory system (Cronqvist et al., 1966; Ingvar & Lassen, 1962). Throughout this period, the addition of electroencephalography (EEG), as an index of neural-evoked potentials, was used in tandem with metrics of rCBF and metabolic demand (Ingvar et al., 1979; Ingvar, Baldy-Moulinier, et al., 1965; Ingvar & Risberg, 1967; Obrist et al., 1963; Skolasinska et al., 1979).

Consequent to these methodological developments, it was now possible to measure and quantify localised changes in rCBF, $rCMRO_2$ and $rCMR_{GLU}$ *in vivo* with concomitant neural activity. The literature continuously demonstrated a temporal and

spatial link between indices of rCBF and metabolic activity in humans (Ingvar et al., 1979; Ingvar, Baldy-Moulinier, et al., 1965; Ingvar & Risberg, 1967; Olesen, 1971; Raichle et al., 1976; Roland & Larsen, 1976), goldfish (Altenau & Agranoff, 1979), monkey (Kennedy et al., 1976) and cats (Freygang & Sokoloff, 1958; Landau et al., 1955; Skolasinska et al., 1979). By the mid-1970s 2D colour-imaging of cerebral topography, in tandem radioactive isotope administration, was used as a visual tool when characterising localised changes in CBF and metabolism at rest and during functional activity (Lassen et al., 1978; Roland & Larsen, 1976). This innovation would form the foundation upon which positron emission tomography (PET) is built. By the end of the 1970s the scientific community had confirmed a spatial and temporal link between local functional activity and rCBF (Ingvar, 1977; Lassen et al., 1978). What they lacked was the mechanistic insight into key signalling molecules and pathways which orchestrated this physiological phenomenon.

1.3.11 NVC Acceptance and Current Techniques

Based on the above summary we can see the progressive appreciation for NVC, which coincided with innovative developments in technical and neuroimaging techniques. Initially, researchers were confined to indirect measures of hemispheric blood volume and speculation of its relation to cerebral functional activity based upon neuroanatomical differences in capillary density. Technical innovation allowed greater inferences in CBF and cerebral metabolism to be made at a global level. Finally, by the 1960's the neuroimaging standards had reached a threshold which allowed association between rCBF and metabolism. It took ≈80 years before confirmation of Roy and Sherrington's (1890) original hypothesis. At present, there

are multiple clinical and research tools employed in the assessment of NVC and CBF such as (1) Transcranial Doppler Ultrasound (TCD, the device used throughout this thesis); (2) Near-infrared spectroscopy (NIRS); (3) Dynamic vessel analysis (DVA); (4) Doppler laser flowmetry; (5) functional Magnetic Resonance-Imaging (fMRI); and (6) Positron emission tomography (PET). Each device presents with its own strengths and limitations and should be used in accordance with the experimental question. The neuroimaging and technical developments of measurement tools has greatly reduced the barrier to entry within NVC-related research. The next section focuses on the key mechanisms involved within this physiological phenomenon.

1.3.12 Overview of NVC mechanisms

Many attempts to delineate the precise mechanistic sequence which describes the NVC response have been made. Early hypotheses posited that reductions in substrates (O_2 and glucose) which support cerebral metabolism coupled with an increase in metabolic by-products initiates a signalling cascade which increases local perfusion to the area (Beishon et al., 2021; Hosford & Gourine, 2019b; S. S. Kety, 1950; Nippert et al., 2018). This rhetoric proposes a metabolic feedback system regulating NVC. Indeed, experimental studies have shown that metabolic by-products (carbon dioxide (CO_2), hydrogen ions (H^+), lactate and adenosine) are vasodilators of cerebral vessels (Freeman & Li, 2016; Ko et al., 1990). However, the transient reduction in O_2 concentration at the site of neural activity is small and unable to account for the dramatic increase in perfusion to the active region (Iadecola, 2004; Raichle & Mintun, 2006). Nevertheless, the magnitude of the NVC response persists in the background of both hyperoxic and hyperglycaemic

administration (Attwell & Iadecola, 2002; Nippert et al., 2018; Raichle & Mintun, 2006; Wolf et al., 1997). Moreover, parenchymal pH undergoes a transient alkalosis, not acidosis, following neural activity due to the rapid CBF-induced washout of CO_2 and H^+ uptake (Chen & Chesler, 1992; Makani & Chesler, 2010; Ueki et al., 1988). Ergo, it was accepted that metabolic feedback signalling was not responsible for initiating the NVC response but may be involved in fine-tuning the haemodynamic response during sustained activation.

It is now recognised that NVC initiates from a series of feedforward signalling pathways consequent to augmented neural activity (Beishon et al., 2021; Cauli & Hamel, 2010; Hosford & Gourine, 2019; Iadecola, 2017; Kaplan et al., 2020; S. S. Kety, 1950; Segarra et al., 2019; Stackhouse & Mishra, 2021). Pre- and post-synaptic activity can modulate cerebrovascular tone through several direct and indirect signalling pathways (Hosford & Gourine, 2019; Iadecola, 2017; Phillips et al., 2015; Segarra et al., 2019). Direct pathways refer to the capacity of neural derivatives to act on adjacent vascular smooth muscle cells (VSMCs) and/or pericytes to initiate a vasoactive response. Indirect pathways refer to neural signalling to interposed cells within the NVU initiating a vasoactive signalling cascade on adjacent vessels. The neural-astrocyte axis is the most widely cited within the NVC response (Howarth, 2014; Muñoz et al., 2015; Otsu et al., 2015; Petzold & Murthy, 2011). The likelihood is that a combination of feedforward and feedback mechanisms work in a time-dependent manner to fine tune the net haemodynamic response during periods of increased neural activity (Beishon et al., 2021; Iadecola, 2017; Segarra et al., 2019). The following paragraphs discuss the involvement of each structure within the NVC response with reference to key signalling pathways.

1.3.13 Neuronal Signalling Within the NVC Response

Glutamate is the main excitatory neurotransmitter in the adult brain (Lourenço et al., 2016). Exocytosis of glutamate from pre-synaptic terminals during augmented neural activity induces localised vasoactive effects on adjacent cerebral vessels (Hosford & Gourine, 2019; Iadecola, 2017; Phillips et al., 2015). Key neural vasoactive molecules include nitric oxide (NO), prostanoid, potassium (K^+) and ATP/adenosine (Iadecola, 2004). Glutamate release activates the ionotropic post-synaptic N-methyl-D-aspartate (NMDAr) and α-amino-3-hydroxy-5-methyl-4-isoxazol-epropionic acid (AMPAr) receptors located on post-synaptic terminals (Iadecola, 2017; McConnell et al., 2017). Activation of post-synaptic NMDA and AMPA receptors incurs an increase in intracellular cytosolic calcium concentration (iCa^{2+}). In situations of sufficient oxygen (O_2) and glucose concentrations, this increase in iCa^{2+} results in the synthesis of both neuronal nitric oxide synthase (nNOS) and cyclooxygenase 2 (COX2), which are precursor enzymes for the potent vasodilators nitric oxide (NO) and prostanoid (i.e., prostaglandin E_2; PGE_2) (Hosford & Gourine, 2019; Iadecola, 2017; Phillips et al., 2015). Additional vasoactive pathways from neural signalling include the post-synaptic release of ATP as well as the release of K^+ into the perivascular space during hyperpolarisation (Iadecola, 2017).

1.3.14 Mechanisms of Neural-mediated Cerebral Vasodilation

NO is a membrane permeant molecule, which diffuses from the post synaptic neuron to the VSMCs. Once within the VSMC, NO activates soluble guanylate cyclase (sGC), promoting the formation of cyclic guanosine monophosphate (cGMP)

(Lourenço & Laranjinha, 2021; Nippert et al., 2018). cGMP dephosphorylates the VSMC myosin light chain complex, inducing VSMC relaxation (Lourenço & Laranjinha, 2021). NO can further hyperpolarise VSMCs through the stimulated release of intracellular Ca^{2+} stores and the subsequent activation of Ca^{2+}-dependent K^+ channels (Lourenço & Laranjinha, 2021; Nippert et al., 2018). PGE_2 hyperpolarises VSMCs through activation of surface K^+ channels as well as binding to membrane bound prostanoid receptors (EP_4) and the subsequent dephosphorylation of the myosin light chain complex (Kaplan et al., 2020; Nippert et al., 2018). K^+ ions are released into the extracellular space during neuronal repolarisation. Extracellular K^+ at concentrations up to 12mM above normal levels hyperpolarises VSMCs through increased conductance of membrane bound K_{IR} channels (Filosa et al., 2006; Nippert et al., 2018). ATP is released from pre-synaptic terminals and is rapidly metabolised to adenosine via extracellular nucleotidases where it hyperpolarises VSMCs through activation of the A_{2A} surface receptors (Dale & Sebastião, 2017; Hosford & Gourine, 2019). All of these signalling pathways coalesce and act on adjacent VSMCs and pericytes to incur localised vasodilation and a consequent increase in rCBF to the activated area.

1.3.15 Perivascular & Subcortical Innervation

The cerebrovascular network is innervated by a milieu of neuronal terminals from extrinsic and sub-cortical origins. Large upstream pial arteries are densely innervated by perivascular nerves from autonomic (superior cervical ganglion, sphenopalatine) and/or sensory ganglion (trigeminal ganglion) (Hamel, 2006). These neural projections are equipped with several neurotransmitters (norepinephrine,

neuropeptide Y, vasoactive intestinal peptide, acetylcholine (ACh), nNOS and substance P) which can modulate vascular tone. The degree to which these extrinsic neural projections partake in the NVC response is currently unknown. Current literature suggests that the extrinsic innervation is essential for the maintenance of resting calibre of large upstream arteries (Drake & Iadecola, 2007; Hamel, 2006; Iadecola, 2004, 2017). Sub-cortical projections from the locus coeruleus, raphe nuclei and nucleus basalis release vasoactive neuromodulators such as ACh, norepinephrine and serotonin (5-HT) respectively (Drake & Iadecola, 2007b; Hamel, 2006). Sub-cortical neurons project to the microvascular network and synapse directly with astrocytic end feet, cortical interneurons and/or endothelial cells (Hamel, 2006; Iadecola, 2004, 2017; Kaplan et al., 2020). The relative contribution of sub-cortical neural projections and/or interneurons in the initiation and/or maintenance of the NVC response is currently undetermined.

1.3.16 Neural-Astrocyte signalling in within the NVC response.

Concomitantly, the release of neurotransmitters and/or neuromodulators following activation of neural activity can activate nearby astrocytes. Astrocytes are intimately positioned within the subcortical space to relay vasoactive messages between the synapse and localised cerebral vessels (Howarth, 2014; Iadecola, 2004, 2017; Muñoz et al., 2015; Petzold & Murthy, 2011). Terminal ends of astrocytes, termed end feet, ensheath the perivascular space of intra-parenchymal arterioles and capillaries. These endfeet are highly specialised and are enriched with membrane bound aquaporin-4, connexin-43, purinergic receptors and K^+ channels (Iadecola, 2004; Petzold & Murthy, 2011). The landmark investigation by Zonta et al., (2003)

demonstrated that astrocytes can detect increases in neural activity and modulate adjacent cerebral vessel tone through increases in i[Ca^{2+}] and the subsequent release of vasoactive molecules. The initial hypothesized mechanism by which astrocytes mediated rCBF was through the uptake of K^+ from post synaptic neurons and subsequent release from astrocytic endfeet, a process termed *K^+ siphoning* (Paulson & Newman, 1987). However, this hypothesis was disproven through experimental work in knockout mice lacking the inward rectifying $K_{IR}4.1$ channel on astrocytes, which demonstrated a fully intact NVC response (Metea et al., 2007).

1.3.17 Mechanisms of Astrocyte-mediated Cerebral Vasodilation

Experimental work by Zonta and Colleagues (2003) showed that astrocytes can modulate adjacent cerebral vessel tone through increases in i[Ca^{2+}] following activation of membrane bound receptors. The release of glutamate from pre-synaptic terminals activates metabotropic glutamate receptors (i.e., mGLUR5) on the astrocytic membrane (Iadecola, 2017; McConnell et al., 2017; Petzold & Murthy, 2011). Once activated, these G-protein-coupled receptors initiate an inositol 1,4,5-triphosphate (IP_3) receptor (IP_3R)-mediated Ca^{2+} cascade which propagates throughout the cell (Muñoz et al., 2015). Elevations in i[Ca^{2+}] levels activate the Ca^{2+}-sensitive enzyme phospholipase A_2. Phospholipase A_2 catalyses the cleavage of the polyunsaturated fatty acid arachidonic acid (AA) from the cellular membrane (McConnell et al., 2017; Petzold & Murthy, 2011). Intracellular AA is then metabolised into either epoxyeicosatrienoic acid (EETs) or prostaglandin E_2 (PGE_2) through the intracellular enzymes cytochrome P450-2C11-epoxygenase (CYP2C11) and cyclooxygenase 1 (COX1), respectively (Petzold & Murthy, 2011). Both EETs

and PGE_2 are released from astrocytic endfeet where they induce vasodilation of adjacent cerebral vessels (Howarth, 2014; Muñoz et al., 2015).

1.3.18 Mechanisms of Astrocyte-Mediated Cerebral Vasoconstriction

Observations of the dynamic relationship between mGLUR activation, astrocytic soma and endfeet $i[Ca^{2+}]$ and adjacent vessel diameter was the first evidence that astrocytes might play a contributing role in NVC (Zonta et al., 2003). In certain instances, AA diffuses out of astrocytes and into VSMC where it elicits vasoconstriction, through 20-hydroxyeicosatetraenoic acid (20-HETE) formation via ω-hydroxylases (Howarth, 2014; Muñoz et al., 2015). Nitric oxide (NO) availability has been shown to play a critical role in modulating the vasoactive direction of astrocytes. NO can bind to the heme moiety and inactivate cytochrome P450 enzymes (Howarth, 2014). This in turn prevent the synthesis of 20-HETE from astrocytes, inhibiting vasoconstrictive signalling. The vasoactive response following the activation of astrocytes has been shown to be influenced by O_2 concentration (Gordan et al., 2008), resting basal tone (Blanco et al., 2008) and extracellular K^+ levels (Girouard et al., 2010). In a similar fashion, elevations of astrocytic $i[Ca^{2+}]$ can be achieved via purinergic signalling (Howarth, 2014; Muñoz et al., 2015). ATP released into the extracellular space by active neurons activates purinergic P2Y and $P2X_7$ receptors located on the astrocytic cellular membrane (Howarth, 2014). Binding of ATP to P2Y and $P2X_7$ receptors initiates a phospholipase C-mediated increase in $i[Ca^{2+}]$, providing further support for the phospholipase A_2-AA pathway.

1.3.19 Time-kinetics of Astrocyte-Mediated Vasodilation

Whether astrocytes contribute to the initiation of the NVC response is often debated. The time-kinetics required for glutamate-mediated astrocyte activation, the development and propagation astrocytic i[Ca^{2+}] waves, synthesis of vasoactive metabolites and the subsequent effect on adjacent VSMCs calls into question whether astrocytes have the capacity to initiate an NVC response (Petzold & Murthy, 2011). Experimental evidence has shown that stimulation of the somatosensory cortex in mice (Takano et al., 2006; Wang et al., 2006), visual cortex stimulation in the ferret (Schummers et al., 2008) and odour stimulation of the olfactory glomeruli in mice (Petzold et al., 2008) result in increases of astrocytic i[Ca^{2+}] at approximately 1-6s (Takano et al., 2006; Wang et al., 2006), 3-4s (Schummers et al., 2008) and 1-2s (Petzold et al., 2008) post-stimulus onset. However, the NVC response typically initiates approximately 1s post-stimulus onset (Tian et al., 2010). The differences in time-kinetics of post-stimulus astrocytic i[Ca^{2+}] highlights task- and/or regional-differences in astrocytic vasoactive signalling. Collectively, these investigations postulate that astrocytes might not be responsible for initiating the NVC response, but may play a role in supporting vasodilation during sustained stimulation (Petzold & Murthy, 2011).

1.3.20 Concluding remarks on NVC

Collectively, these studies support several vasoactive signalling pathways, which contribute to the composite NVC response. The likelihood is that temporal dynamics of the NVC response are pathway specific, where neural and/or astrocytic signalling are responsible for specific domains of the haemodynamic response. The

NVC appears to initiate from neural-mediated signalling, with astrocytes contributing to sustained vasodilation of cerebral vessels. Metabolic feedback signalling is likely involved in the fine-tuning of the haemodynamic response during sustained neural activation. The key mediators within the NVC response are currently under debate. Appraisal of current evidence suggests that nNOS contributes a significant portion of the NVC response (O'Gallagher et al., 2022; Hosford & Gourine, 2019). Blockade of nNOS resulted in an average 64% reduction in the NVC response (Hosford & Gourine, 2019). Interestingly, nNOS inhibition within granular layer of the cerebellum completely abolished the NVC response (Mapelli et al., 2017). This is likely since the granular layer of the cerebellum possesses the highest density of nNOS expressing neurons across all brain regions. In contrast, pharmacological or genetic manipulation of the AA-COX pathway resulted in a 44-57% reduction in the overall NVC response (Hosford & Gourine, 2019). A better understanding of the key mediators in NVC physiology will help identify therapeutic targets in the treatment of neurovascular-related disorders.

1.4 Introduction to High Altitude Physiology

1.4.1 Introduction

HA is a relative term which is often attributed to any location >2100m above sea level (Brown & Grocott, 2013; Hurtado et al., 2012). While commercial expeditions to HA are becoming increasingly popular among habitual lowlanders, exposure to this environment initiates a multisystem stressor, which threatens normal physiological function and survival. Since the landmark investigation of West (1984), who characterised changes in pulmonary gas exchange at the summit of

Everest (8850m above sea-level) there has been a rapid growth in HA-related research. More recent expeditions have explored the physiological response within the mountainous terrains of Nepal, Europe, South America and the United States (Brewster et al., 2021; Caldwell et al., 2017; Clarke et al., 2019; Lefferts et al., 2020; Rasica et al., 2021; Vizcardo-Galindo et al., 2020; Willie, Smith, et al., 2014).

The primary stressor associated with HA exposure is the nonlinear decrease in total atmospheric pressure and subsequent reduction in the partial pressure of ambient oxygen (PO_2), termed hypobaric hypoxia (Chawla & Saxena, 2014; Ke et al., 2017). This hypoxic stress is detected along each step of the oxygen (O_2) cascade, from airway gas diffusion within the respiratory system, to mitochondrial O_2 utilisation at the cellular level (Brown & Grocott, 2013). To mitigate the threat of reduced O_2 availability, human physiological systems respond through a spectrum of time-dependent functional and molecular changes which characterise the acclimatisation process.

1.4.2 Acclimatisation & Maladaptation to High Altitude

The acclimatisation process is integrative, involving gross functional changes within the cardiovascular, respiratory, renal and nervous system(s) (Bärtsch & Gibbs, 2007; Chawla & Saxena, 2014; Dhar et al., 2014; Goldfarb-Rumyantzev & Alper, 2014; Ke et al., 2017; Koller et al., 1991; Milledge, 1979; Naeije, 2010; Palubiski et al., 2020; Riley & Gavin, 2017; Stembridge et al., 2015; West, 1984, 2006, 2012). These functional changes are often illustrated through haematological changes in arterial blood gases and/or circulating molecules (Chakraborti et al., 1985; Crapo et al., 1999; Zouboules et al., 2018). Aberrant acclimatisation to HA

among habitual lowlanders' manifests in the development of acute mountain sickness (AMS), where participants present with symptoms including headache, nausea, vomiting and respiratory difficulties (Brown & Grocott, 2013; Roach et al., 2018; West, 2012). Prophylactic treatment of AMS includes supplemental O_2, descent from HA and treatment with carbonic anhydrase inhibitors (i.e., acetazolamide, methazolamide). If untreated, AMS can progress toward life threatening conditions such as high altitude pulmonary and/or cerebral oedema (HAPE/HACE, respectively) (Brown & Grocott, 2013; West, 2012; Wilson et al., 2014).

The pathogenesis of AMS and maladaptation to HA is currently unknown and likely multifactorial. The development of a physiological screening test or battery of tests, which could be conducted at sea-level to assess individual susceptibility to AMS would be invaluable to the mountaineering community. However, difficulties in study design limit the capacity to delineate the pathophysiology of AMS development in advance, prior to ascent. Sample sizes within HA research are often small due to logistical constraints, with participants experiencing AMS symptoms often removed from study participation due to ethical and/or medical concerns. Moreover, the presence of AMS symptoms relies on self-reported questionnaires (Roach et al., 2018) making it difficult to diagnose and grade the severity of AMS phenotypes. Ergo, AMS severity is often biased towards subjective interpretation with severe AMS participants routinely removed from study participation. Collectively, these factors make it impossible to determine the precise physiological sequalae of events which initiate the AMS cascade.

1.4.2 Acute & Chronic Respiratory Acclimatisation at High Altitude

Time-dependent functional changes within the respiratory system are consistently observed following acute and chronic exposure to HA. Acutely, hypoxia initiates a signalling cascade within the respiratory neural axis which increases ventilatory drive and subsequent ventilatory motor output, a process termed the hypoxic ventilatory response (HVR) (Duffin, 2007; Pamenter & Powell, 2016). The HVR serves to restore O_2 levels through increased alveolar ventilation (Chawla & Saxena, 2014; Duffin, 2007). Reductions in systemic O_2 levels are detected by the carotid body located at the bifurcation of the common carotid arteries, giving rise to the peripheral chemoreflex (Chawla & Saxena, 2014; Duffin, 2007; López-Barneo et al., 2016; Pamenter & Powell, 2016; Pfoh et al., 2016, 2017). The carotid body contains neuron-like, O_2-sensitive, glomus type-1 cells which are depolarized through activation of membrane O_2-sensitive ion channels (Chawla & Saxena, 2014; López-Barneo et al., 2016; Teppema & Dahan, 2010; R. J. A. Wilson & Teppema, 2016). Glomus cells form chemosensory synapses with afferent nerve fibres projecting to the respiratory control centres in the brainstem (López-Barneo et al., 2016; Teppema & Dahan, 2010; R. J. A. Wilson & Teppema, 2016). Once depolarised, glomus cells release neurotransmitters (e.g., ATP, acetylcholine) into the synaptic cleft, initiating an increase in afferent signalling to the respiratory control centre (López-Barneo et al., 2016; Teppema & Dahan, 2010; R. J. A. Wilson & Teppema, 2016). The net result is an increase in ventilatory drive and subsequent hyperventilation. This respiratory phenotype has been demonstrated repeatedly across HA research expeditions and/or laboratory-based studies using hypoxic gas exposure (Ainslie et al., 2008; Ainslie & Burgess, 2008; Ainslie & Poulin, 2004;

Bird et al., 2021; Leacy et al., 2021; Lucas et al., 2011; Pfoh et al., 2016, 2017; Steinback & Poulin, 2007, 2008).

Chronic exposure to HA and/or normobaric hypoxia induces plasticity within the respiratory control network, a process termed ventilatory acclimatisation to hypoxia (VAH) (Pamenter & Powell, 2016; Powell, Dwinell, et al., 2000; Powell, Huey, et al., 2000). The underlying mechanisms suggest an increase in loop gain in signal integration within the respiratory control centre as well as increased O_2-sensitivity at the carotid body, which manifests in an increased ventilatory motor output for a fixed input (Dempsey et al., 2014; Powell, Dwinell, et al., 2000; Powell, Huey, et al., 2000; Wilkinson et al., 2010). This has been shown in animal models of the mouse (Ivy & Scott, 2017), goat (Bisgard et al., 1986; Nielsen et al., 1988; Smith et al., 1986), cat (Barnard et al., 1987; Vizek et al., 1987) and rat (Aaron & Powell, 1993; Dwinell & Powell, 1999; Wilkinson et al., 2010). VAH is often illustrated by an increase in ventilatory output for a given partial pressure of arterial oxygen (PaO_2) stimulus. This has been further demonstrated in human models at HA, which have characterised VAH during chronic HA exposure (Ainslie et al., 2013; Forster et al., 1971; Leacy et al., 2021).

1.4.3 Cardiovascular Acclimatisation at High Altitude

Similar time-dependent responses are observed within the cardiovascular system. Acute exposure to HA elicits an immediate increase in heart rate (tachycardia), myocardial contractility and cardiac output (Bärtsch & Gibbs, 2007; Ke et al., 2017; Naeije, 2010; Riley & Gavin, 2017). This increase in cardiac function is achieved through increased activity of the sympathetic nervous system (SNS)

following peripheral chemoreceptor stimulation and shifts in sympathovagal tone (Bärtsch & Gibbs, 2007; Ke et al., 2017). Increased activity of the SNS has been shown at HA through elevated levels of circulating catecholamines (Kotchen et al., 1973; Mazzeo et al., 1991, 1998). The observed tachycardia and enhanced cardiac output serve to maintain O_2 delivery to active tissue during instances of systemic hypoxia (Naeije, 2010). Acute tachycardia has been observed across laboratory-based studies using hypoxic gas exposure (Pfoh et al., 2016, 2017; Steinback & Poulin, 2007) and HA expeditions (Ainslie et al., 2007; Bird et al., 2021; Lafave et al., 2019; Lucas et al., 2011; Sanborn et al., 2015; Willie, Smith, et al., 2014).

The effects of HA exposure on blood pressure (BP) are often contradictory (Bilo et al., 2019). Several HA studies have demonstrated acute effects on BP (Ainslie et al., 2007; Sanborn et al., 2015) whereas others have shown no effect (Lucas et al., 2011; Willie, Smith, et al., 2014). The disparity is likely attributable to issues regarding study methodology and BP measurement. Many studies incorporate spot measurements of BP in resting conditions, which might not truly reflect representative BP levels. The application of 24 hr ambulatory-monitoring would be more appropriate method of gauging HA-induced effects on systemic BP. Several studies have employed this technique finding significant increases in BP following HA-exposure (Bilo et al., 2015; Parati et al., 2014; Veglio et al., 1999; Wolfel et al., 1994). These 24 hr ambulatory BP investigations provide support for HA-induced increases in BP. The effects of HA on BP are likely the net combination of sympathetically-mediated increase in heart rate, cardiac output and systemic vascular resistance countered in part by the hypoxic-vasodilatory effect on systemic vessels (Bilo et al., 2019).

1.4.4 Renal Acclimatisation at High Altitude

The renal system supports several key functions involved in acid-base regulation, electrolyte balance and haematological changes associated with O_2-carrying capacity following acute and chronic exposure to HA (Goldfarb-Rumyantzev et al., 2014; Pablubiski et al., 2020). Respiratory-induced alkalosis is routinely observed at HA owing to HVR-induced hypocapnia (Brown & Grocott, 2013; Chakraborti et al., 1985; Zouboules et al., 2018). The renal system counteracts this initial respiratory-induced pH deflection with a time-dependent compensatory metabolic acidosis. This ensures arterial pH levels return to homeostatic limits. The compensatory metabolic acidosis is achieved through decreased reabsorption of filtered bicarbonate (HCO_3^-) within the renal tubule. This compensatory mechanism forms the basis of the bicarbonate buffering system and has been consistently illustrated at HA with a reduced concentration of HCO_3^-within the arterial and/or venous blood (Goldfarb-Rumyantzev & Alper, 2014; Zouboules et al., 2018). Moreover, the renal system is responsible for acutely increasing O_2-carrying capacity within the circulatory system through increased secretion of salt and water, causing haemoconcentration and an acute increase in haematocrit (Chawla & Saxena, 2014; Palubiski et al., 2020). Following sustained and chronic exposure to HA the renal system increases production of erythropoietin (EPO) resulting in the synthesis of new red blood cells in bone marrow, increasing haemoglobin [Hb] concentration and O_2-carrying capacity (Palubiski et al., 2020).

1.4.5 High Altitude and Cerebral Blood Flow

Since Severinghaus et al. (1966) first illustrated the acute and chronic effects of HA on CBF in humans, there has been a rapid acceleration in our recognition for how HA influences the cerebrovascular system. HA has a time-dependent effect on cerebral perfusion. Acute exposure to HA incurs an immediate increase in global CBF. This observation has been demonstrated across several HA expeditions in human models (Huang et al., 1987; Jensen et al., 1996; Liu et al., 2017; Lucas et al., 2011; Otis et al., 1989; Reeves et al., 1985; Severinghaus et al., 1966; Willie, Smith, et al., 2014; Liu et al., 2017). The acute increase in CBF serves to maintain cerebral O_2 delivery (CDO_2) during instances of arterial hypoxaemia (Ainslie et al., 2016; Ainslie & Subudhi, 2014; Bailey et al., 2017; Harris et al., 2013; Hoiland et al., 2016). CDO_2 is the net product of CBF and CaO_2 (cerebral arterial oxygen content). Ergo, as CaO_2 decreases during initial HA exposure there is a compensatory increase in CBF.

With time spent at altitude, one observes a return of global CBF levels to measures resembling sea-level values, which follows in accordance with acclimatisation status (Ainslie & Subudhi, 2014; Lucas et al., 2011; Møller et al., 2002; Sanborn et al., 2015; Xu & LaManna, 2006). Reductions in CBF with time spent at HA are thought to occur in conjunction with polycythaemia, alterations in HIF expression, CSF pH buffering, cerebral-capillary angiogenesis, hyperventilatory-induced restoration of PaO_2 and concomitant reductions in $PaCO_2$ (Ainslie & Ogoh, 2010; Ainslie & Subudhi, 2014; Severinghaus et al., 1966; Steinback & Poulin, 2016; Xu & LaManna, 2006). Changes in CBF at altitude are reasoned to be the net product of four major reflex mechanisms: (a) hypoxic ventilatory response, (b) hypercapnic ventilatory response, (c) hypoxic cerebral

vasodilation and (d) hypocapnic cerebral vasoconstriction (Ainslie & Ogoh, 2010; Hoiland et al., 2016).

The major mechanistic pathways which facilitate HA-induced increases in CBF are a series of erythrocyte signalling pathways which serve to increase NO bioavailability (Ainslie et al., 2016; Bailey et al., 2017; Hoiland et al., 2016). During hypoxia, red blood cells (RBCs) transition from their relaxed (oxygenated) 'R' state to a tense (deoxygenated) 'T' state, a process termed allosteric shift. Three major pathways by which 'T' state RBCs can elicit cerebral vasodilation include: (a) S-nitrosohaemoglobin (SNO-Hb)-dependent bioactivity, (b) NO synthesis via nitrite reduction catalysed by deoxyhaemoglobin and (c) purinergic-mediated activation of endothelial nitric oxide synthase (Ainslie et al., 2016; Bailey et al., 2017; Hoiland et al., 2016). A combination of these pathways allows vasodilation of cerebral vessels, an increase in global CBF and maintenance of CDO_2.

1.4.6 High Altitude and CBF Control

While the time-dependent effects of HA on CBF are well recognised, they provide little insight into the regulation or control of CBF at a global and/or regional level. Several investigations have characterised the effects of acute and sustained exposure to HA on CVR using CO_2 inhalation (Jansen et al., 1999; Liu et al., 2018; Lucas et al., 2011) and rebreathing techniques (Ainslie et al., 2007; Fan et al., 2010, 2014) in human models. These investigations yielded mixed reports with respect to the effects of HA on CVR sensitivity. Some studies showed no difference in CVR sensitivity (Jansen et al., 1999) whereas others have shown an increase in CVR sensitivity following HA exposure (Fan et al., 2010, 2014; Liu et al., 2018). More

interestingly, a subset of studies has shown the effects of HA on CVR might be direction-dependent (hypercapnia vs. hypocapnia) (Ainslie et al., 2007; Lucas et al., 2011). Similarly, the effects of HA on CA sensitivity have been explored (Iwasaki et al., 2011; Jansen et al., 2000; Subudhi et al., 2015). All these studies have found a reduction of CA function following exposure to HA. Collectively these results have demonstrated that CBF control mechanics (CVR and CA) are vulnerable during HA exposure and residency. However, at the onset (January 2017) of my doctoral studies there was a paucity of research regarding the effects of HA exposure on NVC. The first study which examined the effects of HA exposure on NVC dynamics was published in November 2017 (Caldwell et al., 2017). Our group attempted to build upon this initial experiment through manipulation of ascent profiles, analysis, and experimental exposure.

1.4.7 Aims & Objectives of Thesis

The primary aims of this thesis were to characterise the effects of different HA exposure paradigms on NVC dynamics within a healthy human cohort. Comparative expeditions were performed employing contrasting ascent profiles (incremental vs. rapid) and maximum altitudes to manipulate stimulus intensity and severity. Additionally, we sought to determine the effect of age and sex on the NVC response due to ongoing disparity within the literature surrounding the interaction between aging and NVC and the potential for sex-dependent differences in NVC *per se*.

