EXAMINING THE EFFECTS OF MODERATE INTENSITY EXERCISE AND HIGH INTENSITY INTERVAL TRAINING ON CEREBRAL BLOOD FLOW AND NEUROVASCULAR SIGNALLING FACTORS DURING CYCLING

BY

RHODRI FURLONG

A thesis submitted to the University of Birmingham for the degree of MASTERS OF SCIENCE BY RESEARCH

School of Sport, Exercise and Rehabilitation Sciences

College of Life and Environmental Sciences

University of Birmingham

September 2018

Abstract

INTRODUCTION: Exercise is thought to induce beneficial effects on the cerebrovasculature through the stimulation of shear stress and cyclic strain, but the optimal exercise regimen to maximally activate these pathways is unknown. *AIM:* To compare moderate intensity cycling (65% $VO2_{max}$ for 30-minutes), clinical HIIT (4 x 4-minute bouts at 85%HR_{max}) and all-out HIIT (4 x 30-second sprints) on cerebral blood flow velocity (CBFv), partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) and representative markers of shear stress and cyclic strain: vascular endothelial growth factor (VEGF) and derivatives of nitric oxide (NO), nitrite (NO_2) and nitrate (NO_3). *METHODS:* Eight physically active (6 male, 2 female) participants completed the study. CBFv was measured as an index of cerebral blood flow using transcranial Doppler ultrasound, whilst total NO_2/NO_3 and VEGF concentrations were quantified from blood plasma. *RESULTS:* Moderate intensity cycling and clinical HIIT significantly increased CBFv (by ~19 cm/s and ~10 cm/s, respectively) and $P_{ET}CO_2$ (~18 mmHg and ~9 mmHg, respectively) compared to all-out HIIT (CBFv, $p = 0.03$, $np^2 = 0.7$; $P_{ET}CO_2$, $p<0.01$, $np^2 = 0.97$). However, increases in CBFv did not translate to significant elevations in total $NO_{2/}NO_3$ for moderate intensity exercise (~19%), whilst clinical and all-out HIIT significantly elevated total $NO_{2/}NO_3$ (by 23% and 26%, respectively) ($p<0.01$, $np^2 = 0.9$). No exercise protocol significantly up-regulated VEGF ($p=0.08$, $np^2 = 0.38$). *CONCLUSION:* Clinical and all-out HIIT are likely to produce the best outcomes for the cerebrovasculature based upon their capacity to significantly elevate total NO_2/NO_3.

Acknowledgements

To my mother and father, for your continuous support in all that I do. Nothing I have achieved would be possible without you both. I hope I can continue to make you proud.

To Dr Rebekah Lucas, Dr Catarina Rendeiro and Sam Weaver, thank you for the time and effort you invested in me and my project. It simply would not have been possible to complete the work without your advice and help.

To Dr Sam Lucas, a special thank you for all of the support you have given me throughout my undergraduate and postgraduate studies. I would not be where I am today without the skills I developed under your tutelage and for that, I am truly grateful.

Table of Contents

List of Illustrations

List of Tables

List of Abbreviations

Akt	*Protein kinase B*
ANOVA	*Analysis of variance*
BBB	*Blood brain barrier*
BOLD	*Blood oxygen level dependent*
CBF	*Cerebral blood flow*
CBFv	*Cerebral blood flow velocity*
ELISA	*Enzyme-linked immunoassay*
eNOS	*Endothelial nitric oxide synthase*
FMD	*Flow-mediated dilation*
HCAR1	*Hydroxycarboxylic acid receptor 1*
HIIT	*High intensity interval training*
HR_{max}	*Heart rate maximum*
ICA	*Internal carotid artery*
KLF2	*Kruppel-like factor 2*
MCA	*Middle cerebral artery*
MCAv	*Middle cerebral artery velocity*
MICT	*Moderate intensity continuous training*
MRI	*Magnetic resonance imaging*
NO	*Nitric oxide*
NO_2	*Nitrite*
NO_3	*Nitrate*
np^2	*Partial eta squared*
$PaCO_2$	*Partial pressure of arterial carbon dioxide*
PEGDA	*Polyethylene (glycol) diacrylate*

$P_{ET}CO_2$	*Partial pressure of end-tidal carbon dioxide*
PET	*Positron emission tomography*
RPE	*Ratings of perceived exertion*
SD	*Standard deviation*
TCD	*Transcranial Doppler ultrasound*
VEGF	*Vascular endothelial growth factor*
VO_2	*Oxygen consumption*
VO_2max	*Maximal oxygen consumption*
W_{max}	*Watt maximum*
x*g*	*Revolutions per minute*

1. Introduction: Overview and problem

The first reports of physical activity's beneficial effects on the human body surfaced approximately 4600 years ago in Ancient China (Lee et al., 2012). Since then, a large amount of epidemiological and experimental evidence has accumulated displaying the positive impact exercise has on the function of the human body, whilst also demonstrating the capabilities of exercise to deter the development of coronary heart disease (Thompson et al., 2003), type 2 diabetes (Bassuk et al., 2005), breast and colon cancer (Warburton et al., 2006), cerebral infarction (Sacco et al., 1998; Lee et al., 1999; Manson et al., 2002; Lee et al., 2003; Buchner, 2007; Zschucke, 2013) and the degeneration of brain tissue (Colcombe et al., 2003). Furthermore, regular exercise has been shown to offset the acquisition of age-related neurodegenerative conditions such as dementia (Heyn et al., 2004; Weuve et al., 2004; Podewils et al., 2005; Larson et al., 2006; Hamer and Chida, 2009), which is increasing in prevalence within the UK (Prince et al., 2014). Currently, the number of people suffering from dementia in Britain is thought to be 645,101 (NHS statistics, 2018) and due to factors such as longevity and physical inactivity, this figure is expected to rise to effect approximately 1 million people by 2025 and upwards of 2 million people by 2050 (Blagosklonny, 2010; Prince et al., 2014). The implementation of preventative strategies to reduce the number of people at risk of developing debilitating neurodegenerative conditions is therefore critical (Lucas et al., 2015; Burley et al., 2016). Given that the current pharmaceutical treatment of illnesses like dementia only offer modest improvements to symptoms (Middleton et al., 2009), alternative lifestyle approaches such as engaging in regular exercise must be considered for their capacity to offset cerebrovascular health decline.