The thesis is structured such that chapter 2 focuses on methodological implications of NVC assessment, in terms of stimulus reliability and reproducibility as well as the interaction between participant demographics and the overall

neurovascular coupling magnitude. The thesis concludes with two distinct experimental chapters examining the role of HA exposure on NVC response dynamics. On conclusion of this thesis, we can confidently determine the effect of HA ascent on neurovascular function in healthy human participants, a question that was previously unaddressed at the onset of my doctoral studies.

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Chapter 2. Variation Within the Visually Evoked Neurovascular Coupling Response of the Posterior Cerebral Artery is not Influenced by Age or Sex

The following chapter is published in the *Journal of Applied Physiology*. I attained initial ethical approval to conduct the investigation. I conducted data collection at both study sites (UCC and MRU). I performed all data and statistical analysis. I developed all relevant tables and figures. Finally, I developed and completed the published manuscript.

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2.1 New & Noteworthy

- Herein we assess the variability within the neurovascular coupling response attributable to age and sex (n=125, 21-66yrs; 41 male). Based on the assessment of posterior cerebral artery responses to visual stimulation, approximately 0-6% of the variance observed within several metrics of NVC response magnitude are attributable to the combination of age and sex. Therefore, observed differences between age groups and or sexes is likely a result of other physiological stressors.

2.2 Abstract

Neurovascular coupling (NVC) is the temporal and spatial co-ordination between local neuronal activity and regional cerebral blood flow. The literature is unsettled on whether age and/or sex affect NVC, which may relate to differences in methodology and the quantification of NVC in small sample sized studies. The aim of this study was to (a) determine the relative and combined contribution of age and sex to the variation observed across several distinct NVC metrics (n=125, 21-66yrs; 41males) and (b) present an approach for the comprehensive systematic assessment of the NVC response using transcranial Doppler ultrasound. NVC was measured as the relative change from baseline (% change) assessing peak, mean, and total area under the curve (tAUC) of cerebral blood velocity through the posterior cerebral artery (PCAv) during intermittent photic stimulation. Additionally, the NVC waveform was compartmentalised into distinct regions: Acute (0-9 seconds), mid (10-19 seconds) and late (20-30 seconds) following the onset of photic stimulation. Hierarchical multiple regression modelling was used to determine the extent of variation within each NVC metric attributable to demographic differences in age and sex. After controlling for differences in baseline PCAv, the R^2 data suggests that 1.6%, 6.1%, 1.1%, 3.4%, 2.5% and 4.2% of the variance observed within mean, peak, tAUC, acute, mid and late response magnitude is attributable to the combination of age and sex. Our study reveals that (a) variability in NVC response magnitude is independent of age and sex in healthy human participants, aged 21-66 years.

2.3 Introduction

2.3.1 The neurovascular unit

The neurovascular unit (NVU) is an anatomical consortium of cellular and extracellular components located within the central nervous system (CNS). The NVU provides structural integrity to the blood-brain barrier (BBB), facilitating the transfer of essential nutrients from the systemic circulation to the CNS, as well as the efflux of noxious metabolic compounds from within the CNS (Stanimirovic & Friedman, 2012; Peridsky et al., 2006; Hawkins & Davis, 2005; McConnell et al., 2017). In addition, the NVU plays a central role in the coordination of regional cerebral blood flow (rCBF). This neurophysiological process is termed neurovascular coupling (NVC), which is the temporal and spatial co-ordination between focal neuronal activity and rCBF (Attwell et al., 2010; Cauli & Hamel, 2010; Girouard & Iadecola, 2006; Hosford & Gourine, 2019; Iadecola, C., 2017; Phillips et al., 2015). The cellular and extracellular pathways that facilitate NVC involve direct neuro-vascular or indirect neuro-glia-vascular communication (Hosford & Gourine, 2019; Phillips et al., 2015).

2.3.2 The importance of cerebral perfusion

The importance of continuous perfusion and sophisticated CBF regulation in maintaining cerebral function is evidenced on the basis that despite only constituting 2% of total body mass, the brain receives approximately 20% of total cardiac output and is responsible for 15-20% of total-body energy consumption at rest (Attwell et al., 2010; Phillips et al., 2015; Drake & Iadecola, 2007; Hawkins & Davis, 2005; Bor-Seng Shuet al., 2011). Neuronal activity has a high metabolic cost, and the brain

possesses limited-to-no energy reserves, and therefore relies on constant perfusion to regulate ion homeostasis, neuroimmune responses, cognitive function, and cerebral metabolic homeostasis (Brown & Ransom, 2007; Attwell & Laughlin, 2001; Arthurs et al., 2000; Huneau et al., 2015; Howarth et al., 2012).

2.3.3 NVC imaging modalities

Several techniques, ranging from functional magnetic resonance imaging (fMRI) (Aizenstein et al., 2004; Ances et al., 2009; Brodtmann et al., 2003; Buckner et al., 2000; D'Esposito et al., 1999; Fabiani et al., 2014; Gauthier et al., 2013; Grinband et al., 2017; Tekes et al., 2005; West et al., 2019; Hesselmann et al., 2001; Huettel et al., 2001), near-infrared spectroscopy (NIRS) (Mehagnoul-Schipper et al., 2002; Ward et al., 2015), dynamic vessel analysis (DVA) (Kneser et al., 2009; Lipecz et al., 2019) and transcranial Doppler ultrasound (TCD) (Leacy et al., 2018; Caldwell et al., 2017; Beishon et al., 2019; Fluck et al., 2014; Nowak-fluck et al., 2018; Madureira et al., 2017; Sorond et al., 2008; Stefanidis et al., 2019; Groschel et al., 2007; Zaletel et al., 2005; Niehau et al., 2001) have been employed to measure and quantify NVC during task and cerebral hemisphere-specific stimuli. Each technique presents with strengths and weaknesses. TCD offers a non-invasive, relatively inexpensive method of quantifying the NVC response by integrating Doppler principles to capture velocity changes in cerebral blood flow of intracranial conduit arteries during region-specific tasks (see figure 1). The intracranial vessels that are traditionally insonated are the middle, anterior and posterior cerebral arteries (MCA, ACA and PCA, respectively).

2.3.4 NVC pathology

NVC impairment, or neurovascular 'uncoupling', has been observed in several pathologies such as Alzheimer's disease, stroke, dementia, hypertension and spinal cord injury (Girouard & Iadecola, 2006; Lecrux & Hamel, 2011; Phillips et al., 2013). Moreover, NVC impairment has been observed with advanced and progressive aging, with studies linking NVC impairment with gait abnormalities, poor locomotor control as well as age-associated cognitive impairment (Tarantini et al., 2015, 2017a, 2017b, 2018, Ungvari et al., 2017; Jor'dan et al., 2020). The aetiology of NVC impairment is posited as the senescence of several key physiological mechanisms associated with aging, such as increased arterial stiffness, cerebral tissue atrophy, vascular and endothelial dysfunction, and increased oxidative stress (Tarantini et al., 2017; D'Esposito et al., 2003; Carvalho & Moreira, PI., 2018; Park et al., 2007; Liu et al., 2017; Muhire et al., 2019).

2.3.5 Current appraisal of age-related NVC literature

The concept of age-related NVC impairment is not without controversy. While many studies support the observation of age-related NVC impairment (Ances et al., 2009; Buckner et al., 2000; Fabiani et al., 2014; Tekes et al., 2005; West et al., 2019; Hesselmann et al., 2001; Mehagnoul-Schipper et al., 2002; Ward et al., 2015; Kneser et al., 2015; Lipecz et al., 2019; Seshardi et al., 2016; fluck et al., 2014; Nowak-Fluck et al., 2018; Zaletel et al., 2005; Niehaus et al., 2001; Tarantini et al., 2017; Akif-Topcuoglu et al., 2009; Balbi et al., 2015; Duncombe et al., 2017; Lourenco et al., 2018; Raemakers et al., 2006; Riecker et al., 2003; Ross et al., 1997) there are several studies which refute this finding (Aizenstein et al., 2004; Brodtmann et al.,

2003; D'Esposito et al. ,1999; Grinband et al., 2017; Huettel et al., 2001; Beishon et al., 2019; Madureira et al., 2017; Sorond et al., 2008; Stefanidis et al., 2019; Groschel et al., 2007; Rosengarten et al., 2003; Panczel et al., 1999; Richter & Richter., 2003; Schroeter et al., 2004). The likely reason for controversy is the inherent physiological heterogeneity during the aging process, coupled with between-study variations in methodology and analytical approaches. Moreover, limited sample size within age-related neurovascular research is a potential contributor to disparate observations.

Several investigations, employing a similar methodology herein have examined the effect of aging on NVC (Beishon et al., 2019; Fluck et al., 2014; Nowak-Fluck et al., 2018; Madureira et al., ,2017; Sorond et al., 2008; Rosengarten et al., 2003; Niehaus et al., 2001; Akif-Topcuoglu et al., 2009; Panczel et al., 1999). Sample sizes have ranged between 19-60 participants, stratified according to age. Owing to small participant pools, it can be difficult to detect age-related differences confidently and accurately within each age profile by making direct group mean comparisons. Within a number of these studies, one can also observe an age-related funnelling effect, whereby recruitment sample size decreases within the older age groups. As a result, it can be difficult to make robust conclusions regarding the precise age-related effects on NVC due to study limitations.

We aimed to overcome such limitations by recruiting a relatively large sample size (n=125), two-fold higher than the comparable literature, whilst avoiding any notable age-related reductions in recruitment, thus allowing us to make definitive conclusions regarding the effect of healthy aging on NVC. Moreover, we avoided the use of arbitrary age ranges as a means of defining the age-related effects on NVC through observed group mean differences. Rather, we employed advanced regression analysis to determine the contribution of age on the variance of each NVC parameter

within our sample population, thus providing a reliable assessment on the capacity of age as a predictor variable for NVC response magnitude. We propose that this is a more robust approach to accurately quantify the degree to which aging predisposes NVC impairment.

2.3.6 Sex-related differences in cerebrovascular control

In addition to aging, there is interest in the extent of sexual dimorphism in cerebral blood flow regulation. Previous literature has demonstrated measurable sex-related differences in cerebral autoregulation, with a greater autoregulatory capacity observed in young and older females compared to age-matched males (Favre & Serrador., 2019; Deegan et al., 2011). Differences in circulating sex hormones have been hypothesized as the likely mediator(s) driving the suggested differences in cerebrovascular function between males and females (Robison et al., 2019; Krause et al., 2006). Whether sex-related differences in cerebrovascular function extend to NVC is conflicting.

2.3.7 Sex-related differences in NVC

Several studies, incorporating fMRI, have observed sex-related differences in NVC. However, the direction of these sex-related differences is inconsistent. Both Kaufmann et al., (2001) and Levin et al., (1998) have shown a significantly greater NVC among males, indexed by the blood-oxygen-level-dependent (BOLD) response to dartboard and binocular photic stimulation, respectively. In contrast, Kastrup et al., (1999) have shown a greater BOLD response to checkerboard stimulation among females. Multiple studies employing TCD have found no sex-related differences in

NVC (Madureira et al., 2017; Akif-Topcuoglu et al., 2009). The disparity in results is reminiscent of that of age-related research and NVC. Importantly, fewer than twenty participants within each sex were recruited within the studies which found sex-related differences in NVC (Kaufmann et al., 2001; Levin et al., 1998; Kastrup et al., 1999). Conversely, more than twenty participants per sex were recruited within the studies which found no sex-related differences in NVC (Madureira et al., 2017; Akif-Topcuoglu et al., 2009) This highlights the importance of adequate sample size recruitment to make definitive conclusions on sex-related differences in cerebral blood flow regulation. Moreover, differences in NVC response are routinely determined by observed differences between males and females. This approach is likely confounded by several physiological differences which exist between males and females. In contrast, our protocol utilised advanced regression analysis to accurately determine the relative contribution of sex on the variance observed in NVC response magnitude. We posit that this approach is better suited to accurately determine the extent to which sex influences NVC response magnitude.

2.3.8 Aim(s) and hypotheses of study

The aim of this study was to (a) use advanced regression analysis to determine the contribution of age and sex on the variance observed within the visually evoked cerebral haemodynamic response of the posterior cerebral artery in healthy humans in a relatively large sample population (n=125; 21-66yrs) and (b) present a systematic approach to elicit and assess the NVC response using non-invasive TCD. We hypothesized that advanced aging would contribute to the variance within NVC response magnitude. In addition, notwithstanding some reports of sex-related

differences in cerebral blood flow regulation, we hypothesized that sex would not contribute to the variance in NVC response magnitude.

2.4 Materials & Methodology

2.4.1 Ethical Approval

This study was a dual-site, collaborative study design with participant recruitment and testing at University College Cork (UCC) and Mount Royal University (MRU). The study abided by guidelines and policy on research ethics with human participants set out by the Declaration of Helsinki, except for registration in a database. Ethical approval was received in advance through the Clinical Research Ethics Committee (CREC; reference ECM 3(m) 10/01/18) for studies at University College Cork. In addition, ethical approval was received in advance through MRU Human Research Ethics Board (HREB protocol 2016-45) for studies at Mount Royal University. All participants were recruited via word-of-mouth, posters and e-communication and they provided verbal and written informed consent prior to voluntary participation in the study.

2.4.2 Participant recruitment and inclusion criteria

Participants were recruited for this comprehensive age and sex-based study *a priori*, and these data are not secondary use from previous studies. We recruited 125 healthy participants (males/females; 41/84; 21-66 yrs; see Table 1) at UCC (n=62) and MRU (n=63). Participants reported for testing in dedicated research laboratories. Following verbal and written consent, but prior to instrumentation and data collection, an extensive health questionnaire was completed and reviewed to assess for any pre-existing contraindications to study involvement. Participants were excluded if they reported any prior or current medical history of neurological, cardiovascular, cerebrovascular and/or pulmonary disease, seizures and/or epileptic

episodes. Female menstrual cycle and/or menopausal status were not controlled for within this investigation.

2.4.3 Instrumentation and Data Collection

Once consent and demographic variables were obtained, participants were instrumented for resting, steady-state measurements under eucapnic conditions while breathing room air. Participants were instrumented with an electrocardiogram (ECG: lead II configuration; ADInstruments Bioamp ML132; Colorado Springs, CO, USA) and finometer (Finapres Med systems M2; Enschede, Netherlands) for non-invasive beat-by-beat measurement of heart rate (HR; beats.min^{-1}) and arterial blood pressure (systolic/diastolic/mean arterial pressure; SBP/DBP/MAP; mmHg), respectively. Commercially available headpieces were used to fixate 2MHz Doppler ultrasound probes either side of the cranium, insonating through the trans-temporal acoustic window. Cerebral blood velocity was measured through the P2 segment of the posterior cerebral artery (PCAv; cm/s) using a Transcranial Doppler Ultrasound system (TCD; Compumedics DWL, Germany & Spencer Technologies, Redmond, WA, USA; UCC and MRU, respectively). The P2 segment was insonated as the perfusion territory of this vessel segment is more proximal to the downstream neuronal pool involved in visual processing, compared with the P1 segment (Panczel et al., 1999). Participants were instrumented with a nasal cannula and peripheral pulse oximeter for non-invasive breath-by-breath measurement of respiratory rate (R_R; breaths^{-1}min), end-tidal CO_2 ($P_{ET}CO_2$; mmHg) and peripheral oxygen saturation (SpO_2; %) respectively, using a capnograph (Capnostream 20p; Medtronic Covidien, USA). Capnography and pulse oximetry were not used at the MRU test site, and as

such the R_R, $P_{ET}CO_2$ and SpO_2 values presented herein pertain to the subset of participants recorded at UCC. Data were recorded, and later analysed offline using PowerLab software v8.0 provided by ADInstruments and LabChart Pro software 8.0 (Colorado Springs, CO, USA).

2.4.4 Experimental Protocol

Minor differences in experimental protocols existed between study sites, which are detailed below. Once instrumentation was completed in a seated position, a ten-minute (UCC; 0m) or three-minute (MRU; 1130m) baseline period was implemented. Participants were instructed to keep their eyes closed for the duration of this period. Data were collected in the seated position, in quiet, lowly-lit rooms to mitigate the effects of external stimuli on recorded parameters. Following this baseline period, participants were exposed to eight (UCC) or five (MRU) consecutive trials of intermittent photic stimulation (30s on/off visual stimulation; VS). Subsequent analysis revealed no difference in NVC response magnitude across all eight VS trials at UCC. Therefore, to better align the experimental protocols between UCC and MRU, the last three VS trials were removed from analysis within the UCC data set, leaving five consecutive intermittent VS trials, which were collected at both locations. VS was elicited using an intermittent strobe light stimulus on a mobile phone app (6Hz; *Strobe* app) which was held directly in front of each participant's face (~15cm). Participants were instructed to look directly at the strobe light and investigators confirmed that participants were following verbal instructions. Participants were verbally cued when to open/close their eyes in a consistent fashion between trials and participants. This method of evoking an NVC

response was used as it is a validated method of assessing NVC (Phillips et al., 2015) (Phillips et al., 2015) and has been previously used by our group without any adverse effects (Leacy et al., 2018; Bader et al., 2021) (Bader et al., 2021; Leacy et al., 2018). Upon completion of VS, participants were asked to sit for a further thirty seconds with their eyes closed. The completion of this thirty-second epoch signalled the end of the experimental protocol. All participants at each site completed the same protocol for the assessment of NVC.

2.4.5 Data Analysis

Data acquisition was performed using LabChart v8.0 software. Data were later analysed offline using Labchart v8.0 with resultant figures developed using GraphPad prism 8 software. We quantified NVC by measuring the difference (Δ%) in mean and peak PCAv (Δmean and Δpeak) achieved during VS compared with the preceding twenty-second epoch prior to VS (20s pre-baseline PCAv). In addition, we compared the Δ total area under the curve (Δ%; ΔtAUC) of the raw PCAv signal during VS with that of a preceding thirty-second epoch prior to VS. The thirty-second baseline period for ΔtAUC analysis was selected to ensure comparable time-domains with VS trials. The magnitude of the NVC response is presented as the percentage change from baseline (Δ%). Moreover, the NVC response (ΔMean) was compartmentalised into three distinct temporal domains during VS: acute (0-9 seconds), mid (10-19 seconds) and late (20-30 seconds) following the onset of VS. This was achieved using a bespoke LabChart macro that calculated a mean PCAv value for each 10-second epoch, performed to assess potential age and/or sex-related mechanistic differences across the time-domains of the NVC response, based on our

understanding of the relative contribution of feedforward and feedback signalling pathways to the net haemodynamic response (Iadecola, C. 2017; Hoiland et al., 2020) (Hoiland et al., 2020; Iadecola, 2017).

Analysis followed a three-step approach. Firstly, given that blood flow and vessel tone are intrinsically linked to the cardiovascular, autonomic and the respiratory system, we sought to examine whether intermittent VS elicited measurable changes in $P_{ET}CO_2$, R_R, SpO_2, HR and MAP. To assess this, we calculated averages for each parameter during VS and compared each with an averaged 20-second pre-baseline epoch. This approach was used to determine stability of the participant throughout testing. Not all parameters were measured for each participant due to technical issues with devices as well as protocol differences between test sites. Secondly, we examined the reproducibility of the NVC response to VS by comparing ΔMean, ΔPeak and ΔtAUC response magnitudes across all VS trials. This approach was used to determine if there was run-down and/or frequency-dependency in the NVC response to intermittent VS. Finally, using simple linear and advanced hierarchical multiple regression modelling, we assessed for the relative and combined effects of age and sex on the variance explained in each NVC parameter (ΔMean, ΔPeak and ΔtAUC) across each temporal region (acute, mid and late) of the NVC waveform.

Waveforms for $P_{ET}CO_2$, R_R, S_pO_2, HR, MAP (Figure 3), PCAv (as seen in figure(s) 1-3), were developed using a bespoke macro(s) on LabChart, which calculated a mean value for each parameter in pre-determined epochs across a user defined region. This process was repeated for all participants (n=125) across all VS trials (n=5) and averaged providing group-averaged waveform(s).

2.4.6 Statistical Analysis

Statistical analysis was performed using SPSS v.28 statistical software. A paired sample t-test was used to assess for significant differences in $P_{ET}CO_2$, R_R, HR and MAP between baseline and visual stimulation. A non-parametric Wilcoxon signed-rank test was used to assess for significant differences in SpO_2, comparing baseline and the VS period. An independent samples t-test was used to determine sex differences between baseline demographics, where necessary a Mann-Whitney U test was used. In all tests, normal distribution was determined using Shapiro-Wilk assessment and visual inspection of normal Q-Q plots. Due to violations in normal distribution, a non-parametric Friedman test was used to assess for statistical differences in ΔMean, ΔPeak and ΔtAUC NVC magnitude across all trials (V1-V5: Δcm/s, Δcm. s^2 and Δ%) with Wilcoxon signed-rank post-hoc analysis when required. Simple linear regression was performed to determine the relationship between age, cerebral haemodynamics and NVC metrics. A hierarchical multiple regression model was used to determine the relative and combined contribution of age and sex on the variance within each NVC parameter, while controlling for the covariate baseline PCAv. In each instance, the NVC parameter was selected as the dependent variable while age and sex were used as independent variables. Prior to final analysis interpretation, the data was checked against eight assumptions when performing a hierarchical multiple regression: (a) The inclusion of a dependent variable measured on a continuous level (NVC metric), (b) The inclusion of two of more independent variables measured on a continuous (age) and/or nominal (sex) level, (c) There should be independence of observations with no 1st-order autocorrelation, as assessed by the Durbin-Watson statistic, (d) There should be a linear relationship between the dependent variable and the independent variables,

both individually and collectively, (e) Homoscedasticity of residuals should be present, (f) Multicollinearity should not be present, as assessed by visual inspection of correlation coefficients and tolerance/VIF values, (g) No significant outliers, high leverage points or highly influential points. These are determined by visual inspection of the computed studentized deleted residuals, leverage point values and Cook's distance values, respectively, (h) Studentized residuals should be approximately normally distributed. This can be assessed in several ways but mostly through inspection of the computed histogram with normality curve superimposed, visual inspection of the P-P plot and/or inspection of the Normal Q-Q plot of the studentized residuals. Statistical significance was set at $p<0.05$.

2.5 Results & Figures

2.5.1 Examination of cardiorespiratory and autonomic variables in response to visual stimulation

No significant differences were observed for $P_{ET}CO_2$ (t (60) = -0.248, p = 0.805; see figure 2c(ii)) or SpO_2 (Z = -1.202, p = 0.229; see figure 2b(ii)) when comparing baseline and VS measures. However, significant differences were observed for R_R (t(60) = 3.36, P<0.001; see figure 2a(ii)), HR (t(122) = 8.435, P<0.001; see figure 2d(ii)) and MAP (t(114) = -3.452, P<0.001; see figure 2e(ii)). Although statistically significant, the magnitude of the changes from baseline for R_R (-1.9 ± 5.36%), HR (-2.1 ± 2.9%) and MAP (0.78 ± 2.2%) during VS were negligible.

2.5.2 Comparison of ΔMean, ΔPeak and ΔtAUC across repeated visual stimulation trials

No significant differences between trials were found for either parameter of ΔMean NVC response magnitude (Δcm/s; X^2(4) = 2.529, p = 0.639; see figure 3b(i)) and (Δ%; X^2(4) = 3.787, p = 0.436; see figure 3b(ii)). Similarly, no significant differences between trials were found for ΔPeak NVC response magnitude (Δcm/s; X^2(4) = 4.613, p = 0.329; see figure 3c(i)) and (Δ%; X^2(4) = 5.084, p = 0.279; see figure 3c(ii)). In contrast, significant main effects between trials were observed for ΔtAUC response magnitude (Δcm.s^2; X^2(4) = 61.218, P<0.001; see figure 3d (i)) and (Δ%; X^2(4) = 59.101, P>0.001; see figure 3d(ii)). Wilcoxon-rank post-hoc analysis revealed significant differences between V1 and V2-V5 for both Δcm.s^2 and Δ% (P<0.001 for all cases, respectively).

2.5.3 Simple regression analysis on the relationship between age, cerebral haemodynamics and NVC metrics

Linear regression analysis determined the relationship between age and mean NVC response ($F_{(1,123)} = 1.900$, $p = 0.171$, $r = -0.123$; see figure 4b), peak NVC response ($F_{(1,123)} = 6.804$, $p = 0.010$, $r = -0.229$; see figure 4c), tAUC NVC response ($F_{(1,123)} = 0.479$, $p = 0.490$, $r = -0.062$; see figure 4d), acute NVC response ($F_{(1,122)} = 0.022$, $p = 0.881$, $r = -0.014$; see figure 4e), mid NVC response ($F_{(1,123)} = 2.608$, $p = 0.109$, $r = -0.144$; see figure 4f), late NVC response ($F_{(1,123)} = 5.266$, $p = 0.023$, $r = -0.203$; see figure 4g) and baseline PCAv ($F_{(1,123)} = 0.271$, $p = 0.603$, $r = 0.047$; see figure 4a).

2.5.4 Hierarchical multiple regression analysis of the variance within Mean, Peak and tAUC NVC magnitude determined by age and/or sex

Full details of the hierarchical multiple regression analysis can be found in table 2. Age and sex (model 3) had no significant effect on the variance within mean NVC response (Δ%; $F_{(1,121)} = .026$, $p = .873$, respectively). R^2 shows that age contributed to 1.6% of the variance within the mean NVC response, while sex had a 0% effect on the variance explained. Using regression coefficient data (see table 2), a one-year increase in age is associated with a reduction in mean NVC response of .059%. Moreover, changing from male to female is associated with an increase in mean NVC response of .189%. Age and sex (model 3) had no significant effect on the variance within peak NVC response ($F_{(1,121)} = .349$, $p = .556$). R^2 values show that age contributes 5.1% of the variance within peak NVC response, while sex has a 0.3% effect on the variance observed. Based on the regression coefficient data (see table

2), a one-year increase in age is associated with a reduction in peak NVC response by .155%. Moreover, changing from male to female is associated with an increase in peak NVC response of .969%. Age and sex (model 3) had no significant effect on the variance within tAUC NVC response (Δ%; $F_{(1,121)} = .776$, p = .380, respectively). R^2 values demonstrate that age contributed to 0.4% of the variance observed within ΔtAUC NVC response, while sex had a 0.6% effect on the variance. Based on the regression coefficient data (see table 2), a one-year increase in age is associated with a reduction in tAUC NVC response by .024%. Moreover, changing from male to female is associated with an increase in mean NVC response of .84%.

2.5.5 Hierarchical multiple regression analysis of the variance within the acute (0-10s), mid (11-20s) and late (21-30s) NVC magnitude determined by age and/or sex

Age and sex (model 3) had a significant effect on the variance within the acute NVC response ($F_{(1,121)} = 3.983$, p = .048). Based on R^2 values, age is responsible for 0% of the variance within the acute NVC response, while sex has a 3.2% effect on the variance. Based on the regression coefficient data (see table 3), a one-year increase in age is associated with a reduction in acute NVC response by .008%. Moreover, changing from male to female is associated with an increase in acute NVC response of 2.094%. Age and sex (model 3) had no significant effect on the variance within the mid NVC response (Δ%; $F_{(1,121)} = .506$, p = .478). Age contributed to 2.1% of the variance within the mid NVC response, while sex had a 0.4% effect on the variance. Based on the regression coefficient data (see table 3), a one-year increase in age is associated with a reduction in mid NVC response by .086%. Moreover, changing from male to female is associated with a decrease of 1.03%

within the mid NVC response. Age and sex (model 3) had no significant effect on the late NVC response ($F_{(1,121)}$ = .138, p = .711). Age contributes 4.1% of the variance within late NVC response, while sex has a 0.1% effect on the variance observed. Based on the regression coefficient data (see table 3), a one-year increase in age is associated with a reduction in Δlate NVC response by .111%. Moreover, changing from male to female is associated with a decrease in Δlate NVC response of .492%.

	Male (n=41)	Female (n=84)	p-value
Age (yrs)	34.90 ± 12.53	39.08 ± 12.11	0.079
Height (m)	1.80 ± 0.07	1.66 ± 0.06***	<0.001
Weight (kg)	84.35 ± 14.23	69.63 ± 14.67***	<0.001
BMI (kg/m^2)	25.84 ± 3.43	25.23 ± 5.18	0.075
PCAv (cm/s)	32.84 ± 5.94	35.52 ± 7.83	0.100
$P_{ET}CO_2$ (mmHg)	37.32 ± 1.95	34.75 ± 3.32*	0.049
R_R ($breaths.min^{-1}$)	14.15 ± 3.42	15.62 ± 3.83	0.588
S_pO_2 (%)	95.31 ± 3.07	97.72 ± 3.11**	0.009
HR ($beats.min^{-1}$)	70.73 ± 12.17	74.48 ± 9.80	0.152
MAP (mmHg)	90.89 ± 16.27	96.82 ± 15.53	0.015

Table 1. Participant demographics. Baseline measures of age (yrs), height (m), weight (kg), body mass index (kg/m^2), posterior cerebral artery velocity (PCAv; cm/s), pressure of end-tidal carbon dioxide ($P_{ET}CO_2$; mmHg), respiratory rate (R_R; $breaths.min^{-1}$), peripheral oxygen saturation (SpO_2; %) heart rate (HR; $beats.min^{-1}$) and mean arterial pressure (MAP; mmHg) are presented. Measures are presented according to sex (male/female) * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ vs male cohort. Values are presented as mean ± SD.

		Model 1		Model 2		Model 3	
	Variable	B	β	B	β	B	β
Mean response (Δ%)	Constant	15.152***		17.205***		16.996***	
	Baseline PCAv	-.027	-.033	-.022	-.028	-.024	-.030
	Age			-.059	-.122	-.060	-.124
	Sex					.189	.015
	R^2	.001		.016		.016	
	F	.138		.991		.664	
	ΔR^2	.001		.015		.000	
	Δ*F*	.138		1.843		.026	
Peak response (Δ%)	Constant	31.056***		36.459***		35.388***	
	Baseline PCAv	-.099	-.086	-.087	-.075	-.097	-.084
	Age			-.155*	-.225	-.160*	-.233
	Sex					.969	.053
	R^2	.007		.058		.061	
	F	.918		3.762*		.349	
	ΔR^2	.007		.051		.003	
	Δ*F*	.918		6.565*		2.611	
tAUC response (Δ%)	Constant	13.325***		14.152***		13.223***	
	Baseline PCAv	-.020	-.031	-.018	-.028	-.027	-.041
	Age			-.024	-.061	-.028	-.073
	Sex					.840	.082
	R^2	.001		.005		.011	
	F	.120		.286		.449	
	ΔR^2	.001		.004		.006	
	Δ*F*	.120		.453		.776	
T2p NVC response (Δs)	Constant	9.756***		12.257***		15.513***	
	Baseline PCAv	.113*	.193	.119*	.203	.149	.253
	Age			-.072*	-0.204	-.055*	-.156
	Sex					-2.947***	-.318
	R^2	.037		.079		.179	
	F	4.754*		5.221*		8.548***	

ΔR^2	.037	.042	.096
ΔF	4.754*	5.513*	14.084***

Table 2. Hierarchical Multiple Regression analysing the contribution of age and sex to the variance within NVC metrics. Mean NVC response (Δ%), Peak NVC response (Δ%), tAUC NVC response (Δ%) are presented within the left column as well as time the peak NVC response (T2p: Δs). The degree to which baseline PCAv (covariate variable) contributes to the variance in each metric is provided, along with the individual and combined effects of age and sex. R^2 refers to the variation within the dependent variable (NVC metric) explained by the independent variable (age and sex). Model coefficients are provided (B and β) which denote the change within the dependent variable following a single unit change within the independent variable. Note: n=125.

		Model 1		Model 2		Model 3	
	Variable	B	β	B	β	B	β
Acute NVC response (Δ%)	Constant	13.336***		13.054***		10.740***	
	Baseline PCAv	-.033	-.046	-.034	-.047	-.055	-.076
	Age			.008	.019	-.004	-.009
	Sex					2.094	.183
	R^2	.002		.002		.034	
	F	.260		.150		1.430	
	ΔR^2	.002		.000		.032	
	Δ*F*	.260		.042		3.983*	
Mid NVC response (Δ%)	Constant	16.595***		19.598***		20.742***	
	Baseline PCAv	-.010	-.010	-.004	-.003	.007	.007
	Age			-.086	-0.144	-.80	-.134
	Sex					-1.035	-.066
	R^2	.000		.021		.025	
	F	.013		1.295		1.029	
	ΔR^2	.000		.021		.004	
	Δ*F*	.013		2.577		.506	
Late NVC response (Δ%)	Constant	14.472***		18.363***		18.907***	
	Baseline PCAv	-.002	-.003	.006	.007	.007	.007
	Age			-.111*	-0.203	-.109*	-.198
	Sex					-.492	-.034
	R^2	.000		.041		.042	
	F	.001		2.615		1.777	
	ΔR^2	.000		.041		.001	
	Δ*F*	.001		5.230*		.138	

Table 3. Hierarchical Multiple Regression analysing the contribution of age and sex to the variance within acute, mid and late NVC metrics. Acute NVC response (0-10s; Δ%), Mid NVC response (11-20s; Δ%) and late NVC response (21-30s; Δ%) are

presented within the left column. The degree to which baseline PCAv (covariate variable) contributes to the variance in each metric is provided, along with the individual and combined effects of age and sex. R^2 refers to the variation within the dependent variable (NVC metric) explained by the independent variable (age and sex). Model coefficients are provided (B and β) which denote the change within the dependent variable following a single unit change within the independent variable. Note: n=125.

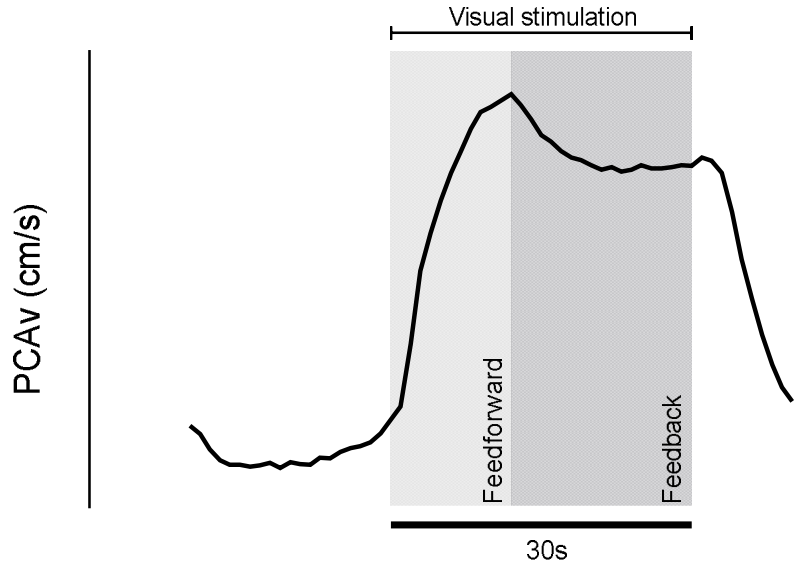

Figure 1. Group averaged PCAv waveform during visual stimulation showcasing the biphasic neurovascular coupling response. This waveform demonstrates the change in PCAv in response to (1) visual stimulation and (2) removal of visual stimulus. The waveform was developed from averaged values across our entire sample population (n=125; n=625 visual stimulation trials). This figure is informed by Hoiland et al., (2020) and Iadecola et al., (2017), highlighting the relative contribution of both feedforward and feedback mechanisms across various time domains of the NVC response. The initial rise in PCAv during visual stimulation is hypothesized as neural feedforward signalling pathways. In contrast, feedback metabolic signalling is thought to contribute to the later sustained elevation in PCAv.

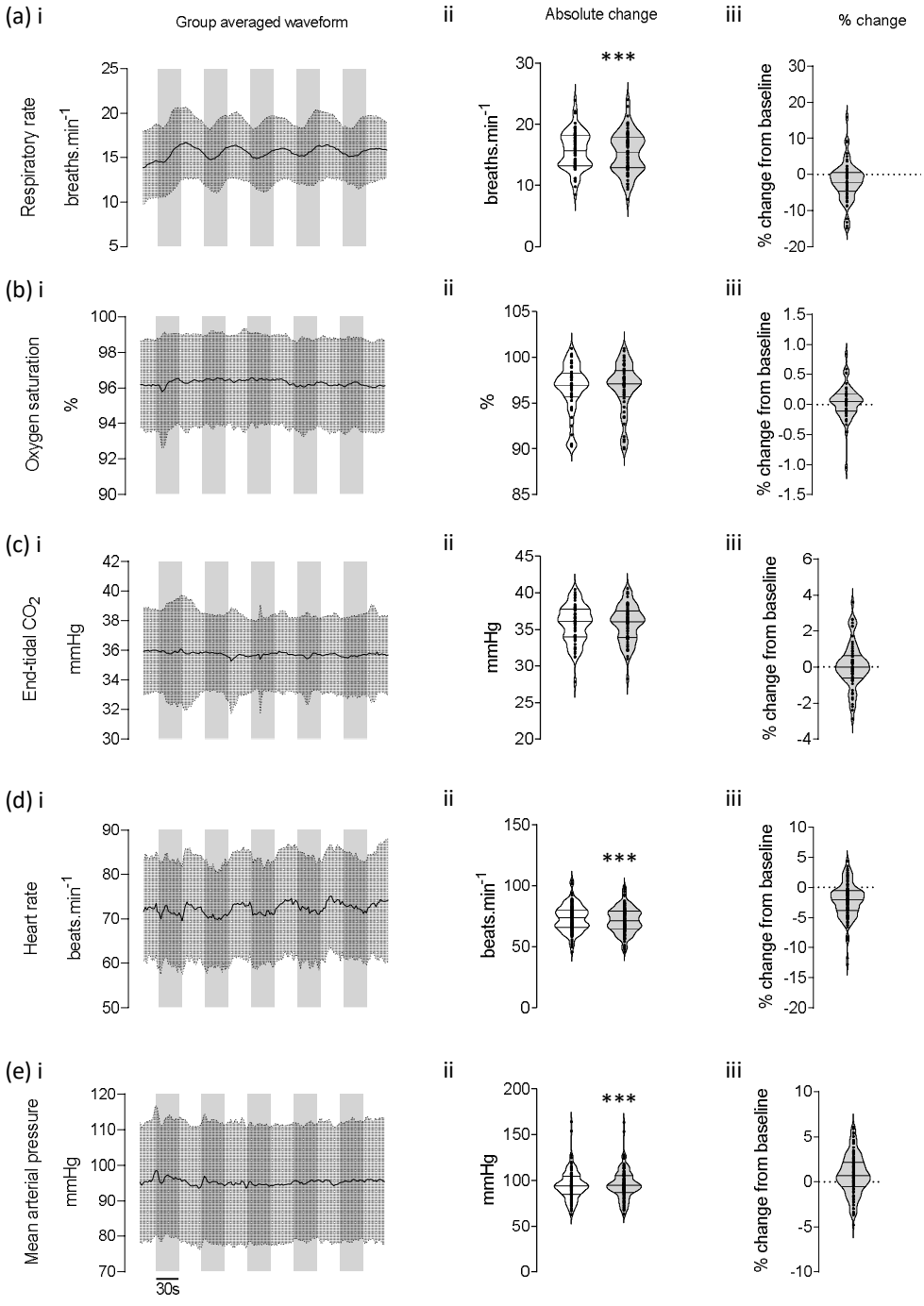

Figure 2. Cardiorespiratory and autonomic variables during baseline and visual stimulation. Total group (n=63 participants) averages for respiratory rate (R_R; breaths.min^{-1}; ai-iii), peripheral oxygen saturation (S_pO_2; %; bi-iii), the partial pressure of end-tidal

CO_2 ($P_{ET}CO_2$; mmHg; ci-iii), heart rate (HR; beats.min^{-1}; di-iii; n=125) and mean arterial pressure (MAP; mmHg; ei-iii; n=125) are provided. Variable averages were calculated and plotted every two-seconds for each participant to provide an average variable waveform for the entire sample population across each visual stimulus (left column) with grey bins representing thirty-second visual stimulation trials. Respective absolute changes (middle column) and relative percentage changes (right column) between baseline and visual stimulation are provided for each variable with baseline and visual stimulation values presented in clear and light-grey boxes, respectively. Data are presented as violin plots showcasing the interquartile range, median, upper and lower limits. *** = $P<0.001$ from baseline. ± SD of the group-averaged waveform is presented as the orange shaded region.

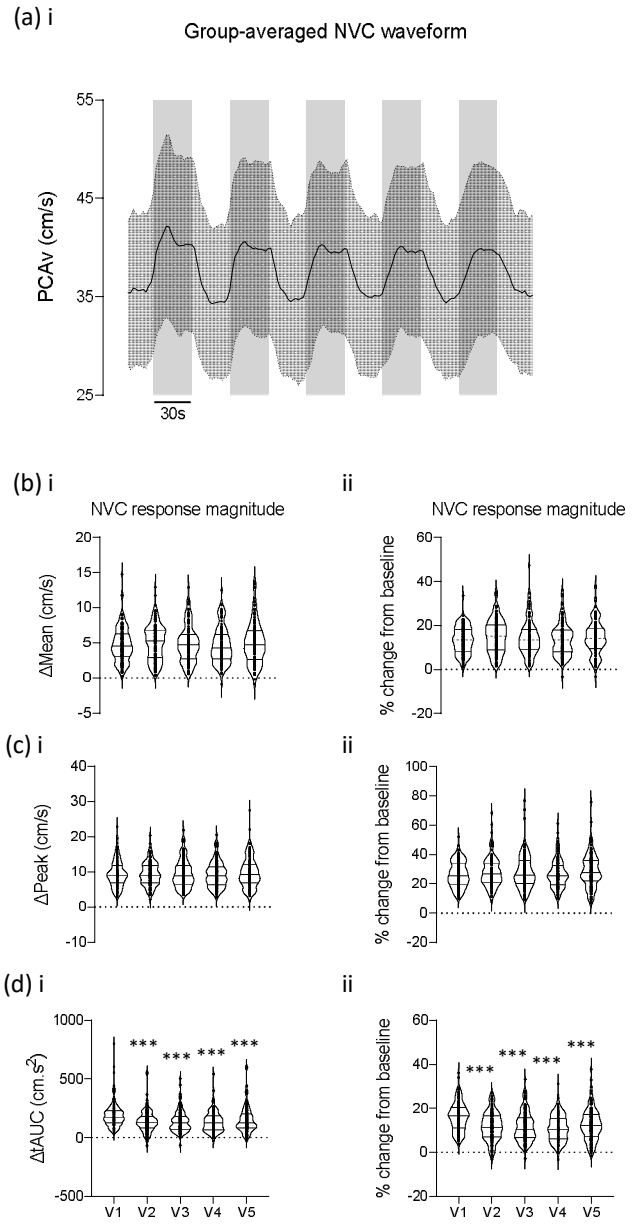

Figure 3. NVC response magnitude across repeated visual stimulation trials. This waveform showcases the group averaged NVC waveform during repeated visual stimulation (a). ΔMean (b(i-ii)), ΔPeak (c(i-ii)) and ΔtAUC (d(i-ii)) NVC response magnitudes across each visual trial are presented (clear boxes). Respective absolute changes for PCAv and tAUC (Δcm/s and $\Delta cm.s^2$, respectively) are presented along the left column whereas the relative percentage change (%) from baseline are presented in

the right column. Data are presented as violin plots showcasing the interquartile range, median, upper and lower limits. *** = P<0.001 from V1. ± SD of the group-averaged waveform is presented as the orange shaded region.

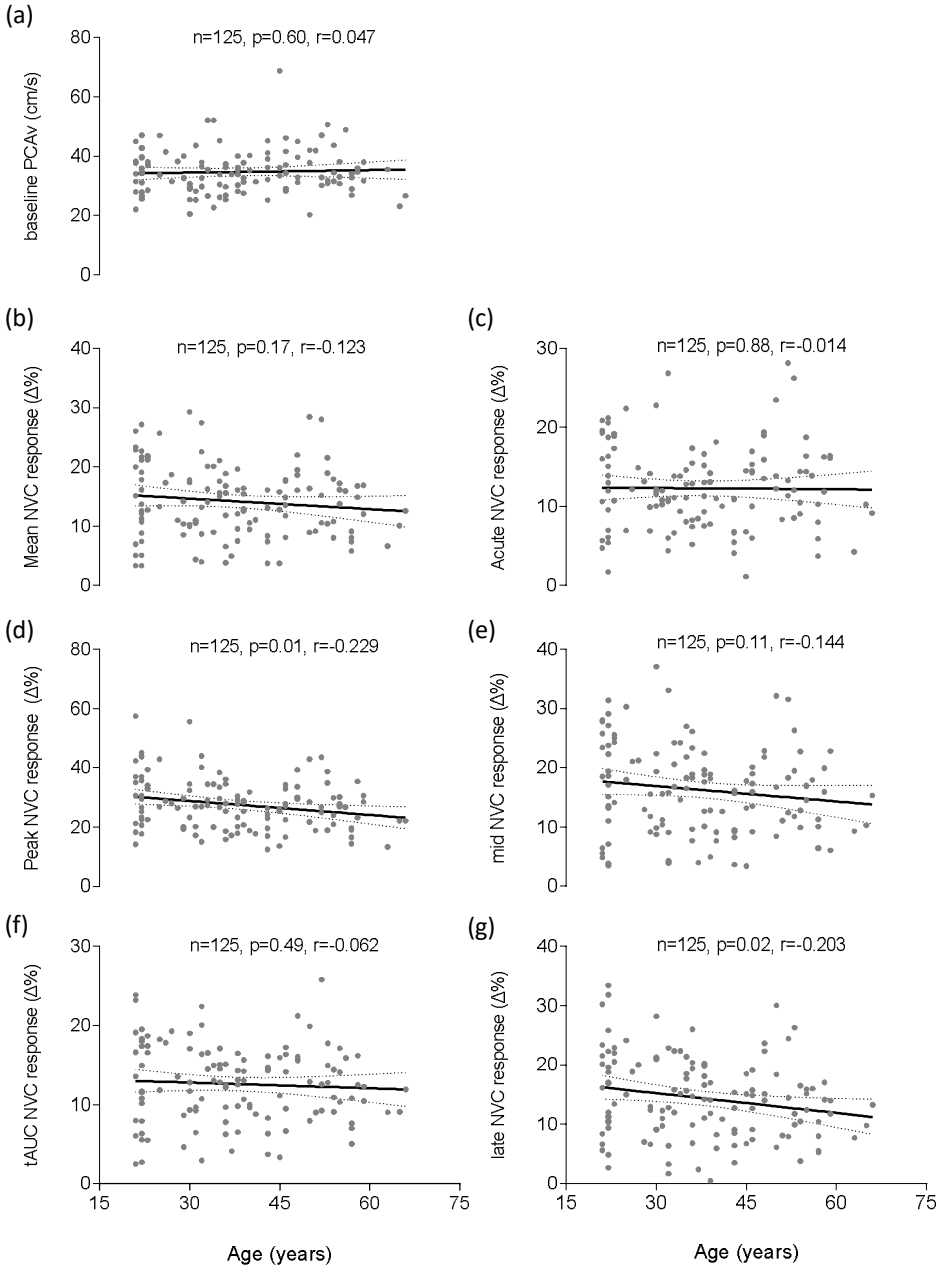

Figure 4. Simple linear regression analysis demonstrating the relationship between age, cerebral haemodynamics and NVC metrics. Linear regressions are presented for the relationship age and (a) baseline PCAv, (b) mean NVC response, (c) acute NVC response, (d) peak NVC response, (e) mid NVC response, (f) tAUC NVC response and (g) late NVC response. NVC metrics are presented as the % change from baseline along the y-xis. Age (years) is presented along the x-axis. Text boxes

imposed on each graph include sample size (n), p-value and Pearson correlation coefficient (r).

2.6 Discussion

2.6.1 Primary Outcomes

The main findings of this study are 1) in healthy participants, aged 21-66yrs, approximately 0-6.1% of the variance observed across several NVC response magnitude metrics is attributable to a combination of age and sex, with implications for the composite response and the distinct temporal domains of the NVC response; and 2) neither age nor sex are useful predictor variables for NVC magnitude.

2.6.2 Demographics

A full breakdown of participant demographics can be found in table 1. There was a small, but significant reduction in baseline $P_{ET}CO_2$ between sexes. Of interest, previously published work from our group has shown graded hyperventilation-induced hypocapnia of a greater magnitude than that seen in the current study has little effect on NVC response magnitude (Bader et al., 2021). There was no observed age-related effect for baseline measurements of PCAv (see figure 4(a)). This finding contrasts with several studies which demonstrate age-dependent reductions in baseline cerebral velocity (Beishon et al., 2019; Nowak-Fluck et al., 2018; Madureira et al., 2017; Stefanidis et al., 2019; Rosengarten et al., 2003; Niehaus et al., 2001; Akif-Topcuoglu et al., 2009). The rationale for age-dependent cerebral hypoperfusion typically relates to increased vascular resistance, cerebral atrophy and subsequent reductions in basal cerebral metabolism.

One possible explanation for the conflicting observation made in this study compared with others is that cerebral atrophy has been shown to be region-specific

to areas of the cerebellum, caudate, hippocampus and associated areas with minimal atrophy observed in primary visual cortex, a territory supplied by the PCA (Raz et al., 2005). Therefore, indices of cerebral hypoperfusion could be sensitive to vessel insonation, with more pronounced hypoperfusion observed in anterior region(s) of the brain, supplied by the MCA. Therefore, the absence of age-dependent cerebral hypoperfusion in our study could be the net effect of maintained cerebral tissue density within the occipital lobe. Furthermore, there is also the inherent possibility that lifestyle and exercise status might have influenced our findings and the findings of others in the field. Ainslie & colleagues (2008) found that increased aerobic fitness had the capacity to attenuate the age-dependent decline in baseline MCAv in males aged 18-79yrs. Future studies should control for aerobic fitness when making between-group comparisons, with respect to age. Finally, cerebral hypoperfusion might be evident in age groups outside the upper age-limit recruited within our sample population (>66yrs).

2.6.3 Age-related effects on NVC

Contrary to our hypothesis, our results revealed that the variance in NVC response magnitude, measured through the PCA in response to VS, was not explained by advanced aging. Simple linear regression analysis demonstrated weak Pearson correlations between each of our NVC metrics and age (see figure 4). This observation was consistent across multiple NVC parameters (ΔMean, ΔPeak and ΔtAUC), for both the entirety of the NVC response and across distinct temporal domains of the NVC waveform (acute/mid/late response magnitude). As shown within tables 2/3, less than 5% of the variance in each NVC parameter was attributed

to age, except peak NVC response (5.1%). This demonstrates the limited capacity of age as a predictor variable for NVC response magnitude and questions the causal role of aging within NVC impairment. As shown within the regression coefficient data (see tables 2 & 3), progressive aging is associated with negligible changes in the magnitude of each NVC metric.

Given the noted disparity in the findings within age-related NVC research, it is important to draw comparisons between our findings and those within the published literature that have employed similar methodological approaches, specifically the use of TCD. Our findings directly contradict several studies that demonstrate age-related decline in the NVC response (Fluck et al., 2014; Nowak-Fluck et al., 2018; Zaletel et al., 2005; Niehaus et al., 2001; Akif-Topcuoglu et al., 2009). In contrast, we extend the views of several other studies that have demonstrated no measurable age-related effect on NVC (Madureira et al., 2017; Sorond et al., 2008; Stefanidis et al., 2019; Rosengarten et al., 2003; Panczel et al., 1999). Adding further to these apparent contradictory findings, a greater NVC response in older compared with younger participants has also been observed (Beishon et al., 2019).

Several methodological aspects are consistent in the literature cited above. Each study recruited from an apparently healthy sample population, free of any cardiovascular, cerebrovascular, and neurological risk factors. The majority focused on NVC assessment through the PCA (Fluck et al., 2014; Nowak-Fluck et al., 2018; Rosengarten et al., 2003; Niehaus et al., 2001; Akif-Topcuoglu et al., 2009; Panczel et al., 1999) by way of multiple visual paradigm(s): checkerboard stimulus, visual search tests, reading tasks and strobe light stimuli. A subset of studies examined NVC through the MCA via frontal-lobe specific cognitive assessment (Beishon et al., 2019; Madureira et al., 2017). Each study incorporated a similar experimental

protocol with an initial baseline period of quiet rest prior to a time dependent NVC assessment encompassing interchangeable periods of stimulus "on/off". Given the inter-study similarities in both experimental set-up and sample groups, the disparity in results is concerning.

Perhaps the discrepancy lies in the minutiae of the TCD application and NVC assessment tool. The PCA can be subdivided into two segments, the P1 and more distal P2. Panczel and colleagues' (1999) elegant work was one of the first to describe the effects of vessel segmentation and task complexity on the NVC response. Their findings showed that NVC response, through the PCA vessel, positively correlated with increased task complexity. Furthermore, their results showed that NVC response through the P2 segment of the PCA was greater than that of the P1 segment. Therefore, perhaps the age-dependent impairment of NVC is related to task complexity and vessel segment insonation, which might explain the notable disparity between studies.

Aging is associated with both structural and physiological changes within the neurovascular unit, which likely play a leading role in NVC impairment (Toth et al., 2017). Growing evidence now supports the causal role for oxidative stress, endothelial and astrocytic dysfunction in age-related neurovascular uncoupling (Tarantini et al., 2015, 2017a, 2017b 2018; Park et al., 2007; Toth et al., 2017). Targeted antioxidative and endothelial treatment therapies have been shown to restore NVC, despite progressive aging (Tarantini et al., 2018; Park et al., 2007; Toth et al., 2014). This supports the growing consensus that physiological maladies, more so than chronological age, act as key drivers of age-related NVC impairment. This view is partially exemplified in human studies by Groschel and colleagues (2007) who found the presence of cardiovascular-atherosclerotic risk factors to be a key

determinant in age-related NVC impairment, compared with age-matched healthy controls. The degree to which each of these physiological stressor's manifests throughout aging varies greatly and thus, variability ensues. With this in mind, we suggest that future research should take into consideration auxiliary indices of redox status and vascular health during the participant recruitment process to help delineate the contributing effects of these stressors on NVC impairment observed in older age groups.

The NVC response involves a concert of feedforward and feedback pathways which operate in a time-dependent manner and coalesce to provide the net haemodynamic response to regional cerebral activation (Hosford & Gourine, 2019; Iadecola, C. 2017; Hoiland et al., 2020). Pioneering work by Hoiland et al., (2020) showed that non-selective nitric oxide synthase (NOS) inhibition decreased peak PCAv response to VS by approximately 20-30% with no effect on the overall mean PCAv response. This provides promising evidence for the role of neural feedforward signalling as a key mediator in the initial peak NVC response, through NO-cGMP-mediated vasodilation, and supports the concept that certain domains of the NVC response can be attributed to specific mechanistic pathways. Whether age-related disruption of these distinct pathways' manifests in downstream NVC temporal-magnitude impairments is unknown. We attempted to discern the extent to which variation in response magnitude across each distinct temporal region can be attributed to age. Overall, age provided limited predictor capacity for NVC response across each distinct temporal region. Approximately 0%, 2.1% and 4.1% of the variance within acute, mid and late NVC response magnitudes, respectively, was attributable to age.

2.6.4 Sex-related research on NVC

Our results reveal that less than 1% of the variance within the NVC response is attributable to sex. This observation was consistent across multiple NVC parameters (ΔMean, ΔPeak and ΔtAUC; see table 2), for both the entirety of the NVC response and across distinct temporal domains of the NVC waveform (acute/mid/late response magnitude; see table 3). The only exception to this observation was a 3.2% R^2 value within the acute NVC response magnitude. Like aging, this finding does not support the narrative that sex affects NVC response magnitude; in contrast, it suggests that NVC operates independent of sex-interaction.

Circulating sex hormones, and their influence on cerebrovascular function, are posited as the underlying mediators driving sex-related differences in CBF regulation (Robison et al., 2019; Krause et al., 2006). There is a significant dearth of literature with respect to the extent of sexual dimorphism on NVC (Duque et al., 2017). Within the available literature, which incorporated similar methodological approaches and sample populations, there are studies that conclude that NVC response magnitude is similar between males and females (Madureira et al., 2017; Akif-Topcuoglu et al., 2009). Both studies utilised TCD for NVC assessment through the MCA (Madureira et al., 2017) and PCA (Akif-Topcuoglu et al., 2009) in response to cognitive tasks and checkerboard stimulation, respectively.

Similar to the age-related observation, we found that the variance in response magnitude across each distinct temporal region was not attributable to sex; 3.2%, 0.4% and 0.1% of the variance in response magnitude within the acute, mid and late response respectively is attributed to sex (see table 3). Of note, hierarchical regression modelling revealed that sex is responsible for approximately 9.6% of the variance within T2p NVC performance. The finding of sex-induced differences in

reduced latency to peak NVC response is novel and interesting. It suggests a more rapid signal integration, from upstream visual stimulus to downstream haemodynamic response, within females. A possible explanation for decreased time to peak NVC response is the reported differences in neural network connectivity between males and females (Gong et al., 2011).

2.6.5 Limitations and future research

Neither circulating female sex hormones or menopausal status were controlled within this investigation. Therefore, we are unable to draw definitive conclusions regarding the potential modulatory role of these on NVC response. There is currently no evidence to suggest an interaction between menstrual cycle and/or menopausal status and NVC magnitude. Pilot data (n=9) employing a similar methodology to that of this study observed no difference in NVC magnitude across the early follicular and mid-luteal phase of the menstrual cycle (Davenport et al., 2015). More encompassing methodologies and extensive research studies are required to accurately determine the role of circulating female sex hormones on NVC magnitude. These studies should include repeated NVC measurements across multiple cycles to account for any intra-individual phase variability in cycle hormonal levels. Herein, the large sample size of female participants (n=84) mitigates potential variability in NVC magnitude associated with ovarian status, although this observation requires further investigation and validation which is beyond the scope of this study.

Prior to this study, there was notable uncertainty in the understanding of age-related effects on neurovascular coupling. Variability in methodological approaches

used to elicit and analyse the NVC waveform, in tandem with the inherent physiological variability between sample populations, likely adds to disparity and limits the capacity to derive concrete conclusions. The upper age limit (66 yrs.) within our sample population is relatively young, compared with other investigations. The observations made herein cannot be extended to older age groups. However, recruitment of participants within older age groups (>70 yrs.) who are free of any confounding ailments and/or physiological maladies is difficult. The field would benefit from the development of comprehensive prerequisite criteria for participant recruitment, complementary physiological measures, and analytical approaches.

2.6.6 Conclusion

In conclusion, our results demonstrate that neither healthy aging nor sex influence the variation observed within the PCA NVC response to VS, in healthy participants aged 21-66yrs. This study supports the argument that NVC impairment is not age-related *per se,* but may be related, when observed, to morbidity which manifest with aging. Ergo, in the absence of age-associated morbidity, NVC response magnitude remains unperturbed, despite progressive aging. Our findings are relevant and useful for smaller, field-based studies examining cerebrovascular function, wherein heterogeneity within the sample population is often observed for various logistical reasons. Our protocol revealed robust, reliable, and reproducible NVC responses that were equivalent in healthy younger and older male and female participants. This study provides useful normative values for future research exploring NVC response magnitude in healthy participants.

2.6.7 Competing Interests

No competing interests to declare.

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2.6.9 Author(s) contribution

All data collection and acquisition were performed at UCC and MRU by JL, EJ, LL, DM, MB, GS, and AL. Data analysis was performed by JL, JAM and EFL. Data interpretation and drafting the work or revising it critically for important intellectual content was completed by JL, DS, NS, TD and KOH. All authors approved the final version of this manuscript and agree to be accountable for all aspects of the manuscript formation and data analysis relating to accuracy and integrity. All persons designated as authors on this manuscript qualify to do so.

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Chapter 3. Neurovascular Coupling Remains Intact During Incremental Ascent to High Altitude (4240m) in Acclimatized Healthy Volunteers

The following chapter is an adaptation of our published manuscript within *Frontiers in Physiology*. Data and statistical analyses were modified for consistency with other chapters in the thesis. I completed data collection throughout ascent. I performed all data analysis and developed relevant figures and tables. Finally, I developed and completed the published manuscript.

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3.1 Abstract

Neurovascular coupling (NVC) is the temporal link between neuronal metabolic activity and regional cerebral blood flow. NVC ensures adequate delivery of nutrients to active regions within the brain. Exposure to high altitude (HA) imparts a multimodal stress which might threaten NVC function through modulation of cerebrovascular tone. The aim of this study was to assess whether incremental ascent to HA affects NVC response magnitude. 10 healthy study participants (4 males, 21.7 ± 1.3yrs, 23.57 ± 2.00kg/m^2, mean ± SD) were recruited as part of a research expedition to the Nepal Himalaya. Measurements of resting posterior cerebral artery velocity (PCAv), NVC and arterial blood draws were performed at Calgary (1130m), Namche (3440m), Debuche (3820m) and Pheriche (4240m). PCAv was measured using transcranial Doppler ultrasound (TCD). Arterial blood draws were sampled from the radial artery and analysed using a portable blood gas analyser. NVC was elicited using an intermittent visual stimulus (VS; Strobe light; 6Hz; 30sec on/off x 3 trials). NVC was indexed as the mean (Δmean) and peak (Δpeak) change in PCAv during VS. In addition, deflections in total area under the curve (ΔtAUC) were used as a measure of NVC. As expected, PaO_2, SaO_2 and $PaCO_2$ were significantly decreased at 3440m, 3820m and 4240m. Arterial pH was statistically unchanged at HA relative to Calgary owing to significant reductions in arterial bicarbonate ($P<0.043$). No significant differences were found for Δmean, Δpeak or ΔtAUC between locations ($P>0.05$). NVC remains intact during incremental ascent to HA (4240m) in healthy acclimatized individuals.

3.2 Introduction

3.2.1 Physiological response to high altitude

High altitude (HA) exposure is a pernicious environment which requires a multimodal physiological adaptation to preserve function and survival. Environmental stressors affixed with HA exposure can include, but are not limited to, severe winds, cold temperatures and decreases in total barometric pressure. Owing to reductions in total barometric pressure, we observe a decrease in total ambient oxygen availability, termed hypobaric hypoxia. Hypobaric hypoxia initiates a cascade of time- and severity-dependent adaptations of the respiratory and renal systems. Routinely observed adaptations include the hypoxic ventilatory response (HVR), HVR-induced hypocapnia and subsequent acid-base disturbance. These stressors require several physiological adaptations to preserve homeostasis. Most noticeably we see an increase in the secretion rate of bicarbonate from within the renal system. This compensatory response acts to restore pH levels within normal limits. This dynamic relationship between stress and adaptation has inspired scientific research for generations into the exact mechanisms and pathways responsible for maintaining homeostasis and health at HA.

3.2.2 Importance of sustained cerebral perfusion

Under normal circumstances the human brain accounts for only 2% of total body weight, and yet is responsible for 20% of total energy consumption within the body (Attwell et al., 2011; Seng-Shu et al., 2011; Subudhi et al., 2014). Brain tissue possesses a high resting metabolic rate (RMR), with limited capacity for glycogen

storage (Willie et al., 2014; Brown & Ransom, 2007). Consequently, the cerebrovasculature requires tight, narrow control of cerebral blood flow (CBF) for the delivery of nutrients and removal of waste metabolites (Drake & Iadecola, 2006; Lecrux & Hamel, 2011). The brain maintains adequate CBF during instances of increased neuronal metabolic demand due to high vascularisation and a sophisticated regulation of regional cerebral blood flow (rCBF) (Phillips et al., 2016).

3.2.3 Cerebrovascular response to high altitude

Regarding the cerebrovasculature, many studies have shown that acute exposure to HA induces an increase in global cerebral blood flow (gCBF), with values returning to sea-level during acclimatisation (Severinghaus et al., 1966; Lucas et al., 2011; Moller et al., 2002). This response stems from the vasodilatory effect of hypoxia below a certain threshold (<40-45 mmHg) (Ainslie & Ogoh, 2009; Brugniaux et al., 2007; Fluck et al., 2015; Willie et al., 2014). The precise mechanisms which drive hypoxia-induced vasodilation are not completely understood, but recent evidence suggests and erythrocyte-mediated nitric oxide pathway. The increase in gCBF is crucial to maintain sufficient cerebral oxygen delivery (CDO_2) at HA (Hoiland et al., 2015). CDO_2 is a product of gCBF and arterial oxygen content (Subudhi et al., 2014). gCBF increases during exposure to HA to compensate for the decrease in PaO_2, maintaining CDO_2.

3.2.4 Neurovascular coupling overview

Although research into the effects of HA on CBF is comprehensive, the impact of ascent to HA on the control of CBF, per se, is less well understood. CBF is controlled via three distinct mechanisms: cerebrovascular reactivity to alterations in blood gases; cerebral autoregulation in response to changes in perfusion pressure; and neurovascular coupling (NVC). NVC pertains to the tight regulation between local neuronal activity and increased regional CBF (rCBF). NVC is controlled via the interplay between astrocytes, neurons and microvessels within the cerebrovasculature (endothelial cells and pericytes) (Girouard & Iadecola, 2006; Filosa et al., 2016; Venkat et al., 2016). Each of these work in concert with one another by eliciting a vasoactive effect on local microvessels within the cerebrovasculature. A validated method of evoking an NVC response is via visual stimulation (VS) of the occipital lobe (Phillips et al., 2016). The resultant NVC response can be monitored by assessment of the posterior cerebral artery (PCA) using a transcranial doppler ultrasound (TCD) (Willie et al., 2011). TCD provides beat-by-beat changes in cerebral blood velocity among other parameters to assess the signal-flow coupling relationship. A dysfunctional NVC response has been observed in several pathological conditions such as stroke, Alzheimer's disease, hypertension, unhealthy aging and spinal-cord injury (Girouard & Iadecola, 2006; Phillips et al., 2016).

3.2.5 High altitude exposure and NVC

Acclimatization to HA and its influence on NVC is fascinating due to contrasting vasoactive stimuli present. Exposure to hypoxia results in hypoxic

vasodilation. However, hypoxia itself triggers the hypoxic ventilatory response (HVR). The HVR manifests increased ventilatory drive raising PaO_2 levels with concomitant decreases in $PaCO_2$ (hypocapnia). The resultant hypocapnia is a potent vasoconstrictor. The cerebrovasculature is particularly sensitive to changes in arterial blood gases, especially $PaCO_2$ (Brugniaux et al., 2007; Ainslie & Ogoh, 2009). Previous literature states that as little as a 1 mmHg increase or decrease from normal $PaCO_2$ levels results in a 3-6% increase or 1-3% decrease in gCBF, respectively (Phillips et al., 2016; Willie et al., 2014). Whether one of these competing stressors elicits a dominant effect on vessel reactivity to stimulus at HA is intriguing as laboratory-based research has shown that hypocapnia reduces NVC response magnitude (Szabo et al., 2011).

3.2.6 Current relevant literature, study aims and hypothesis

The impact of incremental ascent to HA on NVC remains unclear. To our knowledge, only one other study has directly investigated the effect of HA on the NVC response (Caldwell et al., 2017). Caldwell and colleagues observed no significant effects of HA on the NVC response, comparing sea-level measures for NVC to days three and seven at 3800m. We expanded on this previous literature by way of an incremental ascent profile to a higher altitude (4240m). We investigated the effects of four progressively increasing altitudes on the NVC response making direct comparisons between altitudes, within individuals. In addition, we incorporated arterial blood draws within our expedition. This would allow characterisation of arterial blood gases and acid-base status during ascent indexing acclimatisation status. Given that cerebrovascular tone is superiorly sensitive to

changes in $PaCO_2$ compared with PaO_2, we hypothesized that the vasoconstrictive effect of hypocapnia would reduce the NVC response with ascent.

3.3 Materials & Methodology

3.3.1 Participants and Ethical Approval

10 healthy participants (4 males, 21.7 ± 1.3yrs, 23.57 ± 2.00kg/m^2, mean ± SD) were recruited as part of an international research expedition to Everest base camp in the Nepal Himalaya. The expedition was considered a low-risk ascent profile (Zuckerman, 2012; see Figure 1 for ascent profile) consequent to the altitude-differential between rest sites and the rate of ascent. All participants reported no prior history of cardiovascular, respiratory or cerebrovascular disease. Nine subjects were current residents of Calgary (1130m). One subject was an Irish national (0m; sea-level) who had been living in Calgary for five months prior to the research expedition. Several commercially available medications were taken during ascent. Pharmacotherapy were predominantly used as a method of curtailing symptoms associated with exposure to HA (headache, diarrhea, cough, flu-like symptoms). The medications used were: Immodium, Ibuprofen, Neocitran, Advil, Pepto-Bismol and DM expectant syrup. Several of the female participants were taking oral contraception (Yasmin and Tricylen-lo) during the expedition. Diamox (Acetazolamide) was not taken by any participant during the ascent.

This study abided by the Canadian Government Tri-Council policy on research ethics with human participants (TCPS2) and conformed with the standards set by the latest revision of the Declaration of Helsinki, except for registration in a

database. Ethical approval was received in advance through the Mount Royal University Human Research Ethics Board (Protocols 100012 and 100922) and was harmonized with the Nepal Health Research Council (Protocol 109-2017). All participants were recruited via verbal communication and provided written and informed consent prior to voluntary participation in the study. Although this study took place in the context of a large research expedition to altitude, the study design, core research question and data collection were determined *a priori*.

3.3.2 Physiological measures

PCAv and NVC were assessed and later compared between Calgary (1130m), Namche (3440m), Debuche (3820m) and Pheriche (4240m). Within this manuscript, test sites are presented as their respective altitudes within figures and tables. Arterial blood gases (ABGs; PaO_2, SaO_2, $PaCO_2$, arterial bicarbonate [HCO_3^-], base excess and arterial pH) were collected at the same test locations on the same day as NVC assessment. ABGs were predominantly collected at 1130m (n=8). However, two participants had their baseline ABGs taken at Kathmandu (1400m) due to logistical constraints. Ergo, when detailing changes in ABGs throughout this manuscript we have referred to the baseline altitude as 1130m/1400m. In contrast, baseline PCAv and NVC data was recorded entirely at 1130m.

Initial baseline measurements were performed over a three-day period at the Human Physiology Laboratory, Mount Royal University, Calgary, Canada. Following baseline, participants flew to Kathmandu (1400m) where they spent a total of three days. Participants flew from Kathmandu to Lukla (2800m) to begin the research expedition. Several daily measures were obtained each morning beginning

in Kathmandu and continued for the duration of the expedition. The measures collected each morning were: heart rate (HR), arterial blood pressure (Systolic; SBP, Diastolic; DBP), partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$), peripheral oxyhaemoglobin saturation (SpO_2), haemoglobin [Hb] and haematocrit (Hct) (see Table 1).

During ascent, two nights were spent at each test day altitude having trekked between locations (see figure 1). ABGs, PCAv and NVC were measured on the second day at each altitude. This was implemented to mitigate the effects of trekking fatigue on each variable. Participants were tested sequentially in an identical fashion at each location to mitigate the possible effects of circadian rhythm on cerebral haemodynamics.

3.3.3 Instrumentation

Each morning (06:00-08:00), non-invasive fasted daily measures were taken including weight (digital scale; Omron, model OMRHBF514C), haemoglobin concentration ([Hb]; haemoglobinometer; Hemocue HB201+) and haematocrit (hct; hearinized capillary tube, mini-centrifuge; StatSpin, CritSpin microhemaotocrit system, Model M960). The participant was seated in a private and quiet room and provided with white noise through headphones to minimize distraction. Subsequently, respiratory rate and $P_{ET}CO_2$ were measured using a portable, calibrated capnograph (Masimo EMMA, Danderyd, Sweden) with a personal mouthpiece and nose clip. HR and SpO_2 were measured with a portable finger pulse oximeter (Masimo SET® Rad-5, Danderyd, Sweden). Arterial blood pressure (i.e., systolic, diastolic) was measured using an automated blood pressure cuff (Omron,

model BP786n) and used to calculate pulse pressure (systolic-diastolic) and mean arterial pressure (MAP; 1/3 systolic + 2/3 diastolic). Arterial blood draws were assessed using a blood gas analyzer (Abbott iStat, CG4+ and CHEM 8+ cartridges; Mississauga, Ontario, Canada; blood gases corrected for altitude and body temperature). Each morning, participants filled out the Lake Louise acute mountain sickness (AMS) questionnaire to assess self-reported AMS symptoms (see table 1). A non-invasive measurement of instantaneous heart rate (HR) was obtained using an electrocardiogram (ECG: lead II configuration; ADInstruments Bioamp ML132; Colorado Springs CO, USA). PCAv was measured using a Spencer Technologies 2Mhz transcranial Doppler ultrasound (TCD; model MD150B; Redmond, WA, USA). A commercially available headpiece was used to fixate 2MHz Doppler ultrasound probes either side of the cranium, insonating through the trans-temporal acoustic window. Cerebral blood velocity was measured through the P2 segment of the posterior cerebral artery (PCAv; cm/s) using standardized procedures for vessel identification (see Willie et al., 2011). The P2 segment was insonated as the perfusion territory of this vessel segment is more proximal to the downstream neuronal pool involved in visual processing, compared with the P1 segment. NVC was elicited using an intermittent visual stimulus (VS; iPhone “Strobe Light” app; 6Hz). Data was recorded, and later analysed offline using the PowerLab software (v8.0) provided by ADInstruments (model 16SP ML880) and LabChart Pro software 8.0 (Colorado Springs CO, USA).

3.3.4 Experimental Protocol

Immediately following daily measures, participants reported to the designated room for NVC testing. Participants were tested in a quiescent, dark room in the seated position throughout. The duration of the study protocol was ≈60 minutes, including instrumentation. Participants were initially instrumented with the ECG in lead II configuration. A pulse oximeter was placed on the participant's finger to measure beat-by-beat peripheral oxyhaemoglobin saturation for the duration of the protocol. The TCD headpiece was affixed to the participant's cranium to a comfort level determined by the participant. The headpiece was utilized to mitigate probe movement during the protocol. Ultrasound probes were fixated either side of the headpiece, exposing the trans-temporal acoustic window. The trans-temporal window is located approximately 1cm in front of the external auditory meatus and roughly 1-2cm above the zygomatic arch. The P2 segment of the PCA was insonated and monitored throughout. Standard methods for determining the PCA vessel were applied prior to beginning the study (e.g., vessel depth and resting velocity, flow direction, visual responsiveness and carotid compression; see Willie et al., 2011).

Following set-up, the participants sat in a relaxed manner with their eyes closed and room lights turned off for a period of three minutes. This epoch formed the basis of baseline PCAv measures. Following the three-minute baseline period, NVC was assessed using an intermittent visual stimulus (VS; strobe light; 6Hz). The strobe light (iPhone application "Strobe light") was placed approximately six inches in front of the participant's eyes by a member of the research team. Participants were exposed to three consecutive trials of intermittent VS (30s on eyes open/ closed). A member of the research team continuously monitored adherence to the study protocol. Once VS had been completed this signaled the end of the NVC assessment.

This protocol was repeated at each altitude. An example of the group averaged NVC waveform at each altitude is shown in figure 4.

3.3.5 Arterial blood draws

Arterial blood draws were sampled from the radial artery by a trained and registered respiratory therapist (HN) using standard procedures and universal precautions. Blood samples were collected with the participant in the supine position following a period of relaxation.

3.3.6 Data Analysis

Daily measures (HR, SBP, DBP, MAP, $P_{ET}CO_2$ and SpO_2) were recorded as absolute values and compared between locations (see table 1). Similarly, values for PaO_2, SaO_2, $PaCO_2$, arterial [HCO_3^-], base excess and arterial pH were taken as absolute values and compared between locations (see table 1 and figure 2). AMS scores were recorded and averaged at each location and presented in absolute terms (see table 1). Approximate atmospheric pressure (P_{ATM}**)** at each altitude was obtained online (http://altitude.org/) with partial pressure of inspired O_2 (P_IO_2) calculated by multiplying P_{ATM} by 0.21. Baseline PCAv was taken as an average across a three-minute epoch immediately prior to NVC testing. Baseline total area under the curve for PCAv (PCAv tAUC) was calculated across a 30-second period immediately prior to the onset of NVC assessment. To assess for any baseline drift in PCAv and tAUC measures, we compared values for the initial baseline period and between VS trials

(data not shown). No significant differences were found when comparing baseline measures with intermittent rest periods between trials ($P>0.05$).

NVC response magnitude was indexed as the difference (Δ) in mean (ΔMean; cm/s) and peak (ΔPeak; cm/s) PCAv achieved during VS compared with baseline PCAv. Moreover, NVC was indexed as the Δ in tAUC (ΔtAUC; $cm.s^2$) achieved during VS, compared with baseline. Each NVC metric was averaged across the three VS trials and presented as both absolute change (Δcm/s and $\Delta cm.s^2$) from baseline and the relative percentage change from baseline (Δ%). This analytical approach was replicated and compared between altitudes. Furthermore, to ensure that the NVC response was reproducible, we compared response magnitude of each VS trial for all NVC metrics at all locations (see figure 5). NVC waveforms (see figure 4) were developed using a bespoke macro on Labchart.

3.3.7 Statistical Analysis

All statistical analysis was performed using SPSS (IBM statistics, version 28.0). Data were first tested for normal distribution using the Shapiro-Wilk test as well as visual inspection of normal Q-Q plots and dataset histograms. Mauchly's test of sphericity was used to determine whether the differences between each level of the within-subjects factor have equal variance. When data violated the assumption of sphericity an epsilon (**ε**) adjustment was performed. Routinely used ε adjustments include Greenhouse-Geiser or Huynh-Feldt adjustment. In situations of normal distribution, statistically significant differences in group means were made using a one-factor repeated measure analysis of variance (ANOVA) with a Bonferroni post hoc adjustment. When $\varepsilon < 0.75$, the Greenhouse-Geisser adjustment was utilised. In

contrast, when $\varepsilon > 0.75$ the Huynh-Feldt adjustment was used. When the assumption of normal distribution was violated, a non-parametric Friedman test of differences among repeated measures was conducted with a Wilcoxon rank test post hoc analysis between and within locations. Throughout this manuscript, altitude was designated as the within-subjects factor. Effect sizes for the magnitude of the within-subjects effect of altitude on each variable was determined using partial eta squared ($\eta^2 p$) or Kendall's coefficient of concordance (*W*; non-parametric). Simple linear regression was used to determine the relationship between baseline PCAv and $PaO_2/PaCO_2$ index. Statistical significance was assumed at $p<0.05$. During non-parametric assessment, a manual Bonferroni correction was applied for all multiple comparisons.

3.4 Results & Figures

3.4.1 Daily measures

A summary of the statistical observations made for daily measures is provided (see table 1). A significant main effect was found for body weight ($F_{1.937,\ 17.432}$ = 4.837, p = 0.022, η^2p = 0.350). However, post-hoc analysis revealed no significant differences for pairwise comparisons. No main effect was found for BMI ($F_{3,\ 27}$ = 2.065, p = 0.128, η^2p = 0.187) or SBP ($F_{1.891,\ 17.017}$ = 3.232, p = 0.067, η^2p = 0.264). A significant main effect was found for DBP ($F_{3,\ 27}$ = 19.390, p<0.001, η^2p = 0.683). Post-hoc analysis revealed significant differences between 1400m and 3440m (p<0.001), 3820m (p=0.002) and 4240m (p<0.001). A significant main effect was found for MAP ($F_{3,\ 27}$ = 13.613, p<0.001, η^2p = 0.602). Post-hoc analysis revealed significant differences between 1400m and 3440m (p=0.026), 3820m (p=0.006) and 4240m (p<0.001). A significant main effect was found for HR ($F_{3,\ 27}$ = 3.442, p = 0.031, η^2p = 0.277). However, post-hoc analysis revealed no significant differences for pairwise comparisons. A significant main effect was found for SpO_2 ($X^2(3)$ = 25.906, p<0.001, W = 0.864). Post-hoc analysis revealed significant differences between 1400m and 3820m (p=0.015) and 4240m (p<0.001). A significant main effect was found for $P_{ET}CO_2$ ($F_{1.246,\ 11.214}$ = 25.012, p<0.001, η^2p = 0.735). Post-hoc analysis revealed significant differences between 1400m and 3440m (p=0.043), 3820m (p=0.018) and 4240m (p=0.001). Moreover, significant differences were observed when comparing 3440m and 3820m with 4240m (p<0.001 and p<0.001, respectively).

3.4.2 Arterial blood draws

A summary of the statistical observations made for arterial blood draws is provided (see table 2 and figure 2). A significant main effect was found for PaO_2 ($F_{3, 18}$ = 138.270, p<0.001, η^2p = 0.958). Post-hoc analysis revealed significant differences between 1130/1400m and 3440m (p<0.001), 3820m (p<0.001) and 4240m (p<0.001). A significant main effect was found for SaO_2 ($F_{3, 18}$ = 17.313, p<0.001, η^2p = 0.743). Post-hoc analysis revealed significant differences between 1130/1400m and 3440m (p=0.032), 3820m (p=0.003) and 4240m (p<0.001). A significant main effect was found for $PaCO_2$ ($F_{3, 18}$ = 23.123, p<0.001, η^2p = 0.794). Post-hoc analysis revealed significant differences between 1130/1400m and 3440m (p=0.015), 3820m (p=0.016) and 4240m (p<0.001). A significant main effect was found for [HCO_3^-] ($F_{3, 18}$ = 11.342, p<0.001, η^2p = 0.654). Post-hoc analysis revealed significant differences between 1130/1400m and 3820m (p=0.043) and 4240m (p=0.021). A significant main effect was found for base excess ($F_{3, 18}$ = 7.132, p = 0.002, η^2p = 0.543). Post-hoc analysis revealed significant differences between 3440m and 3820m (p=0.022). No significant main effect was found for arterial pH ($F_{3, 18}$ = 2.788, p = 0.070, η^2p = 0.317).

3.4.3 Baseline PCAv and $PaO_2/PaCO_2$ linear regression

A summary for the statistical observations made for the baseline PCAv and $PaO_2/PaCO_2$ relationship is provided (see figure 3). No significant main effect was found for baseline PCAv ($X^2(3)$ = 5.640, p = 0.131, W = 0.188; see figure 3a). A significant main effect was found for $PaO_2/PaCO_2$ index ($F_{3, 18}$ = 30.892, p<0.001, η^2p = 0.837; see figure 3b). Post-hoc analysis revealed significant differences

between 1130m and 3440m (p=0.002), 3820m (p=0.015) and 4240m (p<0.001) for the $PaO_2/PaCO_2$ index.

3.4.4 Inter-visual stimulation reproducibility of the NVC response

Prior to comparing NVC response magnitude between locations, we performed a within-location, inter-visual stimulation analysis of the NVC response to assess reproducibility of the haemodynamic response. A summary of the statistical observations made for NVC reproducibility is provided (see figure 5).

3.4.4.1 1130m

No significant main effect between VS trials was found for ΔMean (Δcm/s; $X^2(2) = 4.200$, p = 0.122, $W = 0.210$ or Δ%; $X^2(2) = 5.515$, p = 0.063, $W = 0.276$; See figures 5ai). No significant main effect between VS trials was found for ΔPeak (Δcm/s; $F_{2, 18} = 0.354$, p = 0.707, $\eta^2p = 0.038$ or Δ%; $X^2(2) = 1.947$, p = 0.378, $W = 0.097$; see figure 5aii). No significant main effect between VS trials was found for ΔtAUC (Δcm.s^2; $F_{1.307, 11.761} = 0.445$, p = 0.570, $\eta^2p = 0.047$ or Δ%; ; $F_{2, 18} = 0.599$, p = 0.560, $\eta^2p = 0.062$; see figure 5aiii).

3.4.4.2 3440m

A significant main effect between VS trials was found for ΔMean (Δcm/s; $F_{2, 18} = 7.523$, p = 0.004, $\eta^2p = 0.455$ and Δ%; $F_{2, 18} = 8.285$, p = 0.003, $\eta^2p = 0.479$; See figures 5bi). Bonferroni post-hoc analysis found a significant difference between V1 and V2 ΔMean response magnitude for both Δcm/s (p = 0.017) and Δ% (p = 0.011).

A significant main effect between VS trials was found for ΔPeak (Δcm/s; $X^2(2)$ = 13.400, p = 0.001, W = 0.670 and Δ%; $F_{2, 18}$ = 12.846, p<0.001, η^2p = 0.588; see figures 5bii). Bonferroni post-hoc analysis found a significant difference between V1 and V2 ΔPeak response magnitude for both Δcm/s (p = 0.001) and Δ% (p = 0.004). In addition, a significant difference was observed between V1 and V3 ΔPeak response magnitude for both Δcm/s (p = 0.042) and Δ% (p = 0.005). No significant main effect between VS trials was found for ΔtAUC (Δcm.s^2; $X^2(2)$ = 4.200, p = 0.122, W = 0.210 or Δ%; $F_{2, 18}$ = 1.807, p = 0.193, η^2p = 0.167; See figures 5biii).

3.4.4.3 3820m

No significant main effect between VS trials was found for ΔMean (Δcm/s; $F_{2, 18}$ = 0.144, p = 0.867, η^2p = 0.016 or Δ%; $F_{2, 16}$ = 0.078, p = 0.926, η^2p = 0.010; See figures 5ci). No significant main effect between VS trials was found for ΔPeak (Δcm/s; $F_{1.257, 11.314}$ = 0.818, p = 0.412, η^2p = 0.083 or Δ%; $F_{1.178, 9.421}$ = 1.396, p = 0.276, η^2p = 0.149; See figures 5cii). No significant main effect between VS trials was found for ΔtAUC (Δcm.s^2; $X^2(2)$ = 2.600, p = 0.273, W = 0.130 or Δ%; $X^2(2)$ = 1.879, p = 0.391, W = 0.094; See figures 5ciii).

3.4.4.4 4240m

No significant main effect between VS trials was found for ΔMean (Δcm/s; $F_{2, 18}$ = 3.483, p = 0.053, η^2p = 0.279 or Δ%; $F_{2, 18}$ = 3.434, p = 0.055, η^2p = 0.276; See figures 5di). No significant main effect between VS trials was found for ΔPeak (Δcm/s; $F_{2, 18}$ = 1.665, p = 0.217, η^2p = 0.156 or Δ%; $F_{2, 18}$ = 1.433, p = 0.264, η^2p = 0.137; See figures 5dii). A significant main effect between VS trials was found for

ΔtAUC (Δcm.s^2; $X^2(2) = 7.400$, p = 0.025, $W = 0.370$ and Δ%; $F_{2, 18} = 6.495$, p = 0.008, $\eta^2p = 0.419$; see figures 5diii). Bonferroni post-hoc analysis found a significant difference between V1 and V2 ΔtAUC response magnitude for both Δcm.s^2 (p = 0.042) and Δ% (p = 0.010).

3.4.5 Between location NVC comparison

A summary of the statistical observations made for between-location NVC comparisons are provided (see figure 6). No significant main effect for location was found for ΔMean (Δcm/s; $F_{3, 27} = 1.946$, p = 0.146, $\eta^2p = 0.178$ or Δ%; $X^2(3) = 4.918$, p = 0.178, $W = 0.164$; see figures 6ai-ii). A significant main effect for location was found for ΔPeak (Δcm/s; $F_{3, 27} = 3.067$, p = 0.045, $\eta^2p = 0.254$ or Δ%; $F_{3, 27} = 6.630$, p = 0.002, $\eta^2p = 0.424$; see figures 6bi-ii). However, Bonferroni post-hoc analysis found no significant pairwise comparisons between locations. No significant main effect for location was found for ΔtAUC (Δcm.s^2; $F_{3, 27} = 1.384$, p = 0.269, $\eta^2p = 0.133$ or Δ%; $F_{3, 27} = 2.438$, p = 0.086, $\eta^2p = 0.213$; see figures 6ci-ii).

Baseline Values for Daily Measures

	1400m	3440m	3820m	4240m	P-value	Effect Size
P_{ATM}	648	509	486	454	-	-
P_IO_2 (mmHg)	136	107	102	95	-	-
AMS	0 ± 1	1 ± 1	1 ± 1	1 ± 1	-	-
Weight (kg)	71.31 ± 14.49	70.30 ± 13.69	70.23 ± 13.68	70.23 ± 13.34	0.022	0.350
BMI (kg/m^2)	23.57 ± 2.00	23.35 ± 1.84	23.33 ± 1.84	23.34 ± 1.78	0.128	0.187
Systolic (mmHg)	112.50 ± 10.77	116.40 ± 8.32	119.40 ± 10.98	118.70 ± 10.68	0.067	0.264
Diastolic (mmHg)	77.90 ± 11.05	86.60 ± 8.41*	89.60 ± 10.45*	87.40 ± 9.42*	<0.001	0.683
MAP (mmHg)	89.43 ± 10.63	96.39 ± 7.32*	99.53 ± 10.13*	97.83 ± 9.27*	<0.001	0.602
HR (bpm)	85.90 ± 8.85	91.60 ± 13.92	84.10 ± 7.49	84.90 ± 10.90	0.031	0.277
SpO_2 (%)	96.40 ± 0.97	91.80 ± 2.49	91.40 ± 2.84*	87.60 ± 2.01*	<0.001	0.864
$P_{ET}CO_2$ (Torr)	32.60 ± 6.77	26.50 ± 2.99*	25.30 ± 2.67*	21.10 ± 1.91*†¥	<0.001	0.735

Table 1 Daily measures. Daily measures collected each morning throughout ascent are presented. Total atmospheric pressure (P_{ATM}), partial pressure of inspired oxygen (P_IO_2; mmHg), acute mountain sickness (AMS), body weight (kg), body mass index (BMI; kg/m^2), systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), mean arterial pressure (MAP; mmHg), heart rate (HR; bpm), peripheral oxygen saturation (SpO_2; %), partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$; torr). * Denotes statistical significance from 1400m. † denotes statistical significance from 3440m. ¥ denotes statistical significance from 3820m. NS, non-significant (P>0.05). Presented as mean ± SD.

Arterial Blood Draw Analysis						
	1130/1400m	**3440m**	**3820m**	**4240m**	**P-value**	**Effect Size**
PaO_2 (mmHg)	84.80 ± 5.35	48.56 ± 8.08*	54.56 ± 6.23*	48.38 ± 4.96*	p<0.001	0.973
SaO_2 (%)	96.80 ± 0.79	84.89 ± 7.87*	88.78 ± 3.73*	85.50 ± 3.66*	p<0.001	0.743
$PaCO_2$ (mmHg)	34.84 ± 4.58	30.23 ± 4.93*	29.57 ± 3.76*	29.05 ± 3.73*	p<0.001	0.794
HCO_3^- (mmol/L)	23.24 ± 2.69	20.99 ± 3.08	19.48 ± 2.01*	19.69 ± 2.80*†	p<0.001	0.654
Base excess (mmol/L)	-1.20 ± 2.78	-3.00 ± 3.08	-4.78 ± 1.86	-4.50 ± 3.07†	0.002	0.543
Arterial pH	7.43 ± 0.02	7.45 ± 0.02	7.43 ± 0.02	7.44 ± 0.02	0.070	0.317

Table 2 Arterial blood draw analysis. Changes in partial pressure of arterial oxygen (PaO_2; mmHg), arterial oxygen saturation (SaO_2; %), partial pressure of arterial carbon dioxide ($PaCO_2$; mmHg), arterial bicarbonate (HCO_3^-; mmol/L), base excess (mmol/L) and arterial pH are presented for each test altitude. * Denotes statistical significance from 1130/1400m. † denotes statistical significance from 3440m. NS, non-significant (P>0.05). Presented as mean ± SD.

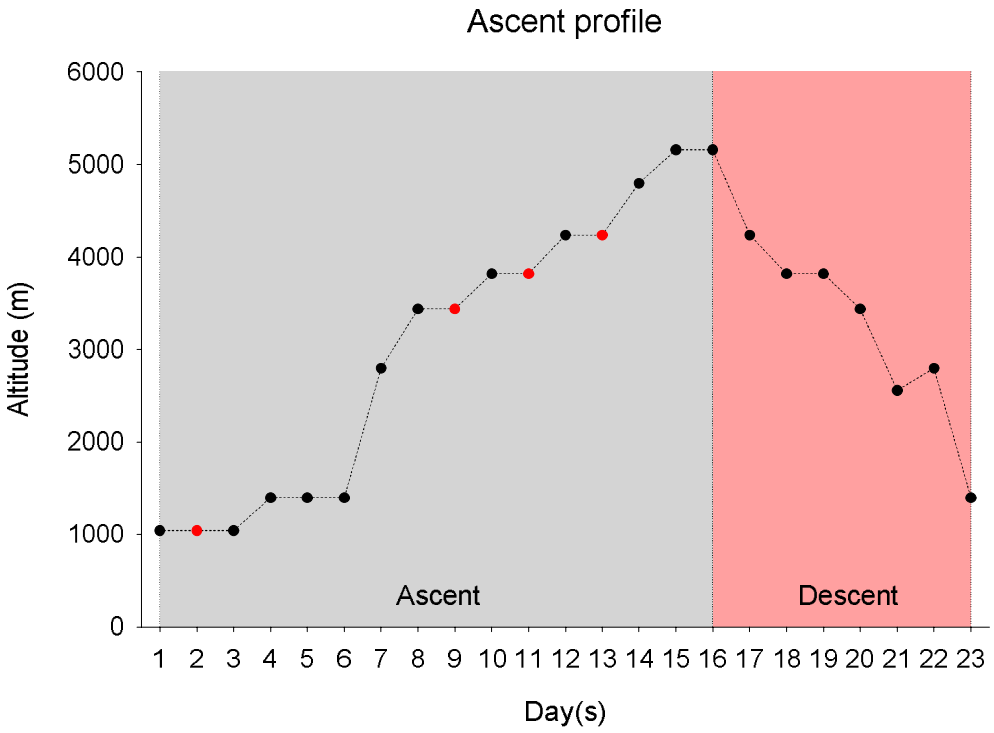

Figure 1 Ascent profile. An illustration of the expedition ascent (grey shaded region) and descent (red shaded region) profile. Altitude (metres above sea-level) is presented along the y-axis with days spent at each altitude presented along the x-axis. Red dots illustrate days of NVC assessment.

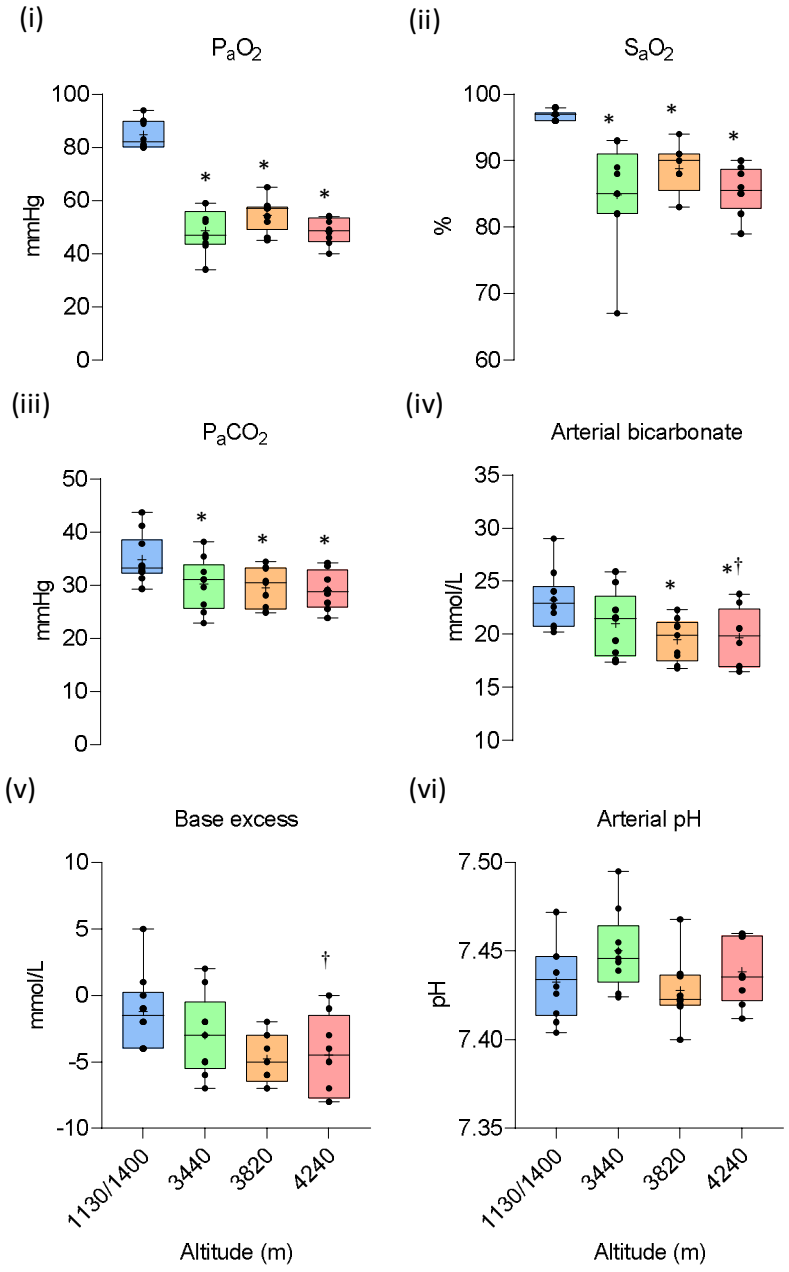

Figure 2 Arterial blood draws. The effect of high altitude exposure on **(i)** partial pressure of arterial oxygen **(**PaO_2; mmHg), **(ii)** arterial oxygen saturation **(**SaO_2; %) ($P<0.032$), **(iii)** partial pressure of arterial carbon dioxide **(**$PaCO_2$; mmHg), **(iv)** arterial bicarbonate (HCO_3^-), **(v)** base excess (mmol/L) and **(vi)** arterial pH is shown. Respective changes in each parameter are shown along the y-axis. Altitude

of test locations are shown along the x-axis. * = denotes statistical significance from 1130/1400m. † = Statistical significance from 3440m. Date are presented as box and whiskers plots showing individual data points, median, 25^{th}-75^{th} percentile with minimum and maximum values.

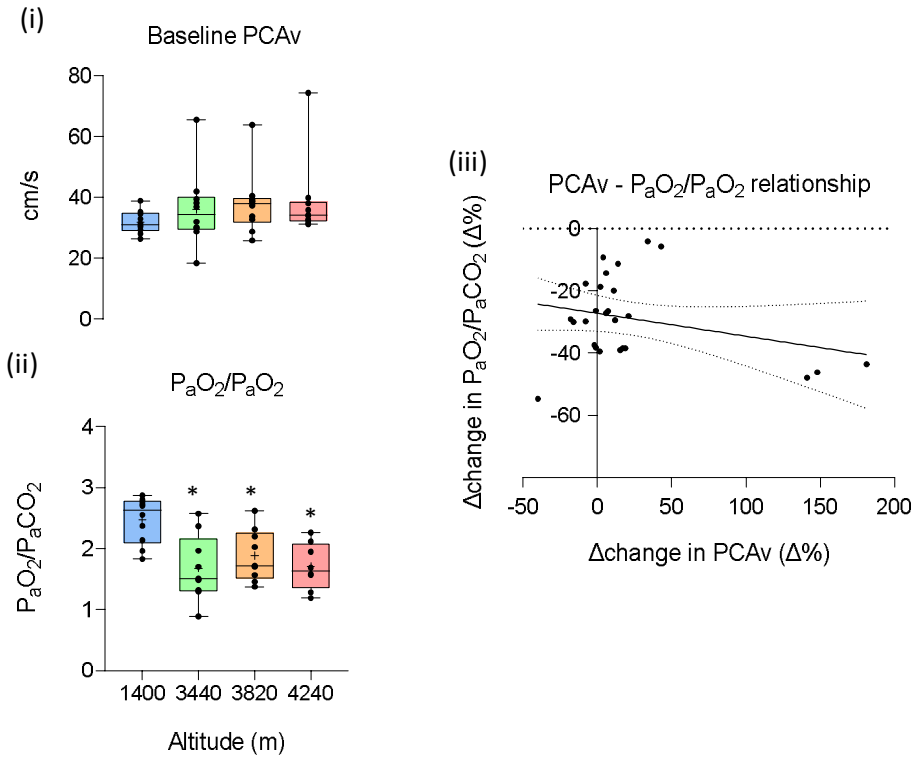

Figure 3 Baseline PCAv and PaO2/PaCO2 relationship. The effect of progressive ascent to high altitude is shown for **(i)** baseline posterior cerebral artery velocity (PCAv), **(ii)** $PaO_2/PaCO_2$ index. Respective changes for each parameter are presented along the y-axis. Each altitude is presented along the x-axis. Data are presented as box and whiskers showing all data points, median, 25^{th}-75^{th} percentile with minimum and maximum values. Moreover, a simple linear regression **(iii)** details the relationship between baseline PCAv and $PaO_2/PaCO_2$ by plotting the percentage changes in each parameter throughout ascent.

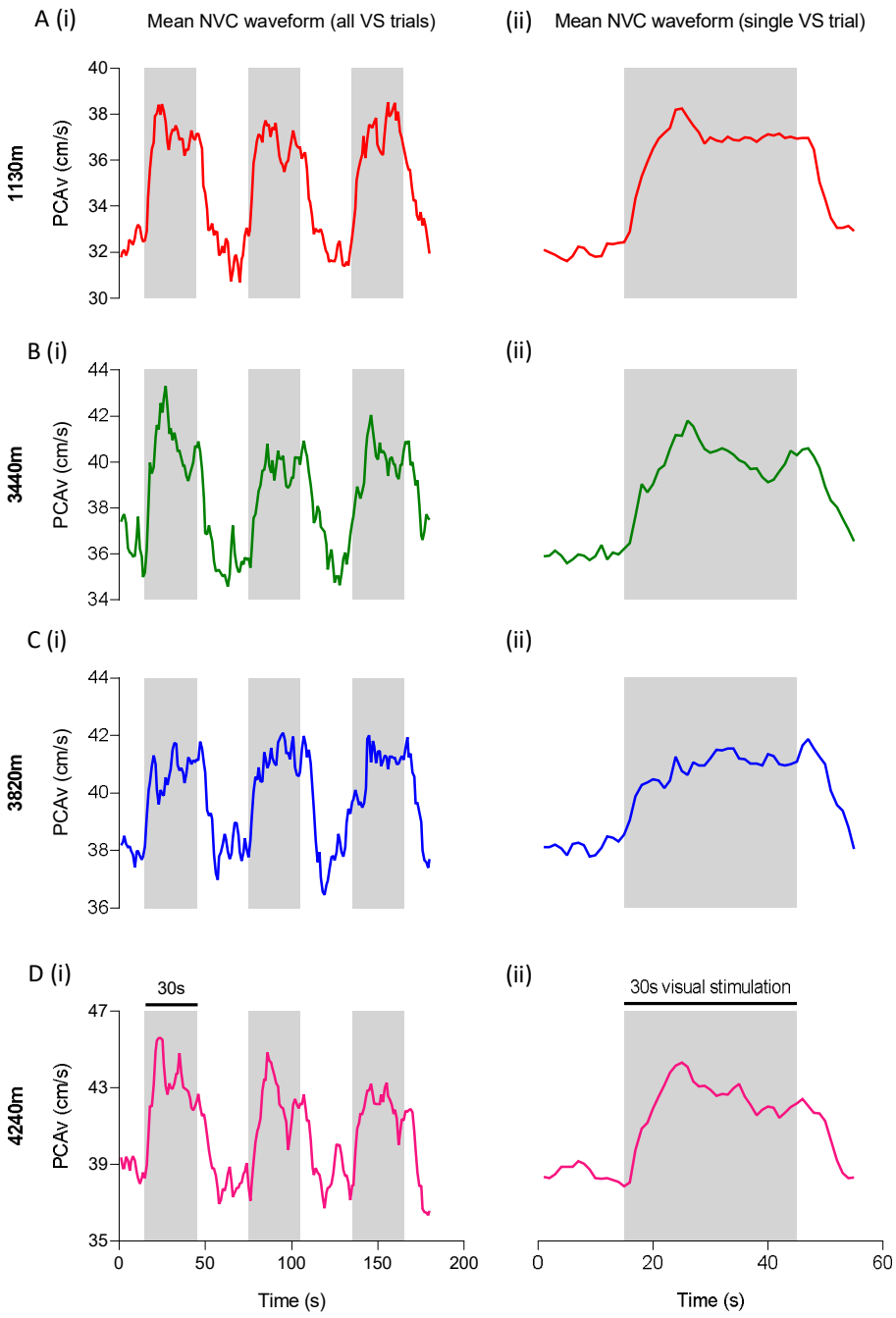

Figure 4 NVC waveforms. Group averaged waveforms of the posterior cerebral artery velocity (PCAv) during each visual stimulus (left column), and the average PCAv response during visual stimulus (right column) are presented for each altitude. Grey

shaded regions denote 30 second periods of visual stimulation. PCAv (cm/s) is presented along the y-axis. Time (seconds) is presented along the x-axis.

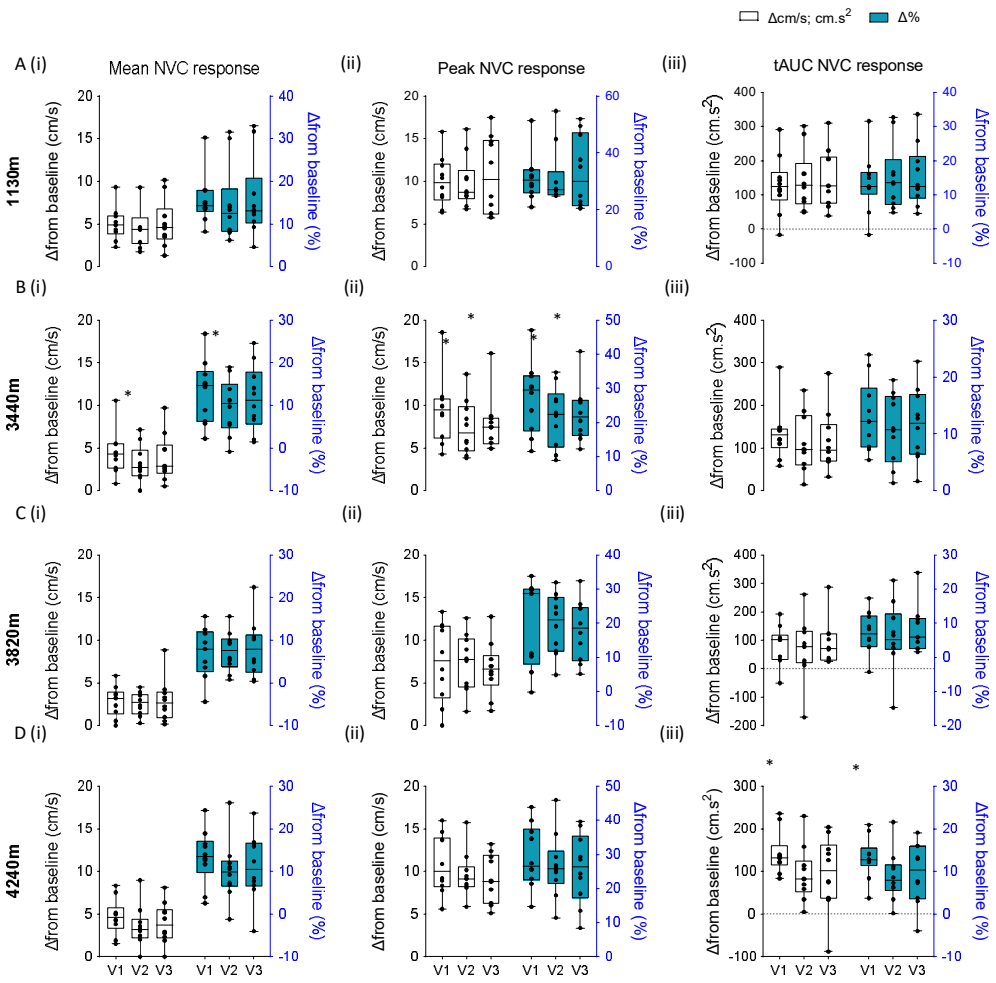

Figure 5 NVC response magnitude stability. This figure illustrates the stability in NVC response magnitude across each metric (Δmean; left column, Δpeak; middle column, and ΔtAUC; right column) at each altitude. NVC response magnitude is presented as a Δcm/s (left y-axis; clear boxes) and Δ% (right y-axis; blue boxes) from baseline respectively. Individual visual stimulation trials (V1-3) are presented along the x-axis. * Denotes statistical significance from corresponding V1 trial. Data are presented as box and whiskers plots showing all data points, median, 25th-75th percentiles with minimum and maximum values.

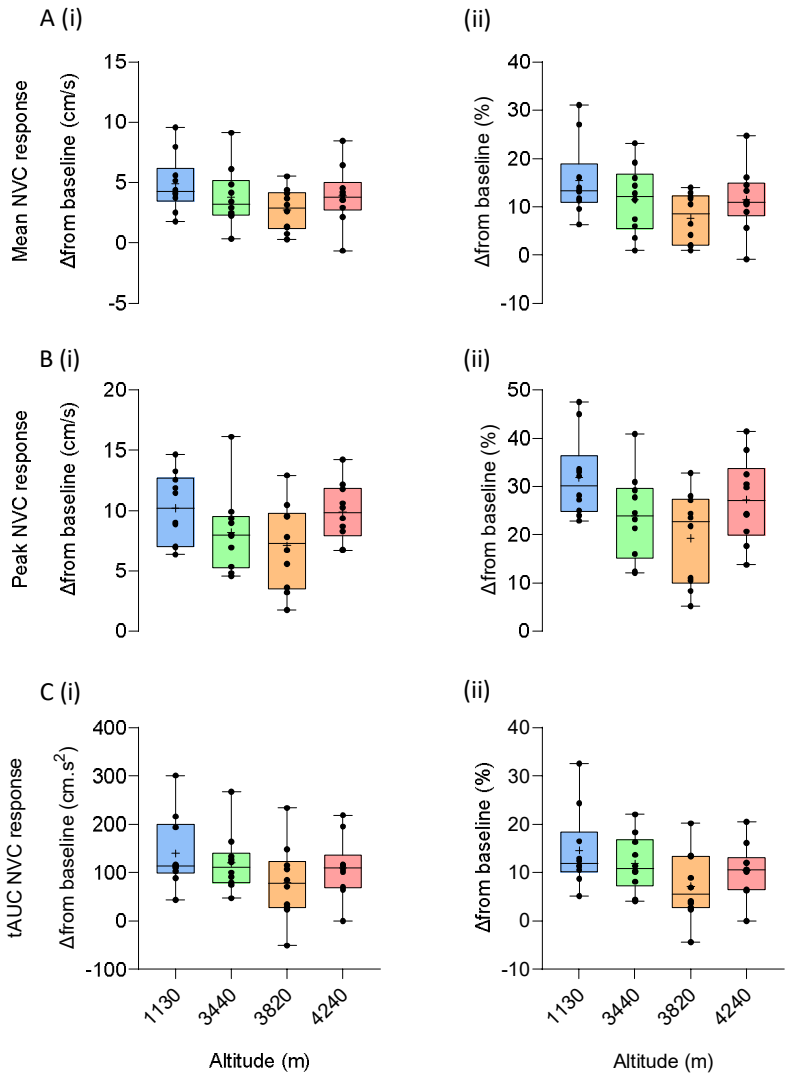

Figure 6 NVC response between altitudes. The magnitude of the haemodynamic response for each NVC metric (Δmean; A(i-ii), Δpeak; B(i-ii), and ΔtAUC; C(i-ii) are presented. Response magnitude is presented as a Δcm.s/Δcm.s^2 (left column) and Δ% (right column) from baseline along the y-axis. Each altitude is presented along the x-axis. Data are presented as box and whisker plots representing showing all data points, median, 25th-75th percentile with minimum and maximum values.

3.5 Discussion

3.5.1 Primary findings

This study investigated the influence of incremental ascent to 4240m on NVC. Our results demonstrate: 1) the NVC response which assessed at each altitude demonstrated remarkable stability and reproducibility; and 2) there was no significant difference in the magnitude of the NVC response between altitudes in healthy acclimatized individuals.

3.5.2 Acclimatisation

Exposure to HA and the attendant drop in total barometric pressure induced a state of hypobaric hypoxaemia. This is evident from the significant decreases in PaO_2 and SaO_2 (see table 2 and figure 2). During prolonged exposure to HA, hypoxia increases activation of the peripheral chemoreceptors, enhancing the HVR (Ainslie & Ogoh, 2009; Willie et al., 2015; Hoiland et al., 2015). The HVR increases ventilatory drive partially restoring PaO_2 and SaO_2 levels toward resting values (Bernardi et al., 2006; Smith et al., 2014). Hyperventilation was evident in our study. Comparing values recorded at 3440m with those obtained at higher altitudes, with further decreases in barometric pressure, we observed no further reductions in PaO_2 and SaO_2, revealing attenuation of a further decline in oxygenation associated with continued ascent.

Secondly, we observed profound hypocapnia, as demonstrated by significant decreases in $PaCO_2$ (see table 2 and figure 2). Hypocapnia serves to blunt both central and peripheral chemoreceptor activation and subsequently reduces

ventilatory drive. Combined, these data reveal the presence of two antagonistic vasoactive stressors: hypoxia and hypocapnia. Hypoxia, below a threshold (<40-45mmHg), has a vasodilatory effect in the systemic circuit (Ainslie & Ogoh, 2009). In contrast, hypocapnia is a potent vasoconstrictor (Brugniaux et al., 2007). The cerebrovasculature is particularly sensitive to changes in arterial blood gases, especially $PaCO_2$ (Brugniaux et al., 2007; Ainslie & Ogoh, 2009). Although prior research has shown that the presence of hypocapnia decreases the NVC response (Szabo et al., 2011), this study was laboratory-based and did not include the hypoxic vasodilatory stress associated with HA. Furthermore, the presence of sustained hypocapnia presents further issues by inducing tissue alkalosis (Krapf et al., 1991).

Hyperventilation-induced hypocapnia triggers an acid-base adjustment in the form of renal compensation. To counteract respiratory alkalosis, and maintain pH within normal limits, a series of compensatory steps are required through increased acid retention coupled with an increase in HCO_3^- excretion via the renal system. The end-product of a relative metabolic acidosis helps to decrease arterial pH towards normal values, thereby preserving acid-base homeostasis (Goldfarb-Rumyantzev & Alper, 2014). The sophisticated and refined nature of this compensatory adaptation is crucial as the vasoconstrictive effects of hypocapnia/alkalosis can decrease CBF (Szabo et al., 2011). Furthermore, a blunted HVR response has been shown to be a strong predictor of AMS (Richalet et al., 2012). We observed evidence of renal compensation by way of significant decreases in arterial [HCO_3^-] (see table 2 and figure 2) in response to hypocapnia, such that arterial pH was maintained constant throughout ascent compared with BL.

3.5.3 Impact of HA on CBF

We found no significant differences in baseline PCAv during ascent (see figure 3). This observation is contrary to what is routinely observed throughout the literature. Exposure to high altitude environments elicits an immediate and time-dependent increase in global cerebral blood flow (gCBF) measures (Severinghaus et al., 1966; Ainslie et al., 2016; Imray et al., 2014; Lucas et al., 2011). This physiological response acts to preserve levels of cerebral oxygen delivery during instances of arterial hypoxaemia (Ainslie et al., 2016; Hoiland et al., 2016; Harris et al., 2012). gCBF returns to baseline measures with time spent at altitude (Sanborn et al., 2015; Liu et al. ,2017; Lucas et al., 2011).

3.5.4 The role of high altitude on NVC

In terms of NVC, we observed that for each of our designated NVC parameters, a significant response was evoked during VS (see figures 5-7). Our results demonstrate the remarkable reproducibility of the NVC response. For between-altitude comparisons, or findings demonstrate that NVC remained intact, as demonstrated by no significant differences in each of the three NVC response parameters between altitudes (see figure 8). These findings are consistent with a previous study (Caldwell et al., 2017), where NVC testing was performed temporally in response to acclimatization at a single altitude. These studies differed in terms of ascent-profile, altitude reached, analysis and stimulus to evoke NVC. However, the finding that NVC remains intact during ascent to HA is consistent.

3.5.5 Methodological limitations

The utilization of TCD for the assessment of CBF has limitations. Although TCD provides excellent beat-by-beat measurement of the intracranial arteries arising from the circle of Willis, it does not provide any indication of pre-existing diameter of the insonated vessel. This is relevant at HA where there are multiple and competing stressors, which influence vessel tone (hypoxic vasodilation and hypocapnic vasoconstriction). There is also recent literature to suggest that there are stimulus-evoked changes in diameter of the PCA as a function of distance from the primary visual cortex (Bizeau et al., 2017). There is therefore an inherent risk of misinterpreting the magnitude of the CBF change (Caldwell et al., 2017). Furthermore, there is a risk of technical error given the sensitivity of transcranial Doppler technique. To account for this, we ensured the same three sonographers were present during each testing session. We also strictly abided by a set of guidelines for insonating the PCA (see Willie et al., 2011). We also acknowledge that TCD provides measurements of velocity rather than flow. However, recent literature has demonstrated CBF velocity to be an appropriate and reliable surrogate for CBF (Secher et al., 2008; Ainslie & Ogoh, 2009).

3.5.6 Conclusion

As expected, exposure to HA elicited an incremental state of hypoxic hypocapnia. However, respiratory alkalosis was adequately combatted via renal compensation (relative metabolic acidosis), preserving arterial pH within normal limits during the ascent profile. Despite the combination of stressors associated with incremental ascent to 4240m, NVC remains remarkably intact. We postulate that

during exposure to HA, NVC could be predominantly sensitive to arterial pH. This study highlights the remarkable innate ability of the cerebrovasculature to adapt to environmental stress through a series of integrative adaptations, with several intrinsically complex systems working in unison to preserve cerebrovascular homeostasis and function. Strengths of this study include the comprehensive manner by which the NVC response was presented, analysed and compared at each altitude. Although we tested NVC at a higher altitude than that employed in previous studies (Caldwell et al., 2017), future studies might incorporate even higher altitudes where HA illness symptomology becomes increasingly prevalent. Whereas our study demonstrates that NVC remains intact during incremental ascent to 4240m in acclimatised volunteers, it is plausible to suggest that responses may be impaired in unacclimatized individuals, during rapid ascent, or perhaps at altitudes higher than 4240m.

3.6 Acknowledgements

We thank all the volunteer research participants for their time and participation, and ADInstruments for their support of this study. We would like to extend our sincerest gratitude to the Sherpa community whose hospitality and expertise in the region made this research possible.

3.7 Author contribution statement

Data collection for PCAv and NVC was completed by JL, CM, JP, GS and TD. Data collection for daily measures was completed by SZ, CN, KOH and TB. Arterial blood draws were completed by HN. Safe ascent to and descent from 4240m

was carried out by MS. Arterial blood draw analysis was completed by SZ. PCAv and NVC analysis/interpretation was completed by JL, KOH and TD. Manuscript, figure and table development/preparation was completed by JL, KOH and TD.

3.8 Disclosure/conflict of interest

The authors have no conflicts of interest to declare.

3.9 Funding

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Chapter 4. Characterising the Protective Effects of Hypobaric Hypoxia on the Neurovascular Coupling Response

The following chapter has been submitted for publication. I completed data collection throughout the expedition. I completed all data analysis and developed relevant figures and tables. Finally, I developed and finalised the manuscript which has been sent for publication.

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4.1 Abstract

Neurovascular coupling (NVC) is the temporal and spatial link between local neuronal activity and regional cerebral blood flow. High altitude (HA) induces acute hypoxic vasodilation of the cerebral vasculature. We aimed to characterise the effects of (a) acute removal of the HA-induced hypoxic vasodilation and (b) rapid ascent to and residency at HA (pre- vs. post-acclimatisation) on NVC response. 12 healthy participants (5 female) were recruited. Arterial blood gases and NVC were measured at baseline (1130m) and on days 2 (<24 hours of HA exposure) and 9 at 3800m. Acute gas challenges were performed using end-tidal forcing, with (a) normoxia and isocapnic hypoxia induced at baseline and (b) poikilocapnic hypoxia and isocapnic hyperoxia induced on days 2 and 9 at 3800m. Posterior cerebral artery velocity (PCAv) was captured using transcranial Doppler ultrasound. NVC was indexed as the peak and mean change in PCAv during intermittent visual stimulation. The NVC waveform was compartmentalised into distinct temporal domains (0-10, 11-20 and 21-30-seconds post-stimulus onset). No significant differences were observed for any NVC metric across all conditions and time points. Our results reveal stability of the NVC response following (a) acute removal of HA-induced hypoxic vasodilation and (b) rapid ascent to and residency at 3800m.

Keywords: Cerebra blood flow, High altitude, Hypoxia, Neurovascular coupling, Transcranial Doppler ultrasound.

4.2 Introduction

4.2.1 High Altitude as a physiological stressor

High altitude (HA) ascent is loosely attributed to any location >2100m above sea-level (Hurtado et al., 2012; Brown & Grocott, 2013). Exposure to this environment, particularly among habitual lowlanders, exerts a multimodal stress which threatens human function and ultimately survival. The chief stressor affixed with HA ascent is the non-linear decrease in total atmospheric pressure and consequent reduction in the partial pressure of ambient oxygen (PO_2), termed hypobaric hypoxia (Chawla et al., 2014; Ke et al., 2017). This hypoxic stress threatens oxygen (O_2) availability along each step of the O_2 cascade, from airway gas diffusion to downstream mitochondrial function (Brown & Grocott, 2013). To ensure adequate O_2 delivery to metabolically active tissue, physiological systems acclimatise through a series of time-dependent molecular and functional changes which characterise the acclimatisation process.

4.2.2 Physiological acclimatisation

The integrative physiological acclimatisation to HA involves gross functional changes within the cardiorespiratory, central nervous and renal system(s) incurring profound haematological adjustments (Palubiski et al., 2020; Luks et al., 2008; Chakraborti, 1985; Bartsch & Gibbs, 2007; Ke et al., 2017; Naeije et al., 2010; Riley et al., 2017; West, 1984,2006,2012, Brown & Grocott, 2013; Goldfarb-Rumyantzev et al., 2014). Moreover, there is now growing appreciation for the genetic involvement within the acclimatisation process. This comes following the discovery

of a subset of transcription factors termed hypoxia-inducible factors (HIF), which become upregulated under hypoxic conditions (Wang et al., 1995; Semenza, 2012; West, 2012). Pathological adaptation to HA is exemplified by the development of acute mountain sickness (AMS), high altitude renal syndrome (HARS), high altitude pulmonary and/or cerebral oedema (HAPE/HACE respectively) (Arestegui et al., 2011; Chewla et al., 2014; Bartsch & Gibbs, 2007; Ke et al., 2017; Brown & Grocott, 2013). Current understanding on the pathogenesis of HA maladaptation is limited due to difficulties in study design and appropriate lab-based models of high altitude exposure.

4.2.3 Cerebrovascular response to high altitude exposure

HA exposure has a time-dependent effect on cerebrovascular function. Cerebral tissue relies upon continuous perfusion to support neurophysiological function. At rest, the brain accounts for approximately 20% of total energy consumption, despite constituting only 2% of total body mass. Following acute exposure to HA and/or hypoxia, there's an immediate increase in global cerebral blood flow (CBF) (Severinghaus et al., 1966; Liu et al., 2017; Ainslie et al., 2016; Imray et al., 2014; Ainslie et al., 2014; Willie et al., 2013; Lucas et al., 2011; Ainslie & Ogoh, 2010; Xu & LaManna, 2006; Wolff, 2000). The relative CBF hyper-perfusion serves to maintain cerebral oxygen delivery during instances of arterial hypoxaemia (Hoiland et al., 2015; Bailey et al., 2016; Ainslie et al., 2014, 2016; Harris et al., 2012; Wolff, 2000). The posited mechanisms of hypoxia-induced cerebral vasodilation are a combination of erythrocyte-mediated pathways which increase nitric oxide (NO) bioavailability (Hoiland et a., 2015; Bailey et al., 2016; Ainslie et al., 2016).

With time spent at HA, we see a reduction in global CBF to levels comparable with sea-level (Sanborn et al., 2015; Steinbeck et al., 2016; Liu et al., 2017; Ainslie et al., 2014; Willie et al., 2013; Lucas et al., 2011; Ainslie & Ogoh, 2010; Xu & LaManna, 2006; Wolff, 2000; Severinghaus et al., 1966). This time-dependent reduction occurs concomitantly with polycythaemia, cerebrospinal fluid pH buffering, HIF expression alterations, cerebro-capillary angiogenesis, hyperventilatory-induced increases in PaO_2 and consequent reductions in $PaCO_2$ (Ainslie et al., 2014; Ainslie & Ogoh, 2010; Xu & LaManna, 2006; Severinghaus et al., 1966; Steinbeck et al., 2016). The extent of CBF modulation is a net product of four reflex mechanisms: (a) hypoxic ventilatory response, (b) hypercapnic ventilatory response, (c) hypoxic cerebral vasodilation and (d) hypocapnic cerebral vasoconstriction (Ke et al., 2017; Hoiland et al., 2015; Ainslie et al., 2014; Harris et al., 2012; Ainslie & Ogoh, 2010). While these observations support altitude-induced changes in global CBF. They provide little insight into the effect of HA exposure on regional control of CBF.

4.2.4 Neurovascular coupling

Neurovascular coupling (NVC) describes the temporal and spatial link between local neuronal activity and regional cerebral blood flow (Phillips et al., 2016; Hosford & Gourine, 2019; Iadecola C, 2017). This process ensures that focal changes in cerebral metabolism are met with a parallel increase in the delivery of glucose and O_2 to the active region, supporting energy-dependent metabolic processes. Both direct neurovascular and indirect neuro-glia-vascular signalling pathways act to modulate tone of the adjacent cerebral vasculature and allow localised increases in

perfusion (Drake & Iadecola, 2006; Cauli & Hamel, 2010; Attwell et al., 2010; Howarth C, 2014; Hosford & Gourine, 2019; Iadecola C, 2017). Cerebrovascular tone is sensitive to a milieu of metabolic and chemical stimuli. Notably, deflections in arterial blood gases (ABGs) as well as intra- and extracellular acid-base conditions can influence functional properties of cerebrovascular smooth muscle cells and endothelium, with subsequent effects on basal vessel calibre. Acidotic and alkalotic conditions induce vasodilation and vasoconstriction of cerebral vessels respectively (Kontos et al., 1977; Mirro et al., 1992; Dietrich et al., 1994; Apkon & Baron, 1995; Zuccarello et al., 2000; Dabertrand et al., 2012; Yoon et al., 2012; Boedtkjer et al., 2018). Blood gas manipulation and transient disruption of acid-base status are consistently observed following HA exposure. The degree to which HA-induced disruption in blood gases and acid-base homeostasis affects NVC is still poorly characterised.

4.2.5 Neurovascular coupling and high altitude

Prior lab-based studies which examined the extent of acute hypocapnia and/or alkalosis on NVC offer mixed reports (Szabo et al., 2011; Bader et al., 2021). One study observed a significant NVC impairment (Szabo et al., 2011), the other demonstrated negligible effects (Bader et al., 2021). It is impossible to draw comparisons from these lab-based experiments as both negate the competing vasodilatory effect of hypoxia, which accompanies HA exposure. Hypoxia is a potent vasodilator of cerebral vessels through increases in NO bioavailability, an integral molecule within the NVC response. Whether hypoxia ensures NVC

homeostasis by mitigating the vasoconstrictive effects of HA-induced hypocapnia and/or respiratory-induced alkalosis is unknown.

To our knowledge only three previous studies have examined the effects of HA exposure on NVC in human participants. Two of these publications were conducted by members of our research group within the Nepal Himalaya, to a maximum altitude of 4240m (Leacy et al., 2018; Lefferts et al., 2020), the other was performed at the Barcroft laboratory (3800m) in California (Caldwell et al., 2017). Their findings concluded that NVC response magnitude was unaffected by ascent to HA. However, owing to the time-differential between initial HA exposure and NVC assessment, in combination with the ascent profile(s) employed, the likelihood is that all participants were fully acclimatised at the point of measurement. Additionally, only one of these studies incorporated arterial blood draws within their methodology and observed normalised arterial pH values during incremental ascent to 4240m (Leacy et al., 2018).

As such, these studies confirmed that NVC remains stable at HA in the background of a normalised pH but provide little insight into the extent of respiratory-induced pH changes at HA on NVC. More importantly, these studies provide little insight into the degree to which hypoxic vasodilation might serve to preserve NVC function at HA, against the background of multiple vasoconstrictive stressors (hypocapnia, respiratory-induced alkalosis).

4.2.6 Study aims and hypotheses

This study aimed to answer two key questions which are currently undetermined within the literature: 1) does the competing hypoxic vasodilatory stimulus act to

offset any hypocapnic/alkalotic-dependent NVC impairment following acute HA exposure? and 2) does respiratory-induced alkalosis following acute exposure to HA induce NVC impairment? We hypothesized that: 1) acute removal of the vasodilatory hypoxic stimulus following exposure to HA would impair NVC response magnitude owing to the vasoconstrictive respiratory-induced hypocapnia and alkalosis and 2) acute exposure to HA impairs NVC, which returns to normal following restoration of arterial pH.

4.3 Materials & Methodology

4.3.1 Ethical Approval, Participant Recruitment & Expedition Protocol

This study abided by research guidelines and policy concerning human participants set out by the Canadian Government Tri-Council and conformed to the latest standards set by the Declaration of Helsinki, except for registration in a database. Ethical approval was received in advance through the University of Calgary Conjoint Human Research Ethics Board (REB18-0374) and harmonized with the Mount Royal University Human Research Ethics Board (Reference number 101879).

12 healthy participants (5 females) were recruited via verbal communication and provided written and informed consent prior to voluntary participation. Previously published work from our research group has shown that neither age and/or sex greatly affect the variance within the NVC metrics presented herein (Leacy et al., 2022). Therefore, demographic heterogeneity observed within this sample population is not a cofounder for NVC assessment. Prior to data collection and study involvement, a pre-medical questionnaire was completed and reviewed to assess for any pre-existing contraindications to study involvement. Participants were excluded from study participation if they reported any prior or current medical history of neurological, cardiovascular, cerebrovascular and/or pulmonary disease. This expedition employed a rapid, acute ascent profile relative to previously published work from our research group (Leacy et al., 2017; Zouboules et al., 2018; see figure 1A). The maximum altitude reached within this research expedition was 3800m. Herein, Calgary (1130m) was used as our baseline altitude. All baseline data were completed within the human physiology laboratory, Mount Royal University,

over a five-day period prior to HA ascent. Once baseline data collection was completed, all participants flew to Las Vegas, Nevada (610m) where they spent one night. HA data collection was conducted at Barcroft research station (3800m), White Mountain, University of California, USA on day(s) 2 (≤ 24 hours high altitude exposure) and 9 of high altitude residency (see figure 1A). Ascent time to Barcroft research station was approximately 6-8 hours from Las Vegas, allowing for acute, rapid exposure to hypobaric hypoxia. The change in altitude (Δm) was approximately Δ3200m from Las Vegas to Barcroft research station.

4.3.2 Instrumentation

Cardiorespiratory and cerebrovascular measures

Participants were instrumented and kept in the seated position throughout. Beat-by-beat measurement of heart rate (HR; bpm) and arterial blood pressure (systolic/diastolic/mean arterial pressure; SBP/DBP/MAP respectively; mmHg) were measured non-invasively using an electrocardiogram (ECG: lead II configuration; ADInstruments Bioamp ML132; Colorado Springs, CO, USA) and finometer (Omron, model BP786n) respectively. Participants were instrumented with a mouthpiece, nose clip and a two-way valve system, the inspiratory port of which was open to either room air or a bespoke end-tidal forcing system (Airforce software, Pneumologix Consulting Ltd., Kelowna, BC, Canada). The forcing system controls inspiratory O_2 and CO_2 gaseous mixtures by a feedback control and error reduction algorithm to achieve targeted end-tidal values. The end-tidal forcing system was calibrated on the morning of each test day. A pneumotachometer, attached proximal to the two-way valve, was used to measure respiratory flow (HR

800L flow head and spirometer amplifier, ADI ML141). Inspiratory minute ventilation ($\dot{V}_I$; L/min) was calculated as the product of breath-by-breath inspiratory tidal volume ($\dot{V}_{TI}$: Litres) and respiratory rate (R_R; min^{-1}). Breath-by-breath peripheral oxygen saturation was measured using a portable finger pulse oximeter secured to the right index finger ($SpO_{2,}$ %; Oximeter pod, ADI ML320).

Beat-by-beat cerebral blood velocity was measured through the posterior cerebral and middle cerebral artery (PCAv and MCAv respectively; cm/s) using a Transcranial Doppler ultrasound (TCD; Spencer technologies, Redmond, WA, USA). Beat-by-beat measurements of cerebrovascular conductance (CVC) and pulsatility index (PI) were continuously recorded for the MCA and PCA vessels. Where possible, the P2 segment of the PCA was insonated as the perfusion territory of this vessel segment is more proximal to the downstream neuronal pool involved in visual processing, compared with the P1 segment (Panczel et al., 1999). Identification and insonation of intracranial arteries were performed using previously described methods within our group (Leacy et al., 2017, 2022; Bader et al., 2021). TCD was conducted by trained researchers with considerable experience in NVC acquisition. A commercially available headpiece was used to fixate both 2MHz Doppler ultrasound probes to either side of the cranium, insonating through the trans-temporal acoustic window. The trans-temporal window was located approximately 1-2 cm anterior to the external auditory meatus and 1-2 cm superior to the zygomatic arch. The side of the cranium and depth of each vessel insonation was recorded at baseline (1130m) and replicated on days 2 and 9 at altitude (3800m).

Regional cerebral oxygen saturation (rCO_2; %) of the frontal cortex was measured using a Near infrared-spectroscopy (NIRS; INVOS cerebral/somatic oximeter; Somanetics corporation, Michigan, USA). rCO_2 was measured on the

forehead within the ipsilateral hemisphere of the insonated MCA vessel. All cardiorespiratory and cerebrovascular measures were continuously recorded throughout the experimental protocol.

Arterial blood draws were obtained on testing days, sampled from the radial artery and analysed using a portable blood gas analyser (Abbott iSTAT, CG4+ and CHEM8+ cartridges; Mississauga, Ontario). Blood draws allowed for precise measurement of partial pressure of arterial oxygen and carbon dioxide (P_aO_2 and P_aCO_2, respectively; mmHg), arterial bicarbonate (HCO_3^-, mmol/L), base excess (mmol/L), arterial pH, haematocrit (%) and haemoglobin (g/L). For precise measurement, blood gases were corrected for body temperature. Arterial blood gases and pH measures were used to infer acclimatisation status within each participant (Bird et al., 2021).

4.3.3 Experimental Protocol

NVC was assessed in the background of two distinct ventilatory conditions: (a) inspiration of ambient air and (b) inspiratory challenge 1 (specific to location, see figure 1B). Inspiration of ambient air constitutes a normoxic (Patm = 670 mmHg, Inspired PO_2 = 139 mmHg) and poikilocapnic hypoxic (Patm = 487 mmHg, Inspired PO_2 = 99) stimulus at baseline and HA, respectively. The contents of *inspiratory challenge 1*, which followed the ambient air assessment was dependent upon the testing location. At baseline, *inspiratory challenge 1* was an isocapnic-hypoxic stimulus (Patm = 490 mmHg, inspired PO_2 = 103 mmHg). The purpose was to emulate $P_{ET}O_2$ to levels anticipated at Barcroft research station (3800m) by manipulation of inspired PO_2. In contrast, *inspiratory challenge 1* on days 2 and 9 at

3800m was an isocapnic-hyperoxic challenge. Again, the hyperoxic gas mixture served to mimic $P_{ET}O_2$ levels observed at baseline (1130m) by manipulation of inspired PO_2. Exposure to the hyperoxic gas was designed to alleviate the hypoxic stress of HA and return participants to an oxygenation status akin to that observed during normoxia at baseline (1130m). The precise procedural and temporal aspects of the experimental protocol used during each ventilatory challenge are detailed below and shown (see figure 1B for illustration of study methodology).

Participants completed an initial three minute unforced baseline period, in the seated position, inspiring ambient air. Participants were instructed to keep their eyes closed for the duration of this period. Upon completion of baseline, participants were exposed to approximately 180 seconds of optical coherence tomography (OCT) 3D retinal imaging (data not shown; separate investigation). Following completion of OCT imaging, and the establishment of a resting baseline, participants were instructed to close their eyes for a further one-minute epoch immediately prior to NVC assessment. NVC was assessed using five repeated trials of intermittent visual stimulation (30s on/off visual stimulation; VS). VS was elicited using an intermittent strobe light stimulus on a mobile phone app (6Hz; *Strobe* app). Participants were instructed to focus their gaze at the strobe light. Participants were verbally queued when to open/close their eyes with a researcher confirming adherence to verbal instructions. This method of inducing an NVC response has been previously used by our group without any adverse effects to the strobe light stimulus (Leacy et al., 2017, 2022; Bader et al., 2021). Upon completion of VS, participants were asked to sit for a further thirty seconds with their eyes closed. The completion of this thirty-second epoch signalled the end of our NVC protocol for each respective gas challenge. This exact temporal and procedural protocol were repeated for each ventilatory challenge

on each test day. During isocapnic hypo- and hyperoxic gas challenges, the three-minute baseline period did not begin until control of end-tidal gases was achieved (≈60s).

4.3.4 Data analysis protocol

Data acquisition was completed using Labchart software (v8.0). Data was later analysed offline with resultant figures developed using Graphpad prism 8 software. All baseline cardiorespiratory and cerebrovascular measures (see table 1) were obtained by calculating an average measure over a one-minute epoch immediately prior to NVC assessment across each experimental condition. Arterial blood data are derived from point measurements taken throughout each test day (see figure 2).

To demonstrate the spatial specificity of the haemodynamic response during VS, the MCAv NVC response during VS was used as a negative control. The perfusion territory of the MCA is not associated with visual integration and processing and as such should not be responsive to VS with only minor fluctuations in cerebral velocity observed owing to resting cardiorespiratory and autonomic variability. The difference (Δ) in mean MCAv (Δcm/s; Δmean) achieved during VS exposure, relative to a twenty-second epoch immediately prior to VS onset was calculated and compared with the PCA. The magnitude of the MCAv NVC response (Δmean) was measured and presented as an absolute change (Δcm/s) and relative change from baseline (Δ%) (see figure 3). Individual and group averaged PCAv and MCAv waveforms (as seen in figures 3-6) across all experimental conditions were derived using a bespoke macro on Labchart.

NVC was measured and analysed in accordance with previously published work from our group (Leacy et al., 2017, 2022). NVC was quantified as the difference (Δ) in mean and peak PCAv (Δcm/s; Δmean and Δpeak, respectively) achieved during VS exposure, relative to a twenty-second epoch immediately prior to VS onset. We further quantified NVC as the difference (Δ) in total area under the curve (Δcm.s^2; ΔtAUC) of the raw PCAv signal, relative to a thirty-second epoch immediately prior to VS onset. The thirty-second baseline period for tAUC analysis was used to ensure time-equivalency between baseline and VS periods when making total area comparisons. The magnitude of the NVC response for each parameter (Δmean, Δpeak and ΔtAUC) is presented as an absolute change (Δcm/s and Δcm.s^2, respectively) and relative change from baseline (Δ%) (see figure 4). Moreover, the NVC response was sub-compartmentalised into three distinct post-stimulus epochs: acute (0-10 seconds), mid (10-20 seconds) and late (20-30 seconds) post VS exposure. The magnitude of the NVC response (Δmean) was measured for each distinct region and presented as an absolute change (Δcm/s) and relative change from baseline (Δ%) (see figure 5).

4.3.5 Statistical analysis protocol

Statistical analysis was completed using statistical software SPSS (IBM statistics software version 26). All normally distributed data are presented as mean ± standard deviation (SD). In comparison, when the assumption of normal distribution was violated, data are presented as median (IQR; 25th-75th percentile). Two core tests were utilised within our statistical analysis: (1) Repeated measures analysis of variance (ANOVA) and (2) Two-way repeated measures ANOVA. The

precise protocol and considerations taken during application of each test are detailed below.

All baseline cardiorespiratory/cerebrovascular measures, arterial blood gases and NVC metrics were first investigated for normal distribution using a combination of Shapiro-Wilk assessment and visual inspection of Q-Q plots. When data satisfied the assumption of normal distribution a repeated-measures ANOVA with Bonferroni post-hoc assessment was performed. The variable of interest was used as the dependent variable and experimental condition used as a within-subjects factor. Mauchly's test of sphericity was used to determine whether the variance of difference between all combinations of the within-subjects factor was equal. When sphericity was violated a Greenhouse-Geisser or Huynh-Feldt adjustment was performed relative to the estimated Epilson (ε). When data violated the assumption of normal distribution, a non-parametric Friedman rank test equivalent was performed. When data violated the assumption of normal distribution, a manual Bonferroni adjustment was made to account for multiple comparisons and reduce the risk of deducing a type 1 error. This was performed by dividing the alpha level (α) by the number of pairwise comparisons made (i.e., 0.05/number of pairwise comparisons). Effect size for repeated-measures ANOVA and Friedman rank test were determined using partial eta squared (N_p^2) and Kendall's W (W) respectively. Statistical significance was set at $p<0.05$.

A two-way repeated measures ANOVA was used to determine spatial specificity of the haemodynamic response to visual stimulation. Two within-subject factors (Experimental condition and vessel) were used whereas the magnitude of the haemodynamic response to visual stimulation was used as the dependent variable. Several assumptions were determined prior to final analysis; 1) no significant

outliers in combination of the within-subjects' factors, 2) The dependent variable should be normally distributed for each combination of the within-subjects' factors and 3) The variance of differences between levels should be equal (examined using Mauchly's test of sphericity). Results were first interpreted for simple main effects for an interaction effect. Where $p>0.05$ for a vessel*condition interaction effect, main effects were interpreted for the two within-subject factors.

4.4 Results & Figures

The following paragraphs represent the key findings. Graphical and tabular illustrations of the key results presented herein can be found in figures 2-7 and table 1, respectively.

4.4.1 Baseline cardiovascular measures

HR was statistically significantly different across conditions (X^2 (5) = 13.095, p=0.023, W = 0.218). HR increased significantly when comparing normoxia (Calgary) with iso-Hx (Calgary) and poi-Hx (White Mountain Day 2, WM2) (median (IQR); 77.06 (66.18-84.92) vs 83.73 (78.30-90.56) & 81.89 (77.66-91.62) respectively; p=0.034 and 0.034, respectively). MAP was statistically significantly different across conditions (X^2 (5) = 17.810, p=0.033, W = 0.297). MAP increased significantly when comparing normoxia (Calgary) with poi-Hx and iso-Hyperoxia (WM2) (median (IQR); 94.98 (89.35-109.19) vs 108.21 (98.90-118.01) & 107.29 (95.64-116.84) respectively; p=0.032 and 0.005, respectively). SBP was statistically significantly different across conditions (X^2 (5) = 17.095, p=0.003, W = 0.298). SBP increased significantly when comparing normoxia (Calgary) with iso-Hx (Calgary) (median (IQR); 129.19 (121.21-145.72) vs 136.96 (132.51-154.52); p=0.007). DBP was statistically significantly different across conditions ($F_{(2.093,\ 23.021)}$ = 6.626, p=0.005, n_p^2 = 0.376). DBP increased significantly when comparing normoxia (Calgary) with iso-Hx (Calgary), poi-Hx & iso-Hyperoxia (WM2 collectively) (mean (SD); 77.07(10.10) vs 81.86(10.54), 88.98(11.44), 89.33(11.26); p=0.016, 0.024 & 0.009 respectively).

4.4.2 Baseline respiratory measures

R_R was unchanged across conditions (X^2 (5) = 5.762, p=0.330, W = 0.096). $\dot{V}_{TI}$ was statistically significantly different across conditions (F ($_{2.272,\ 24.994}$) = 8.191, p<0.001, n_p^2 = 0.427). $\dot{V}_{TI}$ increased significantly when comparing normoxia (Calgary, mean (SD); 0.95 (0.35)) with iso-Hx (Calgary; mean (SD), p-value, 95% CI; 1.22 (0.53), p=0.038, 0.011-0.524), poi-Hx (WM2; 1.72 (0.54), p=0.037, 0.033-1.508), iso-Hyperoxia (WM2; 1.88 (0.70), p=0.026, 0.085-1.777), poi-Hx (White mountain day 9, WM9; 1.85 (0.54), p<0.001, 0.372-1.677) and iso-Hyperoxia (WM9; 1.89 (0.72), p=0.008, 0.210-1.677). Moreover, statistical differences were observed between poi-Hx (WM9; 1.85 (0.54)) and iso-Hx (Calgary; 1.22 (0.53), p=0.037, 0.029-1.246). $\dot{V}_I$ was statistically significantly different across conditions (X^2 (5) = 19.429, p=0.002, W = 0.324). $\dot{V}_I$ increased significantly when comparing normoxia (Calgary) with poi-Hx/iso-Hyperoxia (WM2) and poi-Hx/iso-Hyperoxia (WM9) (median (IQR); 15.55 (7.86-18.10) vs 29.39 (19.94-33.00), 30.02 (19.58-34.31), 26.77 (19.57-32.01) & 28.69 (20.26-29.85) respectively; p=0.011, 0.001, 0.048 & 0.048 respectively).

S_pO_2 was statistically significantly different across conditions (X^2 (5) = 51.190, p<0.001, W = 0.853). The change in S_pO_2 was statistically significant when comparing normoxia (Calgary, median (IQR); 97.70 (96.89-98.20)) with iso-Hx (Calgary; 86.13 (84.63-87.14), p<0.001) & poi-Hx (WM2; 88.36 (84.59-89.37), p=0.003). Iso-Hyperoxia (WM2; 97.93 (97.42-98.44)) was statistically different from iso-Hx (Calgary; 86.13 (84.63-87.14), p<0.001), poi-Hx (WM2; 88.36 (84.59-89.37), p<0.001) and poi-Hx (WM9; 89.24 (86.88-91.06), p=0.002). Iso-Hyperoxia (WM9; 97.44 (95.50-98.34)) was statistically different from iso-Hx (Calgary; 86.13 (84.63-87.14), p<0.001), poi-Hx (WM2; 88.36 (84.59-89.37), p=0.011). PO_2 was

statistically significantly different across conditions (X^2 (5) = 52.952, p<0.001, W = 0.883). The change in PO_2 was statistically significant when comparing normoxia (Calgary, median (IQR); 103.94 (102.47-107.21) with iso-Hx (60.48 (58.18-61.93), p<0.001), poi-Hx (WM2; 68.32 (66.94-70.21), p=0.001) & poi-Hx (WM9; 70.21 (68.06-72.34), p=0.016). Iso-Hyperoxia (WM2; 102.55 (96.75-112.29)) was statistically different from iso-Hx (Calgary; 60.48 (58.18-61.93), p<0.001), poi-Hx (WM2; 68.32 (66.94-70.21), p=0.003). Iso-Hyperoxia (WM9; 101.35 (97.01-105.43)) was statistically different from iso-Hx (Calgary; 60.48 (58.18-61.93), p<0.001), poi-Hx (WM2; 68.32 (66.94-70.21), p=0.016). PCO_2 was statistically significantly different across conditions (X^2 (5) = 52.368, p<0.001, W = 0.873). Poi-Hx (WM2, median (IQR); 19.15 (17.55-20.85)) was statistically different when compared with iso-Hx (Calgary; 27.29 (23.92-32.80), p=0.002). Poi-Hx (WM9; 17.38 (15.85-19.02)) was statistically different when compared with normoxia (Calgary; 21.17 (19.93-23.12), p<0.001), iso-Hx (Calgary; 27.29 (23.92-32.80), p<0.001) and iso-Hyperoxia (WM2; 20.03 (18.11-21.48), p=0.011). Similarly, iso-Hyperoxia (WM9; 17.25 (15.80-19.13)) was statistically different when compared with normoxia (Calgary; 21.17 (19.93-23.12), p=0.003), iso-Hx (Calgary; 27.29 (23.92-32.80), p<0.001) and iso-Hyperoxia (WM2; 20.03 (18.11-21.48), p=0.048).

$P_{ET}O_2$ was statistically significantly different across conditions (X^2 (5) = 51.190, p<0.001, W = 0.853). The change in $P_{ET}O_2$ was statistically significant when comparing normoxia (Calgary, median (IQR); 87.79 (84.18-90.70)) with iso-Hx (Calgary; 51.59 (51.05-51.91), p<0.001), poi-Hx (WM2; p<0.001) & poi-Hx (WM9; p=0.016). Iso-Hyperoxia (WM2; 85.94 (80.33-93.09)) was statistically different when compared with iso-Hx (Calgary; 51.59 (51.05-51.91), p<0.001) & poi-Hx (WM2; 52.49 (51.63-54.88), p=0.002). Iso-Hyperoxia (WM9; 85.22 (82.05-90.34))

was statistically different when compared with iso-Hx (Calgary; 51.59 (51.05-51.91), p=0.016) & poi-Hx (WM2; 52.49 (51.63-54.88), p=0.002). $P_{ET}CO_2$ was statistically significantly different across conditions (X^2 (5) = 49.095, p<0.001, W = 0.818). Poi-Hx (WM2, median (IQR); 31.60 (30.06-33.45)) was statistically different compared with iso-Hx (Calgary, 35.74 (33.56-39.68), p=0.016). Poi-Hx (WM9; 29.21 (27.67-30.30)) was statistically different compared with normoxia (Calgary; 36.10 (33.82-38.53), p<0.001), iso-Hx (Calgary; 35.74 (33.56-39.68), p<0.001) & iso-Hyperoxia (WM2; 32.53 (31.30-35.09), p=0.002). Iso-Hyperoxia (WM9; 30.36 (28.39-31.08)) was statistically different compared with normoxia (Calgary; 36.10 (33.82-38.53), p=0.002) & iso-Hx (Calgary; 35.74 (33.56-39.68), p<0.001).

4.4.3 Baseline cerebrovascular measures

No significant main effect was found for MCA_v ($F_{(1.884, 18.839)}$ = 0.728, p=0.488, n_p^2 = 0.068), MCA_{cvc} ($F_{(1.923, 19.226)}$ = 0.771, p=0.472, n_p^2 = 0.072), MCA_{sys} ($F_{(1.790, 17.896)}$ = 0.814, p=0.446, n_p^2 = 0.075), MCA_{dia} ($F_{(2.031, 20.307)}$ = 1.227, p=0.315, n_p^2 = 0.109), PCA_v ($F_{(1.865, 20.518)}$ = 1.448, p=0.257, n_p^2 = 0.116), PCA_{cvc} ($F_{(1.735, 19.082)}$ = 0.952, p=0.392, n_p^2 = 0.080) & PCA_{sys} ($F_{(2.250, 24.752)}$ = 0.964, p=0.404, n_p^2 = 0.081). MCA_{PI} was statistically significantly different across conditions ($F_{(2.205, 22.052)}$ = 5.024, p=0.014, n_p^2 = 0.334). The change in MCA_{PI} was statistically significant when comparing normoxia (Calgary, mean (SD); 1.00 (0.12)) with poi-Hx (WM2, mean(SD), p-value, 95% CI; 0.83 (0.13), p=0.013, 0.029-0.283) and iso-Hyperoxia (WM2; 0.83 (0.13), p=0.010, 0.033-0.276). PCA_{PI} was statistically significantly different across conditions (X^2(5) = 16.571, p=0.005, W = 0.276). The change in

PCA_{PI} was statistically significant when comparing normoxia (Calgary, median (IQR); 0.94 (0.84-1.05)) with poi-Hx (WM2; 0.75 (0.71-0.88), p=0.016) and iso-Hyperoxia (WM2; 0.75 (0.72-0.86), p=0.023). PCA_{dia} was statistically significantly different across conditions ($F_{(2.381, 26.192)} = 4.353$, p=0.018, $n_p^2 = 0.284$). The change in PCA_{dia} was statistically significant when comparing normoxia (Calgary, mean (SD); 24.96 (5.86)) with poi-Hx (WM2, mean (SD), p-value, 95% CI; 31.77 (5.54), p=0.038, -13.342-(-)0.273). NIRS was statistically significantly different across conditions ($X^2(5) = 38.698$, p<0.001, $W = 0.704$). The change in NIRS was statistically significant when comparing iso-Hyperoxia (WM2, median (IQR); 76.37 (68.75-80)) with iso-Hx (Calgary; 65.4 (63.33-70), p<0.001), poi-Hx (WM2; 66.85 (58.79-69.75), p=0.001) & poi-Hx (WM9; 64.63 (58.63-70.63), p=0.001). Iso-Hyperoxia (WM9; 76.5 (68.5-78)) was statistically different when compared with iso-Hx (Calgary; 65.4 (63.33-70), p=0.002), poi-Hx (WM2; 66.85 (58.79-69.75), p=0.006) & Poi-Hx (WM9; 64.63 (58.63-70.63), p=0.008).

4.4.4 Arterial blood gases and pH

Changes within arterial blood data are illustrated in figure 2. P_aO_2 was statistically significantly different across time-points ($X^2(2) = 20.667$, p<0.001, $W = 0.861$). The change in P_aO_2 was statistically significant when comparing 1130m (Calgary, median (IQR); 82.00 (79.00-86.25) with 3800m day2 (51.00 (49.25-55.50), p<0.001) and day 9 (54.50 (50.50-58.75), p=0.013). P_aCO_2 was statistically significantly different across time-points ($F_{(1.132, 12.456)} = 18.556$, p<0.001, $n_p^2 = 0.628$). The change in P_aCO_2 was statistically significant when comparing 1130m (mean (SD); 36.46 (5.21)) with 3800m day 2 (mean(SD), p-value, 95% CI: 33.10

(3.35), p=0.002, 1.32-5.40) and 3800m day 9 (mean(SD), p-value, 95% CI: 30.32 (1.81), p=0.003, 2.24-10.04).). 3800m day 2 (33.10 (3.35)) was statistically different when compared with 3800m day 9 (mean(SD), p-value, 95% CI; 30.32 (1.81), p=0.015, -5.40(-)1.32). HCO_3^- was statistically significantly different across time-points ($F_{(2, 22)} = 24.197$, $p<0.001$, $n_p^2 = 0.687$). The change in HCO_3^- was statistically significant when comparing 1130m (mean (SD); 23.65 (2.33)) with 3800m day 2 (mean (SD), p-value, 95% CI: 21.95 (2.36), p=0.012, 0.37-3.01) and 3800m day 9 (mean (SD), p-value, 95% CI: 19.61 (1.46), p<0.001, 2.13-5.96). 3800m day 2 (21.95 (2.36)) was statistically different when compared with 3800m day 9 (mean (SD), p-value, 95% CI: 19.61 (1.46), p=0.006, 0.69-4.00). Base excess was statistically significantly different across time-points ($F_{(2, 22)} = 24.264$, $p<0.001$, $n_p^2 = 0.688$). The change in base excess was statistically significant when comparing 1130m (mean (SD); -0.75 (2.05)) with 3800m day 2 (mean (SD), p-value, 95% CI: -2.38 (2.56), p=0.033, 0.12-3.13) and 3800m day 9 (mean (SD), p-value, 95% CI: -4.92 (1.56), p<0.001, 2.40-5.93). 3800m day 2 (-2.38 (2.56)) was statistically different when compared with 3800m day 9 (-4.92 (1.56), p=0.007, 0.73-4.36). Arterial pH was not statistically significantly different across time-points ($F_{(2, 20)} = 1.439$, p=0.261, $n_p^2 = 0.126$). Haematocrit was statistically significantly different across time-points ($F_{(2, 22)} = 27.073$, $p<0.001$, $n_p^2 = 0.711$). The change in haematocrit was statistically significant when comparing 1130m (mean (SD); 43.33 (3.77)) with 3800m day 9 (mean (SD), p-value, 95% CI: 46.66 (3.11), p<0.001, -4.65-(-)2.02). 3800m day 2 (43.75 (3.11)) was statistically different when compared with 3800m day 9 (46.66 (3.11), p<0.001, -4.329-(-)1.51). Haemoglobin was statistically significantly different across time-points ($F_{(2, 20)} = 22.202$, $p<0.001$, $n_p^2 = 0.689$). The change in haemoglobin was statistically significant when comparing 1130m

(mean (SD); 147.91 (13.31)) with 3800m day 9 (mean (SD), p-value, 95% CI: 158.55 (11.03), p<0.001, -15.22-(-)6.05). 3800m day 2 (149.55 (10.64)) was statistically different when compared with 3800m day 9 (158.55 (11.03), p<0.001, -13.51-(-)4.49).

4.4.5 Region-specific haemodynamic response

No significant vessel*condition simple main effects were found for 1130m (Δcm/s; $F_{(1,10)} = 0.242$, p=0.633, $n_p^2 = 0.024$ & Δ%; $F_{(1,10)} = 4.131$, p=0.070, $n_p^2 = 0.292$). Main effects found a significant effect for vessel insonation (Δcm/s; $F_{(1,10)} = 65.082$, p<0.001, $n_p^2 = 0.867$ & Δ%; $F_{(1,10)} = 187.767$, p<0.001, $n_p^2 = 0.949$). The magnitude of the haemodynamic response during VS was significantly greater within the PCA, compared with the MCA, during normoxia (p<0.001) and iso-Hx (p<0.001) conditions at 1130m. No significant vessel*condition simple main effects were found for 3800m day 2 (Δcm/s; $F_{(1,11)} = 0.775$, p=0.397, $n_p^2 = 0.066$ & Δ%; $F_{(1,11)} = 1.985$, p=0.187, $n_p^2 = 0.153$). Main effects found a significant effect for vessel insonation (Δcm/s; $F_{(1,11)} = 71.116$, p<0.001, $n_p^2 = 0.866$ & Δ%; $F_{(1,11)} = 71.667$, p<0.001, $n_p^2 = 0.867$). The magnitude of the haemodynamic response during VS was significantly greater within the PCA, compared with the MCA, during normoxia (p<0.001) and iso-hyperoxia (p<0.001) conditions at 3800m day 2. No significant vessel*condition simple main effects were found for 3800m day 9 (Δcm/s; $F_{(1,10)} = 0.099$, p=0.759, $n_p^2 = 0.010$ & Δ%; $F_{(1,10)} = 0.367$, p=0.558, $n_p^2 = 0.035$). Main effects found a significant effect for vessel insonation (Δcm/s; $F_{(1,10)} = 64.923$, p<0.001, $n_p^2 = 0.867$ & Δ%; $F_{(1,10)} = 94.954$, p<0.001, $n_p^2 = 0.905$). The magnitude of the haemodynamic response during VS was significantly greater within the PCA,

compared with the MCA, during normoxia ($p<0.001$) and iso-hyperoxia ($p<0.001$) conditions at 3800m day 9.

4.4.6 Neurovascular coupling across experimental conditions

NVC response magnitude was not statistically different across conditions for each metric; ΔMean (Δcm.s; $F_{(2.383, 26.218)} = 1.457$, $p=0.251$, $n_p^2 = 0.117$ & Δ%; $F_{(2.14, 23.54)} = 0.917$, $p=0.419$, $n_p^2 = 0.077$), ΔPeak (Δcm.s; $F_{(2.243, 24.671)} = 1.424$, $p=0.260$, $n_p^2 = 0.115$ & Δ%; $F_{(2.01, 22.07)} = 1.21$, $p=0.317$, $n_p^2 = 0.099$), ΔtAUC (Δcm.s^2; $F_{(2.297, 22.969)} = 1.641$, $p=0.214$, $n_p^2 = 0.141$ & Δ%; $X^2(5) = 9.857$, $p=0.079$, $W = 0.179$), ΔAcute NVC response (Δcm.s; $X^2(5) = 6.532$, $p=0.258$, $W = 0.119$ & Δ%; $X^2(5) = 5.048$, $p=0.410$, $W = 0.084$), ΔMid NVC response (Δcm.s; $X^2(5) = 5.130$, $p=0.400$, $W = 0.093$ & Δ%; $X^2(5) = 10.273$, $p=0.068$, $W = 0.187$), ΔLate NVC response (Δcm.s; $F_{(5, 50)} = 1.749$, $p=0.141$, $n_p^2 = 0.149$ & Δ%; $F_{(2.207, 22.069)} = 2.267$, $p=0.123$, $n_p^2 = 0.185$).

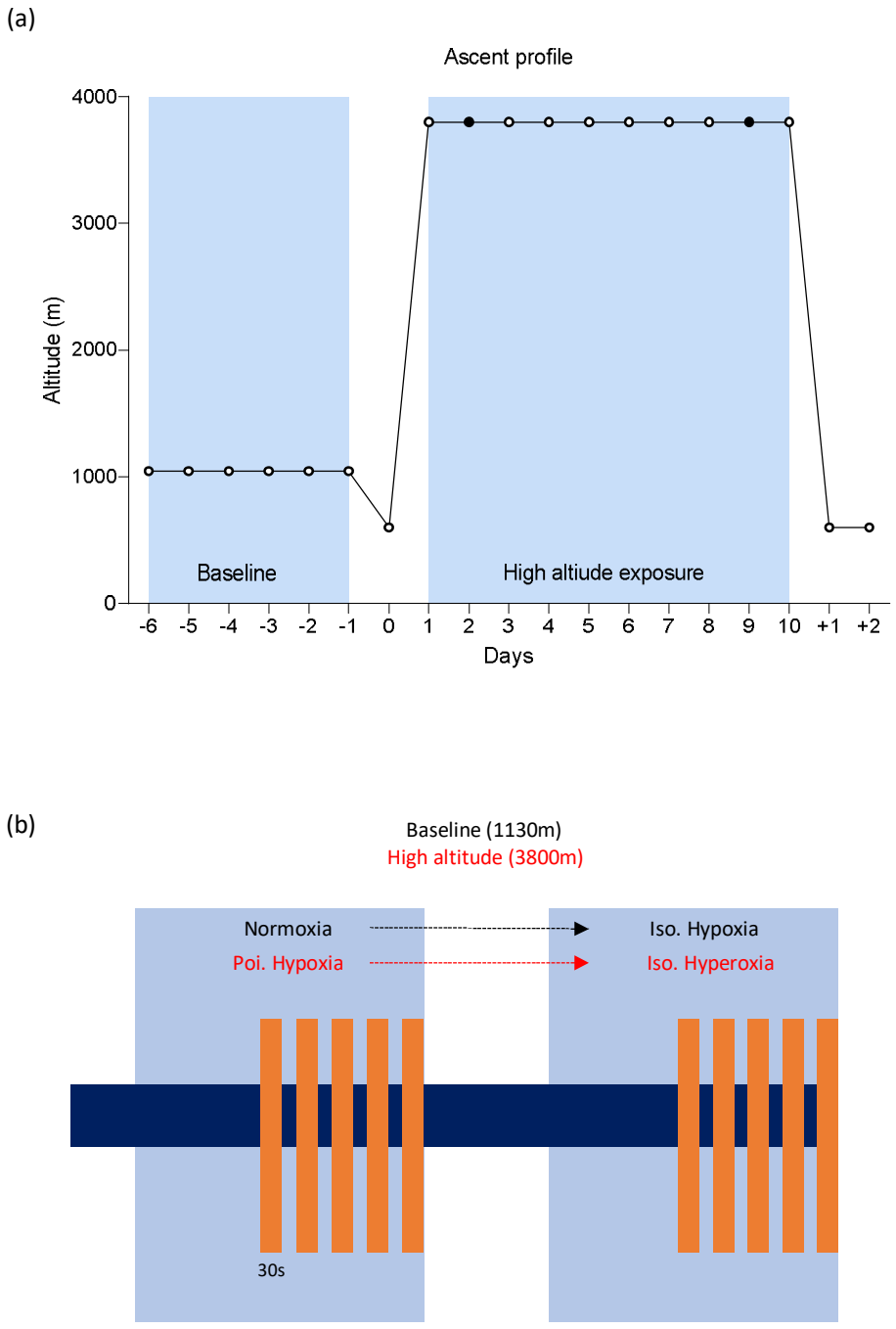

Figure 1 Ascent profile & study methodology. Illustrations of the ascent profile & inherent study methodology used within this research expedition are provided (A and B, respectively). **A:** Baseline measures were collected over a five day period at 1130m (Calgary, Canada) followed by an overnight stay at 600m (Las Vegas, Nevada). Participants sojourned by car from 600m to 3800m (Barcroft research station, California, USA) over a 6-8 hour period. Measurements were taken on day 2 and 9 of high altitude

exposure (3800m; black dots) before returning to Las Vegas. **B:** An illustration of the precise methodology employed is provided. Prevailing gas conditions for sea-level and high altitude test days are provided in black and red, respectively. Orange shaded boxes denote periods of 30 second visual stimulation.

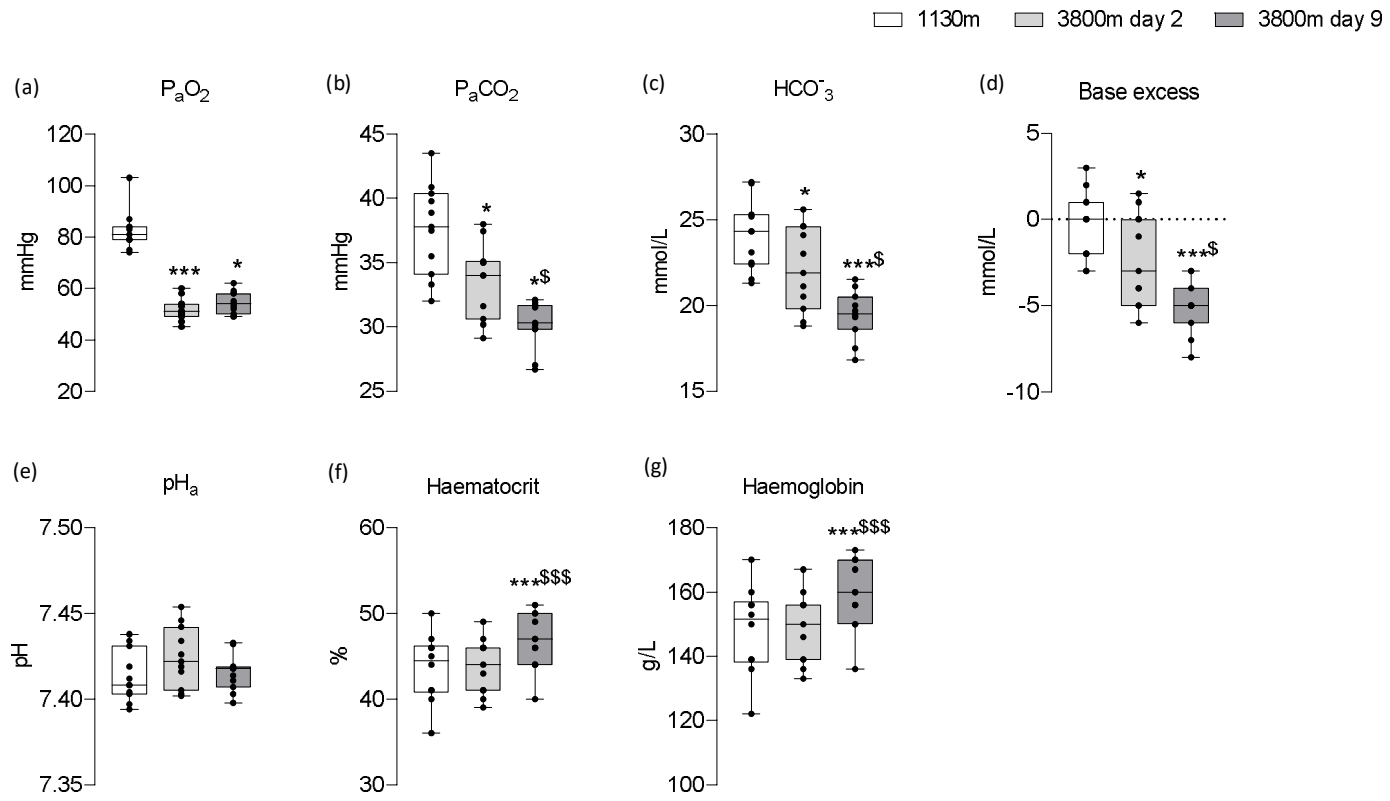

Figure 2 Arterial blood draw data across locations. Measurements of arterial oxygen concentration (PaO_2; **a**), arterial carbon dioxide concentration ($PaCO_2$; **b**), arterial bicarbonate (HCO_3^-; **c**), base excess (**d**), arterial pH (**e**), Haematocrit (**f**) and Haemoglobin (**g**) are provided across test days. Measurements for

1130m, 3800m day 2 and 3800m day 9 are provided in clear, light grey and dark grey boxes respectively. Data is presented in boxplot format showcasing mean ± SD. */*** = $p<0.05/<0.001$ from 1130m respectively; $^{\$/\$\$\$}$ = $p<0.05/<0.001$ from 3800m day 2 respectively

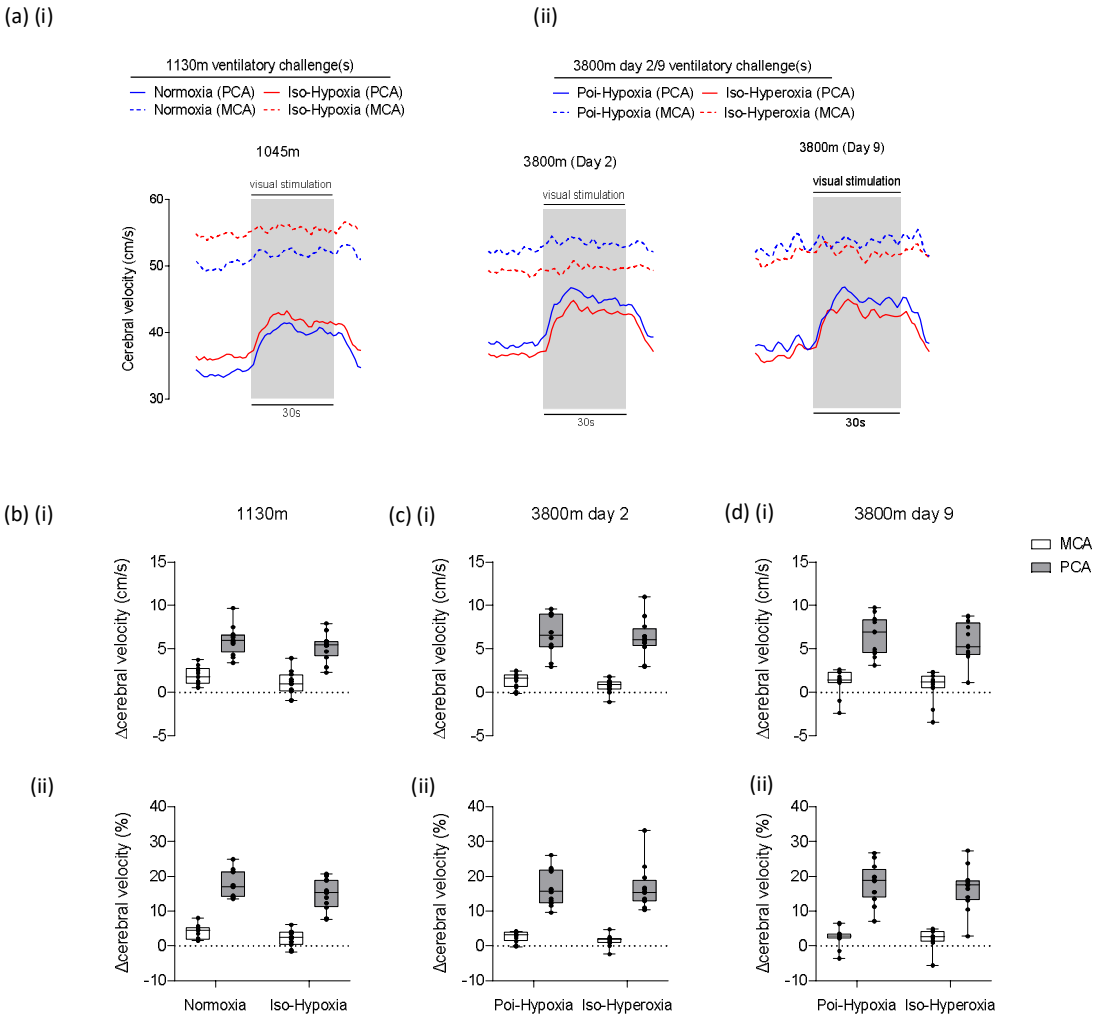

Figure 3 Spatial specificity of the visually evoked haemodynamic response. (A) Group averaged waveforms during visual stimulation (grey region) for middle and posterior cerebral arteries (MCA/PCA respectively) are provided for each gas challenge at 1130m (i) and 3800m (ii). Cerebral velocity is presented along the y-axis. MCA waveforms are presented as dotted lines. PCA waveforms are presented as continuous lines. **(B-D)** The magnitude of the haemodynamic response, within the MCA (clear boxes) and PCA (grey boxes), to visual stimulation is presented. Experimental conditions are presented along the x-axes. Y-axes represent the magnitude of the response as a change in absolute units (Δcm/s; top row) and as a relative change from baseline (Δ%; bottom row). Data is presented in boxplot format with mean ± SD. *** = P<0.001 from gas-matched MCA.

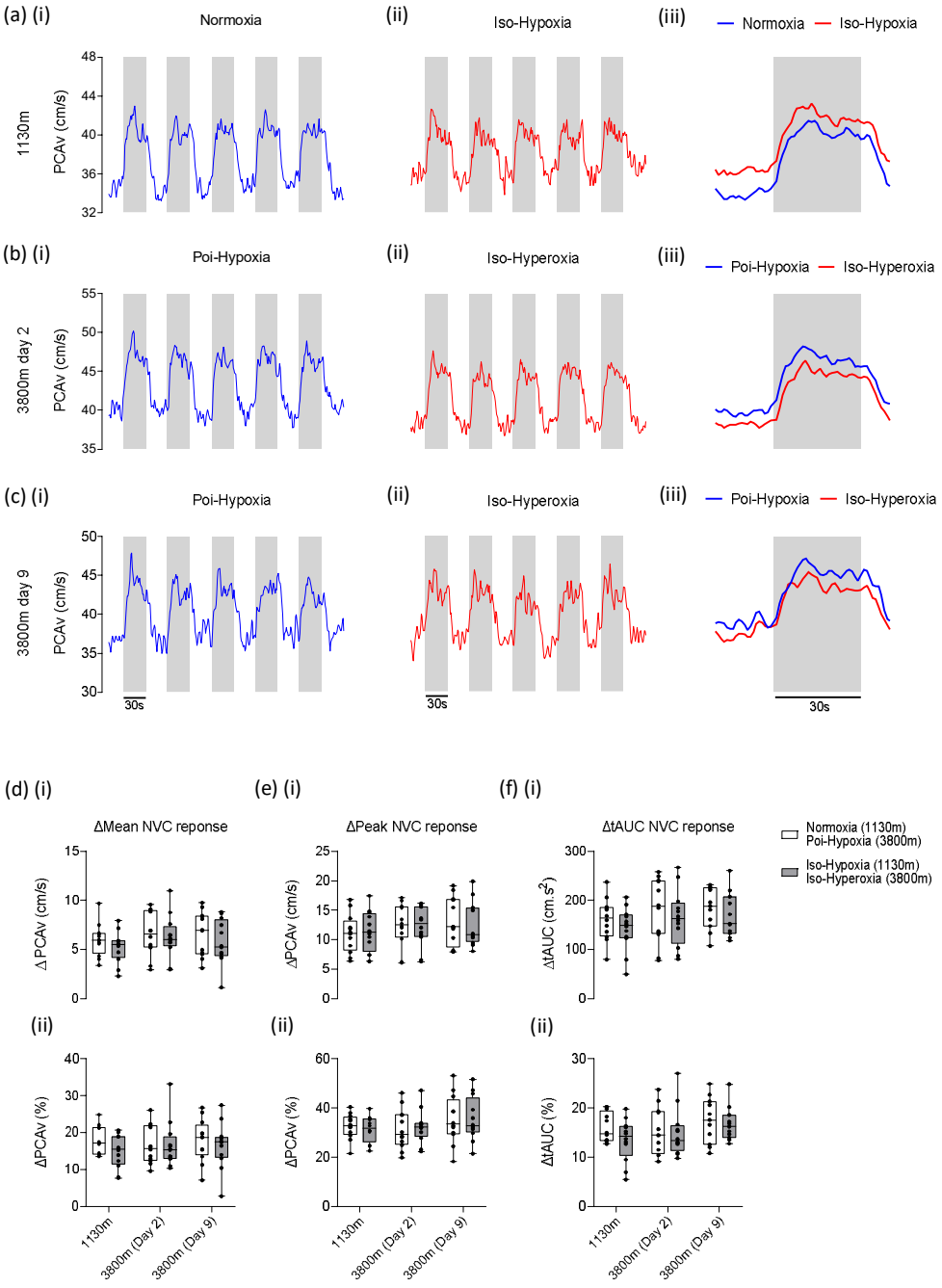

Figure 4 Neurovascular coupling waveform(s) and response magnitude between experimental conditions. (a-c) Group averaged waveforms of the posterior cerebral artery velocity (PCAv) during each visual stimulus (a-ci-ii), and the average PCAv response during visual stimulus (a-ciii), are presented for each experimental condition. PCAv is presented along the y-axes. **(d-f)** The magnitude of the haemodynamic response to visual stimulation is presented for each NVC metric; ΔMean (di-ii); ΔPeak (ei-ii); ΔtAUC (fi-ii). Test locations

are presented along the x-axes. Y-axes represent the magnitude of the response as a change in absolute units (Δcm/s; top row) and as a relative change from baseline (Δ%; bottom row). Data is presented in boxplot format with mean ± SD.

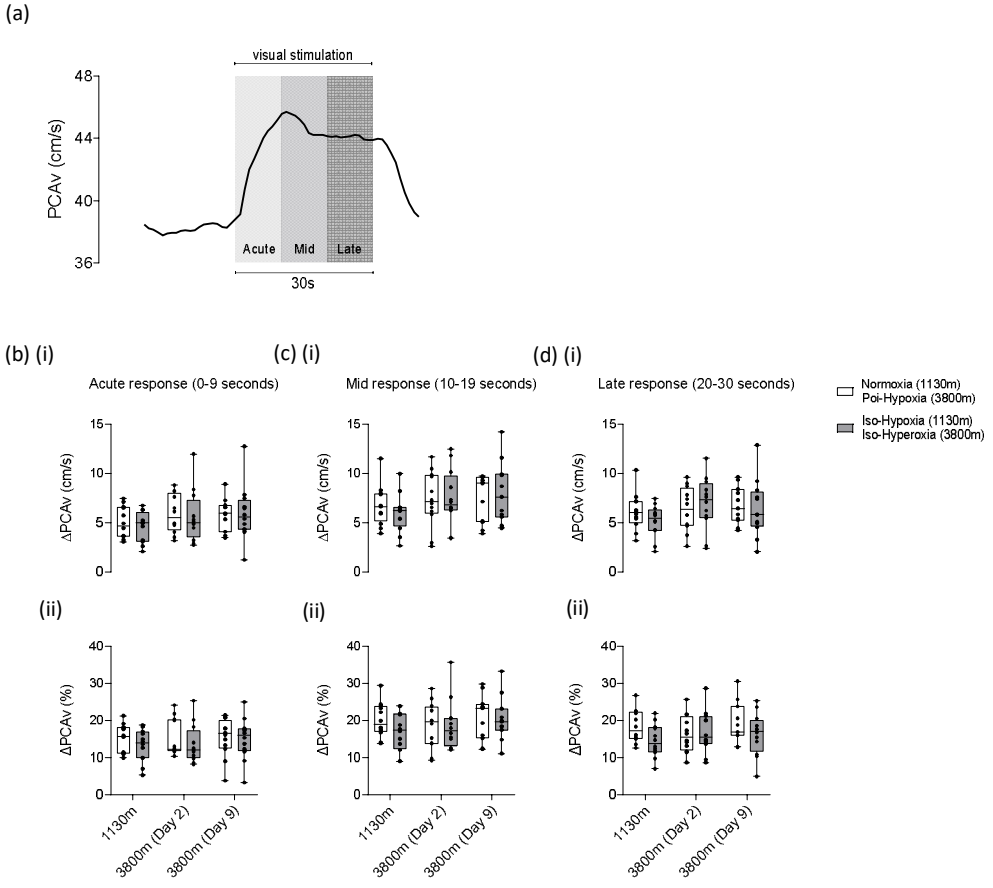

Figure 5 Temporal-dependent regional analysis of the neurovascular coupling response across experimental conditions. (a) Group averaged haemodynamic response within the posterior cerebral artery during visual stimulation. Distinct temporal regions of the haemodynamic response, relative to the onset of visual stimulation, are illustrated. PCAv is presented along the y-axis **(b-d)** The magnitude of the haemodynamic response to visual stimulation is presented for each temporal region; ΔAcute (bi-ii); ΔMid (ci-ii); ΔLate (di-ii). Test locations are presented along the x-axes. Y-axes represent the magnitude of the response as a change in absolute units (Δcm/s; top row) and as a relative change from baseline (Δ%; bottom row). Data is presented in boxplot format with mean ± SD.

Figure 6 Individual posterior cerebral artery velocity waveforms during repetitive visual stimulation across all conditions. An illustration of the individual NVC response within the

posterior cerebral artery is provided for all experimental conditions. Grey shaded regions denote 30-second periods of visual stimulation. Subject ID and PCAv are presented along the y-axes. Intra-individual y-axis ranges are consistent across experimental conditions. Experimental conditions are grouped according to test location (1130m, 3800m day 2 and day 9).

Baseline cardiorespiratory and cerebrovascular measures									
		Calgary		White Mountain Day 2		White Mountain Day 9		One-way RM ANOVA/Friedman test	
		Normoxia (n=12)	Iso-Hx (n=12)	Poi-Hx (n=12)	Iso-Hyperoxia (n=12)	Poi-Hx (n=12)	Iso-Hyperoxia (n=12)	p-value	Effect size
Cardiorespiratory	**HR (bpm)**	77.06 (66.18-84.92)	83.73 (78.30-90.56)*	81.89 (77.66-91.62)*	78.25 (75.03-87.79)	82.95 (75.24-92.81)	77.74 (76.45-92.27)	**0.023**	0.218
	MAP (mmHg)	94.98 (89.35-109.19)	100.84 (97.22-114.71)	108.21 (98.90-118.01)*	107.29 (95.64-116.84)*	106.89 (95.64-116.84)	103.49 (90.49-117.59)	**0.003**	0.297
	SBP (mmHg)	129.19 (121.21-145.72)	136.96 (132.51-154.52)*	138.89 (132.63-156.41)	141.81 (128.59-157.36)	135.36 (126.72-149.88)	131.36 (122.73-154.10)	**0.003**	0.298
	DBP (mmHg)	77.07 ± 10.10	81.86 ± 10.54*	88.98 ± 11.44*	89.33 ± 11.26*	86.79 ± 9.96	84.56 ± 11.72	**0.005**	0.376
	R_R (min^{-1})	15.55 (12.86-17.84)	17.80 (15.01-19.76)	16.47 (12.37-19.72)	14.49 (13.26-15.62)	14.42 (11.82-19.59)	15.29 (12.65-18.09)	0.330	0.096
	V_{TI} (L)	0.95 ± 0.35	1.22 ± 0.53*	1.72 ± 0.54*	1.88 ± 0.70*	1.85 ± 0.54*$^{\&}$	1.89 ± 0.72*	**0.001**	0.427
	$\dot{V}_I$ (L/min)	15.55 (7.86-18.10)	20.74 (11.93-28.88)	29.39 (19.94-33.00)*	30.02 (19.58-34.31)*	26.77 (19.57-32.01)*	28.69 (20.26-29.85)*	**0.002**	0.324
	SpO_2 (%)	97.70 (96.89-98.20)	86.13 (84.63-87.14)*	88.36 (84.59-89.37)*	97.93 (97.42-98.44)$^{\&\%}$	89.24 (86.88-91.06)$^{\$}$	97.44 (95.50-98.34)$^{\&\%}$	**<0.001**	0.853
	$P_{ET}O_2$ (mmHg)	87.79 (84.18-90.70)	51.59 (51.05-51.91)*	52.49 (51.63-54.88)*	85.94 (80.33-93.09)$^{\&\%}$	55.42 (53.86-58.19)*	85.22 (82.05-90.34)$^{\&\%}$	**<0.001**	0.853
	$P_{ET}CO_2$ (mmHg)	36.10 (33.82-38.53)	35.74 (33.56-39.68)	31.60 (30.06-33.45)$^{\&}$	32.53 (31.30-35.09)	29.21 (27.67-30.30)*$^{\&\$}$	30.36 (28.39-31.08)*$^{\&}$	**<0.001**	0.818
Cerebrovascular	**MCAv (cm/s)**	50.63 ± 13.12	57.52 ± 19.71	55.66 ± 11.15	52.53 ± 9.19	54.51 ± 11.24	55.46 ± 11.62	0.488	0.068
	MCA_{CVC}	0.52 ± 0.13	0.54 ± 0.15	0.51 ± 0.11	0.48 ± 0.08	0.53 ± 0.10	0.55 ± 0.11	0.472	0.072
	MCA_{PI}	1.00 ± 0.12	0.92 ± 0.12	0.83 ± 0.13*	0.83 ± 0.13*	0.93 ± 0.18	0.88 ± 0.16	**0.014**	0.334
	$MCAv_{Sys}$	84.44 ± 24.08	90.96 ± 29.20	85.70 ± 17.29	80.58 ± 14.95	89.26 ± 14.75	87.29 ± 15.94	0.446	0.075
	$MCAv_{Dia}$	33.61 ± 7.86	38.71 ± 12.54	39.77 ± 9.12	37.32 ± 7.17	38.97 ± 8.38	39.63 ± 8.74	0.315	0.109
	PCAv (cm/s)	37.25 ± 8.55	40.88 ± 11.72	44.33 ± 8.11	37.67 ± 13.56	42.39 ± 8.08	42.86 ± 8.04	0.257	0.116
	PCA_{CVC}	0.38 ± 0.08	0.38 ± 0.09	0.40 ± 0.05	0.38 ± 0.06	0.40 ± 0.05	0.42 ± 0.06	0.392	0.080
	PCA_{PI}	0.94 (0.84-1.05)	0.83 (0.79-1.08)	0.75 (0.71-0.88)*	0.75 (0.72-0.86)*	0.79 (0.69-1.09)	0.79 (0.65-0.96)	**0.005**	0.276
	$PCAv_{Sys}$	60.53 ± 12.42	63.65 ± 14.92	66.00 ± 10.41	62.28 ± 11.78	65.98 ± 12.93	65.47 ± 12.67	0.404	0.081
	$PCAv_{Dia}$	24.96 ± 5.86	27.46 ± 7.96	31.77 ± 5.54*	30.08 ± 5.78	29.93 ± 5.15	30.73 ± 5.59	**0.018**	0.284
	NIRS (%)	72 (70-77.5)	65.4 (63.33-70)	66.85 (58.79-69.75)	76.37 (68.75-80)$^{\&\%}$	64.63 (58.63-70.63)$^{\$}$	76.5 (68.5-78)$^{\&\%@}$	**<0.001**	0.704

Table 1 Participant baseline cardiorespiratory and cerebrovascular measures across experimental conditions. Group-averaged baseline values for cardiorespiratory and cerebrovascular variables are provided for each experimental condition. Experimental conditions are grouped according to test location (Calgary; White Mountain Day 2 or 9). Data which were normally distributed are presented as mean ± SD. In contrast, data which violated normal distribution are presented as median (25^{th}-75^{th} percentile). P-values for repeated measures analysis of variance (parametric data) or Friedman ranks test (non-

parametric data) are provided. Effect sizes refer to partial eta squared (N_p^2; parametric data) or Kendall's W (*W*; non-parametric data). * = p<0.05 from Normoxia; & = p<0.05 from Iso-Hx (Calgary); % = p<0.05 from Poi-Hx (White Mountain Day 2); $ = p<0.05 from Iso-Hyperoxia (White Mountain Day 2); @ = p<0.05 from Poi-Hx (White Mountain Day 9).

4.5 Discussion

The major findings of this study are that neither (1) removal of the hypoxic vasodilatory stimulus at HA nor (2) acute and sustained exposure to 3800m affect NVC of the PCA in healthy participants. An illustration of the stability of the NVC response across all conditions for each participant is shown in figure 6.

4.5.1 Changes within the cardiorespiratory and cerebrovascular parameters across all experimental conditions

Table 1 characterises the changes in cardiorespiratory and cerebrovascular parameters across all experimental conditions. Several key physiological adaptions to acute and sustained exposure to HA can be observed.

When compared with room air at 1130m (normoxia), acute and sustained exposure to HA induced a systemic and central hypoxia evidenced by significant reductions in SpO_2, $P_{ET}O_2$ and NIRS (see table 1). To mitigate the risk of hypoxic insult, a series of integrative cardiorespiratory and cerebrovascular adaptations are initiated. The reduction in PaO_2 levels associated with HA exposure is detected by the carotid body and gives rise to the peripheral chemoreflex. O_2-sensitive type I glomus cells housed within the carotid body increase afferent signalling to the respiratory control centres during hypoxic conditions (Wilson & Teppema, 2016; Parmenter & Powell, 2016; Lopez-Barneo et al., 2015; Dempsey et al., 2013; Teppema et al., 2010; Powell et al., 2000). The net result is an increase in ventilatory drive which partially restores O_2 levels. This response is known as the hypoxic ventilatory response (HVR) and is evidenced herein by significant increases in inspiratory tidal volume ($\dot{V}_{TI}$) and minute ventilation ($\dot{V}_I$) following acute exposure

and sustained residency at HA (see table 1). A similar respiratory phenotype is observed following acute exposure to Iso-Hx at 1130m (see table 1). Notably, the change $\dot{V}_I$ is driven predominantly by changes in respiratory volume and not rate. No further increases in $\dot{V}_I$ were observed between days 2 and 9 at 3800m. This is likely a result of the temporal heterogeneity in ventilatory acclimatisation to chronic hypoxia within the sample population.

It's well recognised that HA is a potent cardiac stressor. Acute exposure to HA elicited an immediate increase in heart rate (tachycardia) (Batsch et al., 2007; Naeije et al., 2010; Ke et al., 2017; riley et al., 2017). This immediate response ensures preservation of O_2 delivery to systemic tissue during hypoxic conditions. When compared with normoxia at 1130m there was an immediate and significant increase in HR following acute Iso-Hx exposure at 1130m and on day 2 at HA with no significant differences found on day 9 at HA (see table 1). The mechanisms which drive this cardiac response are multifaceted. A large proportion is attributed to the shift in sympathovagal balance following hypoxic activation of the peripheral chemoreceptors (Batsch et al., 2007; Naeije et al., 2010; Ke et al., 2017; riley et al., 2017).

Blood pressure (BP) response to HA exposure is often varied across research expeditions. BP responses to HA are reportedly dependent upon 1) the net interaction between hypoxic vasodilation and sympathetic vasoconstriction of systemic vessels, 2) absolute altitude of exposure, 3) individual physiological responses to HA and 4) duration of stay at altitude (Riley et al., 2017; Ke et al., 2017). When compared with normoxia at 1130m, our results show a significant increase in DBP and MAP following acute exposure to HA with no significant differences observed by day 9 at HA (see table 1). Moreover, no significant changes in SBP were observed during

acute exposure to and residency at HA (see table 1). The disparate response to BP found herein was likely a result of the inter-individual response to hypoxia coupled with the large variability within each BP parameter (SBP, DBP and MAP). The increase in BP parameters following acute exposure to HA agrees with previous literature which examined correlative changes in BP and AMS following acute and prolonged exposure to 3700m in healthy men (Liu et al., 2014).

No significant differences were found for indices of cerebral blood velocity (MCAv and PCAv) across all experimental conditions (see table 1). This observation was unexpected as dramatic and acute increases in cerebral blood velocity following acute exposure to HA and hypoxia are well recognised (Severinghaus et al., 1966; Wilson et al., 2009; Ainslie & Ogoh, 2010; Ainslie et al., 2014). Stability in resting cerebral blood velocity might suggest that the interaction between hypoxic vasodilation and hyperventilation-induced hypocapnic-vasoconstriction are in equilibrium such that the net effect on CBF is unchanged. However, this explanation would not apply to the Iso-Hx condition at 1130m where $P_{ET}CO_2$ levels were clamped from normoxic conditions. Baseline variability in both MCAv and PCAv might explain the non-significant difference between normoxia and Iso-Hx conditions.

4.5.2 Changes in arterial blood draws throughout the expedition

Changes within ABGs following acute exposure and residency at HA are illustrated in figure 2. The observed changes detail the integrative physiological adaptation to HA and mirror previous work from our group (Leacy et al., 2018). Acute exposure to HA elicited an immediate reduction in PaO_2 consequent to the

environmental hypobaric hypoxia (see figure 2a). Evidence of the respiratory reflex (HVR) to hypoxia is evidenced by significant reductions in $PaCO_2$ on days 2 and 9 at HA (see figure 2b). The stepwise reductions in $PaCO_2$ between days 2 and 9 despite a fixed HA stimulus illustrate plasticity within the respiratory control neural axis, known as ventilatory acclimatisation to hypoxia. Following chronic exposure to hypoxia (as observed during HA exposure), there is an increase in both O_2-sensitivity at the level of the carotid body as well as enhanced central integration within the respiratory control centres (Bisgard et al., 1986; Vizek et al., 1987; Barnard et al., 1987; Nielsen et al., 1988; Dwinell & Powell, 1999; Powell et al., 2000; F. Powell, 2007). The net effect is an increase in ventilatory output for a given PaO_2 stimulus. We infer evidence of ventilatory acclimatisation to sustained hypoxia with concurrent reductions in $PaCO_2$ at 3800m.

Metabolic compensation is necessary owing to the profound respiratory response detailed above. A relative metabolic acidosis is achieved through the renal system and involves significant reductions in arterial HCO_3^- levels through enhanced urine secretion (see figure 2c) (Palubiski et al., 2020; Zouboules et al., 2018; Goldfarb-Rumyantzev et al., 2014). This integrative respiratory-renal interaction facilitates the maintenance of arterial pH levels within narrow limits (see figure 2e). Lastly, following chronic exposure (day 9) to HA there is an increase in both haematocrit and haemoglobin levels (see figures 2f and 2g). This response is well recognised and is consequent to a time-dependent increase in erythropoiesis. This protective mechanism acts to increase circulatory O_2 carrying capacity mitigating the risk of hypoxic injury with chronic exposure to HA. In summary, ABGs provide a detailed illustration of the multi-system integrative physiological response to HA which acts to preserve homeostasis.

4.5.3 Region-specific haemodynamic response to intermittent visual stimulus

As expected, the haemodynamic response to intermittent visual stimulation was region-specific. The magnitude of the haemodynamic response is significantly greater within the PCA compared with the MCA across all experimental conditions (see figure 3). Throughout this investigation, the MCA was used as a negative control for quality control purposes and assurances of technical proficiency. The physiological explanation for the stimulus-dependent haemodynamic response relates to the perfusion territory of both vessels. At a broad level, the MCA perfuses regions of the frontal and temporal lobes, whereas the PCA perfuses posterior regions of the brain, including the occipital lobe, where visual processing occurs. As such, we would not expect a haemodynamic change within the MCA vessel during visual stimulation.

4.5.4 NVC response following acute removal of systemic and central hypoxia at high altitude

An important consideration when examining the role of HA exposure on NVC is the degree to which hypoxia might act to preserve NVC vasodilatory capacity countering the influence of multiple cerebral vasoconstrictors (hypocapnia, alkalosis). Lab-based investigations have demonstrated significant NVC impairment following acute hyperventilation-induced hypocapnia and alkalosis (Szabo et al., 2011). Unlike HA literature, Szabo and colleagues (2011) investigation was not confounded by the competing effects of hypoxia during hypocapnia and subsequent pH changes. Hypoxia is a well-recognised cerebral vasodilator. hypoxic-vasodilation of cerebral vessels is mediated by a series of erythrocyte-signalling pathways

consequent to the allosteric shift from their relaxed ('R') to active ('T') state (Hoiland et al., 2105; Ainslie et al., 2016). Deoxyhaemoglobin serves to increase nitric oxide (NO) bioavailability through S-nitrosohaemoglobin dependent bioactivity, deoxyhaemoglobin-mediated reduction of nitrite to NO and ATP-mediated activation of endothelial nitric oxide synthase (Hoiland et al., 2105; Ainslie et al., 2016).

To date, the extent to which hypoxia protects NVC function at HA is unknown. Using hyperoxic inspiratory gas mixtures, our investigation removed the systemic and central hypoxic stimulus on days 2 and 9 at HA. We reasoned that this experimental condition would alleviate the hypoxic vasodilation and hence facilitate a pro-vasoconstrictive state at HA through relative hypocapnia and allow us to examine the extent to which hypoxia-dependent vasodilation protects NVC function at HA. Importantly, indices of peripheral ($P_{ET}O_2$, SpO_2) and cerebral oxygenation (NIRS) were comparable between normoxia at 1130m and Iso-hyperoxia challenges on days 2 and 9 at HA (see table 1). This observation provides assurances that the competitive vasodilatory stimulus at HA has been acutely removed. However, as shown in figures 4 and 5 no significant differences were found when comparing NVC indices across conditions. This observation suggests that hypoxic vasodilation does not protect against NVC impairment in the background of a HA-induced relative hypocapnia. This is the first study to characterise the potentially protective effects of hypobaric hypoxia on NVC.

4.5.5 NVC response during acute, and sustained high altitude exposure

The stability in NVC following exposure to HA was in agreement with previous comparable literature which employed TCD for the assessment of NVC at HA (Caldwell et al., 2017, Leacy et al., 2018, Lefferts et al., 2020). The previous publications employed incremental (Leacy et al., 2018, Lefferts et al., 2020) and acute (Caldwell et al., 2017) ascent profiles reaching peak altitudes of 4240m (Leacy et al., 2018, Lefferts et al., 2020) and 3800m (Caldwell et al., 2017). NVC was assessed using a milieu of stimulus paradigms ranging from visual stimulation (Caldwell et al., 2017, Leacy et al., 2018) to region-specific cognitive tasks (Caldwell et al., 2017; Lefferts et al., 2020) while insonating the PCA (Caldwell et al., 2017; Leacy et al., 2018) and the ACA/MCA (Lefferts et al., 2020) respectively. With respect to the previous literature, the time-differential between HA exposure and NVC assessment, or the ascent profile employed did not allow for examination of the respiratory-induced acid-base disturbance during acute HA exposure on NVC.

The rate of ascent within each incremental study (Leacy et al., 2018; Lefferts et al., 2020) was slow, allowing sufficient time for full acclimatisation and metabolic compensatory mechanisms to occur throughout ascent. This was exemplified within Leacy et al., (2018) where arterial blood draw analysis revealed a stable arterial pH with progressive ascent to 4240m. Arterial blood draws were not performed within Lefferts et al., (2020) but based upon similarities within the ascent profile, one could reason that participants were fully acclimatized to HA. Caldwell et al., (2017) incorporated a rapid ascent profile, akin to the ascent profile used herein. Participants ascended from a baseline altitude of 344m to 3800m in an acute fashion inducing a profound HA exposure stimulus. NVC measurements were recorded on days 3 and 7 following HA residency. Arterial blood gases were not recorded during this

expedition to characterise arterial pH status at the time of NVC assessment. It is likely that the time differential (3 days) between initial exposure and NVC assessment was sufficient to allow full metabolic compensation of respiratory-induced alkalosis.

Our hypothesis was based on our understanding of how acid-base disturbances affect cerebrovascular function. It's well recognised that alterations in pH can manipulate cerebrovascular tone in a direction-dependent manner (Dietrich et al., 1994; Apkon et al., ,1997; West et al., 1999; Chul Kim et al., 2004). For example, respiratory-induced alkalosis is a profound cerebral vasoconstrictor and routinely observed at HA because of the HVR. Under normoxic conditions, respiratory-induced alkalosis induces an immediate reduction in CBF (Raichle & Plum, 1972; Mirro et al. ,1992; Szabo et al., 2011; Bader et al., 2021). There's still some debate as to whether this vascular reactivity is driven through pH- and/or CO_2-mediated mechanisms (Yoon et al., 2012). Changes in cerebrovascular tone and consequently CBF are achieved through functional manipulation of membrane bound ion channels/receptors, signalling molecules and intracellular enzymes within vascular smooth muscle and endothelial cells (West et al., 1999; Aalkjaer & Peng, 1997; Wray et al., 2004; Celotto et al., 2008; Yoon et al., 2012; Boedtkjer et al., 2018). At the point of data collection herein, only one lab-based study had demonstrated that hyperventilation-induced respiratory alkalosis significantly impaired NVC in human participants (Szabo et al., 2011). Our hypothesis explored whether similar observations to Szabo and colleagues (2011) would be found consequent to the respiratory-induced alkalosis following acute exposure to HA.

Our investigation attempted to overcome the temporal issues raised within each of the previous HA publications by employing an acute ascent profile (Δ3200m

in approximately 6-8hrs) with NVC assessment completed within ≤24 hrs of HA exposure. We hypothesized that metabolic compensation of the respiratory-induced alkalosis would not have occurred within this timeframe. This methodological approach would allow us to characterise the pre- vs. post-acclimatisation effects on NVC indices. As shown in figure 4 and 5, no NVC impairment was observed between experimental conditions, including normoxia at 1130m and days 2/9 of HA exposure. A possible explanation for this observation is evident within the arterial blood analysis (see figure 2). Group-averaged data reveals that arterial pH was normalised at the point of NVC measurement on days 2 and 9 of HA exposure The correction of pH by means of increased bicarbonate excretion within 24h was unexpected (Bird et al., 2021).

Therefore, our study did not assess NVC against the backdrop of respiratory alkalosis, which we anticipated would result in impairment in NVC. However, a follow-up study by our research group utilised an acute stepwise hyperventilation strategy to achieve progressive hypocapnia and subsequent respiratory alkalosis (Bader et al, 2021 NVC in response to intermittent VS was measured through the PCA at each hypocapnic stage. Given the acute nature of the hyperventilation, it is likely that NVC was assessed against the background of an acute respiratory-induced alkalosis. The results demonstrated negligible effects of stepwise hypocapnia on NVC parameters. This observation was in direct contradiction to the findings of Szabo et al., (2011). Further research is required in this area to better elucidate the impact of acid-base regulation on NVC.

Overall, these investigations offer mixed reports on the effects of systemic pH disturbance on NVC performance. Of interest, both studies demonstrated deleterious effects of hyperventilation-induced hypocapnia and subsequent alkalosis on the peak haemodynamic response to visual stimulation. However, the impairment observed in Bader et al., (2011) was minor and although observed at -5 Torr ($_{ET}CO_2$), it was not further impaired in -10 Torr. Given our growing mechanistic understanding for the temporal involvement of feedforward and feedback pathways within the NVC response (Hoiland et al., 2020; Iadecola et al., 2017) it is possible that stressors including respiratory induced pH deflections, might target specific components of the NVC response rather than affect the magnitude of the response in its entirety.

4.5.6 Summarising the effects of high altitude exposure on NVC

Overall, the current study and the existing literature suggests that NVC remains intact throughout HA exposure and that hypoxic vasodilation does not protect against HA-induced NVC impairment. In reconciling the existing state of the literature, two schools of thought can be proposed. The first suggests that there is no interaction between HA as a stressor and the physiological pathways integral to NVC function in healthy participants. The second view highlights the utmost importance of NVC to neurophysiological function despite the profound environmental and physiological stress which accompanies HA exposure. Other facets of CBF regulation (cerebrovascular reactivity and/or autoregulation) have shown significant vulnerability to HA exposure, which demonstrates that CBF control mechanisms are not impervious to manipulation (Jansen et al., 2000; Iwasaki et al., 2011; Lucas et

al., 2011). However, NVC shows remarkable stability in the face of HA stress, and acute and chronic blood gas and acid-base manipulation.

4.5.7 Study Limitations and future recommendations

The major findings reported herein are applicable within the confines of the study methodology and parameters measured. The application of TCD examines NVC response within the large conduit cerebral arteries which project along the cortical surface and reside within the subarachnoid space. Whether these observations extend to the deeper cerebral microvascular network is unknown. However, this would require more advanced neuroimaging techniques which are neither readily available nor portable for the purposes of HA expedition research.

4.5.8 Conclusion

In conclusion, the NVC response to intermittent photic stimulation within the PCA remains intact despite (a) removal of the hypoxic vasodilatory stimulus at HA and (b) acute exposure and residency at 3800m. This work highlights remarkable functional stability within the neurovascular network despite exposure to profound physiological stressors.

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4.5.10 Author contribution statement

JKL, TAD & KOH designed the experiments. JKL, DPB, NJ, CB, BH, TV, GF, AR, GA, CR performed data acquisition throughout the research expedition. JKL analysed the data in consultation with EFL, TAD and KOH. JKL drafted the manuscript with critical revision by KOH and TAD. All authors reviewed the manuscript before submitting for publication.

4.5.11 Declaration of conflicting interests

The author(s) declare no conflicts of interest with respect to the research investigation, authorship and/or publication of this manuscript.

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Chapter 5. Discussion of Key Thesis Findings, Recommendations for Future Research and Conclusions

5.1 Summary of major findings

Based on the observations made within this thesis we deduce that: (1) Neither age nor sex greatly affect the variability within the visually evoked NVC response in human participants aged 21-66yrs and (2) The visually evoked NVC response remains intact despite acute and incremental exposure to HA. All conclusions were made within healthy human participants. This thesis addressed several knowledge gaps concerning the effects of HA exposure on NVC.

5.2 Contribution to the field

Collectively, our findings have made a valuable contribution into the effects of both healthy aging and sex (chapter 2) and HA on NVC (chapter 3 and 4).

- There is notable disparity in the collective recognition of how aging affects cerebrovascular control. Several lines of investigation support age-related impairment of NVC, whereas an equal number of studies refute this rhetoric. Our investigation points towards methodological issues in study design and analytical approach as the likely contributors to this noted disparity. The assortment and comparison of participants across arbitrary age ranges as a means of deducing age-related dysfunction is incorrect and vulnerable to bias. We overcame this issue with sophisticated regression analysis in order to characterise the true extent of variability within NVC attributable to age and/or sex. This approach eliminates the risk of expressing and potentially

misinterpreting group-mean differences commonly observed within the literature. Our comprehensive investigation found that neither age nor sex greatly contributed to the observed variance across several NVC metrics. The significance of this observation is useful for small, field-based investigations (e.g., high altitude expeditions) wherein demographic heterogeneity can be observed within the sample population.

- At the onset of this thesis there were no prior publications that characterised the effects of HA on NVC. All previous investigations focused on either cerebrovascular reactivity (CVR) and/or cerebral autoregulation (CA) as an index of cerebrovascular function. At present (circa. December 2022), there are only four publications that have quantified the effects of HA exposure on NVC., two of which are presented herein (chapter 3 and 4). On this basis, our research group has made a substantial contribution to the understanding of how NVC responds across multiple exposure paradigms to HA. Employing well-designed experiments, we demonstrated that NVC remains intact despite incremental (chapter 3) and acute (chapter 4) exposure to HA (4240m and 3800m respectively). Furthermore, our group demonstrated that acute removal of systemic and central hypoxia at HA does not affect dynamics of the NVC response (chapter 4). Collectively, our work demonstrates a remarkable stability in NVC despite the profound physiological stress of HA. These investigations provide a framework for future experimental assessment of NVC at HA.

5.3 Recommendations for future research

To date, the effects of HA exposure on NVC have only been characterised to a peak altitude of 4240m (Leacy et al., 2018; Lefferts et al., 2020). Therefore, the conclusion that NVC remains stable during progressive HA exposure can only be applied to an altitude of 4240m. The current lack of evidence for altitudes >4240m is largely attributable to logistical difficulties in study design. There is only one research laboratory where NVC can be appropriately investigated above 4240m (The Pyramid laboratory, Nepal Himalaya, 5050m). It would be interesting to assess whether an inflection point or threshold exists, above which NVC function begins to deteriorate. This could arise for several reasons: (1) physiological difficulties in acclimatisation, (2) chronic sleep disturbances and (3) progressive disruption of arterial blood gases and acid-base homeostasis. Additionally, indices of cognitive function and AMS should be recorded in tandem to determine whether overall health and performance at HA is associated with NVC stability.

As discussed in chapter 4, our investigation employed an acute ascent profile to 3800m in an attempt to capture pre- vs. post-acclimatisation measures of NVC. Our research group characterises acclimatisation status based upon pH maintenance. Unexpectedly, the metabolic compensation (increased renal secretion of HCO_3^-) occurred more rapidly than anticipated. This negated our capacity to measure and compare the effects of acclimatisation status on NVC. Future studies should consider two possible solutions to assess this hypothesis. The first involves supplementation with an exogenous HCO_3^- source throughout HA ascent and residency. In theory this supplementation should curtail/inhibit the acclimatisation process by preventing metabolic compensation of pH. The second approach involves better control of time under HA exposure. Researchers should consider staggering ascent times of smaller

participant cohorts to reduce the risk of redundant time spent under exposure of each participant. This strategy could be used to characterise the importance of acclimatisation status on NVC stability.

Finally, there is still an abundance of work required from a methodological standpoint when employing the TCD technique. The capacity to discern between accurate vs. inaccurate insonation of cerebral vessels renders the field vulnerable to high variability and limited reproducibility in research findings. More work is required to better characterise poor responders vs. inaccurate vessel insonation in human participants. The issues with reproducibility are best highlighted within the age-related research cited herein. Multiple studies employed identical methodologies with respect to sample population, vessel insonation, experimental stimulus and yet found conflicting results. In order to progress the field, we need to improve our capacity to detect poor technical application of TCD. Several solutions which can be implemented within the study methodology and data analysis are proposed:

- Examination of baseline variability in cerebral blood velocity (CBv) compared with the magnitude of the haemodynamic response to region specific tasks could be a useful method of discerning true NVC responses vs. changes in CBv associated with baseline cardiorespiratory and autonomic variability.

- The insonation of auxiliary vessels which may be used as negative controls. An example could be bilateral MCA insonation during hemisphere specific tasks. The magnitude of the haemodynamic response within each vessel should be quantified and compared. Theoretically, the haemodynamic

response should be greater within the vessel perfusing the activated hemisphere.

- The development of a prerequisite catalogue of NVC metrics to be analysed. To date there is limited inter-study coherence in analytical approach. Several studies focus on examination of the peak haemodynamic response, whereas others focused on the mean haemodynamic response to cognitive specific tasks. The field would greatly benefit from the development of a standardised battery of indices relevant to the NVC response. This would facilitate greater cross comparison between studies and undoubtedly improve reproducibility within the field.

- The development of a methodological framework would undoubtedly improve reproducibility within the field. Inter-study experimental set-up varies across: (1) inter-stimulus epoch length, (2) stimulus selection (checkerboard, strobe light, executive function tasks, motor paradigms), (3) inter-stimulus epochs (30s on/off vs. 30s/60s on/off), (4) auxiliary physiological measures recorded (Finometer, ECG, capnography, spirometry and (5) participant position (sitting, supine). The development of an experimental handbook which details the recommended experimental set-up for each of the above parameters, including expected mean haemodynamic responses across healthy participants would greatly advance the field.

The inclusion and/or development of the aforementioned would greatly improve technical proficiency and reproducibility within the field.

5.3 Limitations

Several limitations must be acknowledged when interpreting the results presented herein.

- The utility of TCD as a measurement tool has several inherent limitations. It does not provide a direct measurement of flow, but instead quantifies changes in CBv as an indirect surrogate for flow. Although this is routinely expressed as a limitation, there is now considerable evidence that demonstrates the linearity between cerebral blood flow and velocity. Therefore, the application of TCD during HA expeditions is extremely useful due to its portability and non-invasive nature compared with other methods of CBF assessment. It is therefore appropriate that this technique was employed relevant to the nature of the study design and research question. Additionally, the principles which govern the Doppler principle assume a constant diameter of the insonated vessel. This might increase the risk of misinterpreting the vasoactive response during conditions where changes in vessel calibre are expected. Previous experimental work has shown that hypoxia and/or HA exposure causes vasodilation of resting calibre in cerebral vessels. This could pose a threat to the application of TCD as a measurement technique.

- High altitude studies pose several logistical difficulties in terms of sample size and available techniques. Our sample size was comparable with the available literature (chapter 3; n=10, chapter 4; n=14). In addition, controlling for the effects of physical exertion, sleep disturbances and substantial dietary changes and dehydration during HA expeditions are often neglected.

- The conclusions made herein are specific to the occipital territory and PCA vessel using intermittent visual stimulation. Therefore, we have no insight into whether HA exposure and aging/sex might affect NVC and neurovascular function at the microvascular level, or across other regions of the cerebral tissue (frontal, temporal and parietal lobes). However, more advanced neuroimaging techniques would be required to ascertain these answers which is beyond the capacity of TCD. Several investigations have attempted to characterise the magnitude of the NVC response within the MCA vessel territories. However, consequent to the large perfusion territory of the vessel it is difficult to determine the appropriate stimulus which would induce a reliable and reproducible NVC response.

5.5 Conclusion

In conclusion, this thesis demonstrates that NVC within the occipital territory remains stable across multiple exposure paradigms to HA in healthy human participants. This finding is contradictory to other facets of CBF control (CVR and CA), which demonstrated significant vulnerability to HA exposure. The disparity appears to showcase the relative importance of maintaining NVC during HA exposure, in comparison with CVR and/or CA. Aberrant NVC control has been observed across several pathologies associated with poor cognitive and neurophysiological function. Therefore, its maintenance could be considered paramount despite the multimodal stress associated with HA. Moreover, this thesis concludes that variability within the NVC response is not directly attributable to demographic (age and/or sex) heterogeneity in healthy human participants aged 21-

66yrs. This observation has important application for future field based NVC research.

Conference Proceedings and Oral Communications

1. **Jack K. Leacy.,** Lavoie, L., Johnson, E., Sheehan, D., O'Halloran, K., Sharma N, & Day, T. (2017). MRU active office study: The effects of an active office workstation on the magnitude of neurovascular coupling. Abstr. Royal Academy of Medicine in Ireland section of biomedical sciences annual meeting 2017, Trinity college Dublin, June 22nd.

2. **Jack K. Leacy.,** Lavoie, L., Johnson, E., Sheehan, D., O'Halloran, K., Sharma N, & Day, T. (2017). MRU active office study: The effects of an active office workstation on the magnitude of neurovascular coupling. Abstract New horizons symposium 2017, University College Cork, December 7th.

3. Bruce CD, Saran G, Pfoh JR, **Jack K. Leacy**, Mann C, Peltonen J, Zouboules S, O'Halloran KD, Sherpa MT and Day TA (2018). Steady state chemoreflex drive does not predict voluntary breath hold duration during acclimatization to altitude**.** Okanagan Cardiovascular and Respiratory Symposium, March 15-17, 2018. Silver Star Mountain Resort, Vernon BC.

4. **Jack K. Leacy,** Shae M. Zouboules, Heidi Nysten, Carli R. Mann, Joel D.B. Peltonen, Cassandra E. Nysten, Tom D. Brutsaert, Ken D. O'Halloran, Mingma T. Sherpa and Trevor A. Day (2018). The Neurovascular Coupling Response Remains Intact During Incremental Ascent to High Altitude (4370m) in Acclimatized Healthy Volunteers. DNNI/CNSC, Brain connections 2018 public event, March 10, 2018. University College Cork.

5. **Jack K. Leacy**, Shae M. Zouboules, Heidi Nysten, Carli R. Mann, Joel D.B. Peltonen, Cassandra E. Nysten, Tom D. Brutsaert, Ken D. O'Halloran, Mingma T. Sherpa and Trevor A. Day (2018). The Neurovascular Coupling Response Remains Intact During Incremental Ascent to High Altitude (4370m) in Acclimatized Healthy Volunteers. Experimental Biology Symposium, April 21-25, 2018. San Diego, CA, USA.

6. **Jack K. Leacy**, Shae M. Zouboules, Heidi Nysten, Carli R. Mann, Joel D.B. Peltonen, Cassandra E. Nysten, Tom D. Brutsaert, Ken D. O'Halloran, Mingma T. Sherpa and Trevor A. Day (2018). The Neurovascular Coupling Response Remains Intact During Incremental Ascent to High Altitude (4370m) in Acclimatized Healthy Volunteers. New Horizons 2018, University College Cork, 6th December.

7. **Jack K. Leacy,** Linares AM, Zouboules SM, Rampuri, Z, Herrington B, Mann L, Soriano JE, Thrall S, Bird J, Kalker A, Brutsaert TD, O'Halloran KD, Day TA (2019). Cardiorespiratory hysteresis during incremental high-altitude ascent-descent quantifies the magnitude of ventilatory acclimatization.

Abstr. Extreme Physiology. September 2-4, 2019. Portsmouth, UK.

8. **Jack K. Leacy**, David P. Burns, Nicholas Jendzjowsky, Connor Braun, Brittney Herrington, Richard Wilson, Tyler Vermeulen, Glen Foster, Alexander Rosenberg, Garren Anderson, Caroline Rickards, Eric F. Lucking, Ken D. O'Halloran, Trevor A. Day (2021). Investigating the effects of acute exposure to high altitude (3800m), and O_2 manipulation, on neurovascular coupling. Experimental Biology symposium (e-conference), April 27th, 2021.

9. **Jack K. Leacy**, David P. Burns, Nicholas Jendzjowsky, Connor Braun, Brittney Herrington, Richard Wilson, Tyler Vermeulen, Glen Foster, Alexander Rosenberg, Garren Anderson, Caroline Rickards, Eric F. Lucking, Ken D. O'Halloran, Trevor A. Day (2021). Investigating the effects of acute exposure to high altitude (3800m), and O_2 manipulation, on neurovascular coupling. Future of Physiology symposium (e-conference), April 19-22nd, 2021.

10. **Jack K. Leacy**, David P. Burns, Nicholas Jendzjowsky, Connor Braun, Brittney Herrington, Richard Wilson, Tyler Vermeulen, Glen Foster, Alexander Rosenberg, Garren Anderson, Caroline Rickards, Eric F. Lucking, Ken D. O'Halloran, Trevor A. Day (2021). Investigating the effects of acute exposure to high altitude (3800m), and O_2 manipulation, on neurovascular coupling. Experimental Biology symposium (e-conference), April 27th, 2021. Short communication.

11. **Jack K. Leacy**, David P. Burns, Nicholas Jendzjowsky, Connor Braun, Brittney Herrington, Richard Wilson, Tyler Vermeulen, Glen Foster, Alexander Rosenberg, Garren Anderson, Caroline Rickards, Eric F. Lucking, Ken D. O'Halloran, Trevor A. Day (2021). Investigating the effects of acute exposure to high altitude (3800m), and O_2 manipulation, on neurovascular coupling. International Society for arterial chemoreception (ISAC), June 28th, 2022. Short communication

Teaching Contributions

Throughout my doctoral training I have availed of the opportunity to contribute to teaching and learning across undergraduate and postgraduate programmes (listed below). This has provided me with invaluable experience into scientific teaching and learning at a third level institution.

1. PL3001 – BSc Medical and Health Sciences (Invited lectures)
2. CG6002 - MSc Stroke Rehabilitation (Invited lecture)
3. MD3003/MD3016 - BSc Paramedic studies (module coordinator 2018-2022)
4. DS7100 - PG Oral surgery (Invited lecture)
5. Biol 3205 – Human Physiology II (Invited lecture, Mount Royal University)

List of Publications During Doctoral Training

1. **Leacy, J. K**., Johnson, E. M., Lavoie, L. R., Macilwraith, D. N., Bambury, M., Martin, J. A., Lucking, E. F., Linares, A. M., Saran, G., Sheehan, D. P., Sharma, N., Day, T. A., & O'Halloran, K. D. (2022). Variation within the visually evoked neurovascular coupling response of the posterior cerebral artery is not influenced by age or sex. *Journal of applied physiology (Bethesda, Md.: 1985)*, *133*(2), 335–348. https://doi.org/10.1152/japplphysiol.00292.2021

2. Bader, T. J., **Leacy, J. K**., Keough, J., Ciorogariu-Ivan, A. M., Donald, J. R., Marullo, A. L., O'Halloran, K. D., Jendzjowsky, N. G., Wilson, R., & Day, T. A. (2021). The effects of acute incremental hypocapnia on the magnitude of neurovascular coupling in healthy participants. *Physiological reports*, *9*(15), e14952. https://doi.org/10.14814/phy2.14952

3. **Leacy J. K.** (2021). Lessons from on high: arterial CO_2, not pH, is the key mediator of cerebrovascular function. *The Journal of physiology*, *599*(18), 4247–4248. https://doi.org/10.1113/JP281999

4. Bird, J. D., **Leacy, J. K.,** Foster, G. E., Rickards, C. A., Wilson, R., O'Halloran, K. D., Jendzjowsky, N. G., Pentz, B. A., Byman, B., Thrall, S. F., Skalk, A. L., Hewitt, S. A., Steinback, C. D., Burns, D., Ondrus, P., & Day, T. A. (2021). Time course and magnitude of ventilatory and renal acid-base acclimatization following rapid ascent to and residence at 3,800 m over nine days. Journal of applied physiology (Bethesda, Md: 1985), 130(6), 1705–1715. https://doi.org/10.1152/japplphysiol.00973.2020

5. Marullo, A. L., **Leacy, J. K.,** O'Halloran, K. D., & Day, T. A. (2021). Ascending the gut-brain axis: does the microbiome affect acclimatization to high altitude? *Experimental physiology*, *106*(3), 583–584. https://doi.org/10.1113/EP089406

6. **Leacy, J. K.,** Linares, A. M., Zouboules, S. M., Rampuri, Z. H., Bird, J. D., Herrington, B. A., Mann, L. M., Soriano, J. E., Thrall, S. F., Kalker, A., Brutsaert, T. D., O'Halloran, K. D., Sherpa, M. T., & Day, T. A. (2021). Cardiorespiratory hysteresis during incremental high-altitude ascent-descent quantifies the magnitude of ventilatory acclimatization. *Experimental physiology*, *106*(1), 139–150. https://doi.org/10.1113/EP088488

7. **Leacy, J. K.,** O'Halloran, K. D., & Day, T. A. (2020). Simulating the space station: a launch pad for new explorations in integrative physiology. *The Journal of physiology*, *598*(12), 2285–2286. https://doi.org/10.1113/JP279848

8. **Leacy, J. K.,** & O'Halloran, K. D. (2020). Corticomotor control of airway calibre in obstructive sleep apnoea syndrome. *Experimental physiology*, *105*(2), 234–235. https://doi.org/10.1113/EP088336

9. **Leacy, J. K.,** Day, T. A., & O'Halloran, K. D. (2020). Carbonic anhydrase inhibition and chemoreflex control of breathing: A litmus test for methazolamide as a viable alternative to acetazolamide. Experimental physiology, 105(2), 230–231. https://doi.org/10.1113/EP088238

10. **Leacy, J. K.,** Day, T. A., & O'Halloran, K. D. (2019). Is alkalosis the dominant factor in hypoxia-induced cognitive dysfunction? *Experimental physiology*, *104*(10), 1443–1444. https://doi.org/10.1113/EP087967

11. **Leacy, J. K.,** & O'Halloran, K. D. (2019). Cortical control of upper airway calibre: It's the thought that counts! *Experimental physiology*, *104*(6), 789–790. https://doi.org/10.1113/EP087681

12. **Leacy, J. K.,** Zouboules, S. M., Mann, C. R., Peltonen, J., Saran, G., Nysten, C. E., Nysten, H. E., Brutsaert, T. D., O'Halloran, K. D., Sherpa, M. T., & Day, T. A. (2018). Neurovascular Coupling Remains Intact During Incremental Ascent to High Altitude (4240 m) in Acclimatized Healthy Volunteers. *Frontiers in physiology*, *9*, 1691. https://doi.org/10.3389/fphys.2018.01691

13. Bruce, C. D., Saran, G., Pfoh, J. R., **Leacy, J. K.,** Zouboules, S. M., Mann, C. R., Peltonen, J., Linares, A. M., Chiew, A. E., O'Halloran, K. D., Sherpa, M. T., & Day, T. A. (2018). What Is the Point of the Peak? Assessing Steady-State Respiratory Chemoreflex Drive in High Altitude Field Studies. *Advances in experimental medicine and biology*, *1071*, 13–23. https://doi.org/10.1007/978-3-319-91137-3_2

14. Huff, A., Day, T. A., English, M., Reed, M. D., Zouboules, S., Saran, G., **Leacy, J. K.,** Mann, C., Peltonen, J., O'Halloran, K. D., Sherpa, M. T., & Pitts, T. (2019). Swallow-breathing coordination during incremental ascent to altitude. *Respiratory physiology & neurobiology*, *265*, 121–126. https://doi.org/10.1016/j.resp.2018.06.005

15. Cates, V. C., Bruce, C. D., Marullo, A. L., Isakovich, R., Saran, G., **Leacy, J. K.,** O Halloran, K. D., Brutsaert, T. D., Sherpa, M. T., & Day, T. A. (2022). Steady state chemoreflex drive captures ventilatory acclimatization during incremental ascent to high altitude: Effect of acetazolamide. *Physiological reports*, *10*(23), e15521. https://doi.org/10.14814/phy2.15521

Additional Physiology Training

- **Clinical Neurophysiologist (September 2020 – present):** In conjunction with my doctoral studies, I have operated as a clinical neurophysiologist within the Department of Pain Management & Neuromodulation, Mater Private Hospital, Cork. My major responsibilities include examination of the somatosensory nervous system across several chronic pain phenotypes. This experience has afforded me the opportunity to apply some of the physiological understanding I have developed throughout my doctoral training. Our ambition is to develop an active clinical research unit focused on improving our understanding of chronic pain and the pathophysiology which drives this debilitating disease.

Current Active Research Projects

- **Characterising neurovascular function and its interaction with cognitive status in mild cognitive impairment and subtypes of dementia:** Dementia and neurodegenerative disease(s) are associated with an increased burden, both physically and psychologically, on both the patient and their support network. There is growing evidence which supports a causal role for neurovascular dysfunction in the pathogenesis of neurodegenerative disease(s). However, there is a dearth of literature which characterises neurovascular dysfunction between neurodegenerative cohorts in human participants. The purpose of this study is to perform non-invasive assessments of neurovascular function, cerebral blood flow control and cognitive status in clinical cohorts of mild cognitive impairment and dementia subtypes (vascular, frontotemporal, Lewy bodies and Alzheimer's), compared with age-matched controls. Non-invasive neurovascular assessments will be performed using transcranial Doppler ultrasound (TCD), in tandem with electroencephalogram (EEG). Cognitive function is assessed electronically using a battery of region-specific cognitive tasks. The findings of this study allow us to investigate functional differences in cerebral haemodynamics, brain blood flow control processes, spatial and temporal differences in neural recruitment patterns and

amplitudes.

- **Characterising the effects of female sex hormones on cerebral blood flow control:** Aberrant CBF control is becoming increasingly implicated within neurodegenerative and cerebrovascular pathologies. Cerebrovascular disease is a major cause of morbidity and mortality among women, with increased risk among post-menopausal women. Sex differences in the prevalence of cerebrovascular disease indicates a possible role for ovarian sex hormones in the health and function of the cerebrovascular system. There is currently a dearth of literature which characterises the effects of circulating sex hormones and menopausal status on CBF control among women. The purpose of this study is to characterise the implications of menstrual and menopausal status on CBF control within healthy female participants. Non-invasive assessments of NVC, CVR and CA will be assessed during the late follicular and mid-luteal phase of a given menstrual cycle using TCD. Quantitative analysis of circulating sex hormones (oestrogen and progesterone) will be determined on the same test day. This investigation could provide important insight into the implications of menstrual and menopausal status on CBF control and overall cerebrovascular health in women.

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