Increased levels of physical activity and exercise have been shown to deter cerebrovascular disease. It has been specifically found that exercise is capable of combatting age-related decrements in cognition (Colcombe and Kramer, 2003; Bolduc et al., 2013) as well as preventing cerebral hypoperfusion; an age-related risk-factor that dramatically increases the likelihood of an individual developing Alzheimer's disease or dementia (Bolduc et al., 2013; Phillips et al., 2014; Lucas et al., 2015; Barha et al., 2016). In addition to the neuroprotective effects exercise exerts on the brain, it is also known to be a cost effective, sustainable and widely accessible strategy (Phillips et al., 2014) that can induce a vast array of positive cerebrovascular structural adaptations such as, elevated levels of angiogenesis, neurogenesis and synaptogenesis (Isaacs et al., 1992; Churchill et al., 2002; Vaynman and Gornez-Pinilla, 2006), as well as enhanced cerebral blood flow (CBF) (Colcombe et al., 2003; Ainslie et al., 2008), cerebrovascular reactivity (Brown et al., 2010), neuroplasticity (Voss et al., 2013) and hippocampal size (Erickson et al., 2011). Furthermore, reports of improved brain function

via enhanced cognition (Colcombe and Kramer, 2003; Lautenschlager et al., 2008) and memory (Erickson et al., 2011) are conjointly associated with regular physical activity.

It is therefore abundantly clear, that exercise is capable of inducing advantageous structural, functional and neuroprotective adaptations within the brain; all of which support its promotion as a favourable strategy that can drive significant improvements in brain health (Philipps et al., 2014; Lucas et al., 2015). However, despite knowing the beneficial impact exercise has on the brain, our understanding of the underlying molecular mechanisms and pathways that mediate the positive exercise-induced cerebrovascular adaptations are poorly understood (Bolduc et al., 2013; Lucas et al., 2015; Barha et al., 2016). Consequently, our ability to target and exploit these mechanisms through exercise intervention is impeded (Voss et al., 2013; Philips et al., 2014; Barha et al., 2016). Therefore, to enable exercise to be fully utilised as a preventative strategy in the fight against neurodegeneration and as a treatment in existing cases of poor brain health, our knowledge of the mechanisms driving beneficial changes within the cerebrovasculature must be improved.

2. Literature review:

2.1 Shear stress and cyclic strain; potential mechanisms underlying positive exercise-induced cerebrovascular adaptation.

Shear stress and cyclic strain (pulsatile stretch, circumferential wall stress, tangential wall stress) have been proposed as the likely mechanisms that drive beneficial changes within the cerebrovasculature in response to exercise (Padilla et al., 2011; Bolduc et al., 2013; Lucas et al., 2015; Green et al., 2017). The first of these mechanisms, shear stress, is stimulated by the frictional force blood flow exerts on the endothelium of an artery (Nierbauer and Cooke, 1996; Lu and Kassab, 2011). This in turn activates molecular pathways that are capable of inducing positive endothelial and vascular adaptation through the up-regulation of neurovascular signalling factors such as vascular endothelial growth factor (VEGF) (Egginton et al., 1998; Prior et al., 2004; Suhr et al., 2007; dela Paz et al., 2011) and nitric oxide (NO) (Bolduc et al., 2013; Green et al., 2017). VEGF production is associated with the mobilisation and proliferation of endothelial cells, as well as the activation of angiogenic signalling cascades that stimulate for the construction and formation of new blood vessels (Prior et al., 2004). NO on the other hand, is a potent vasodilatory molecule that permits increased blood flow through a given artery when released (Prior et al., 2004).

It has been shown that NO and VEGF share very similar signalling cascades that both begin with the activation of Kruppel-like factor 2 (KLF2) (dela Paz et al., 2011; Bolduc et al., 2013); a shear stress detecting protein that plays an important role in the regulation of endothelial responses to

increased blood flow (Bolduc et al., 2013). Additionally, both NO and VEGF signalling cascades require phosphorylation of protein kinase B (Akt) (Iemitsu et al., 2006; Bolduc et al., 2013) prior to the enhanced expression of endothelial nitric oxide synthase (eNOS) and VEGF, resulting in increased NO bioavailability (Bolduc et al., 2013; Green et al., 2017) and the activation of angiogenic signalling cascades respectively (Wang et al., 2003; Iemitsu et al., 2006).

2.1.1 The acute and chronic effects of exercise-induced shear stress

As mentioned above, the acute stimulation of shear stress and accompanying elevation in NO bioavailability can rapidly increase vessel diameter (Prior et al., 2004); a phenomenon termed flow-mediated dilation (FMD) that has been demonstrated within peripheral and cerebral conduit arteries in response to a single bout of exercise (Tinken et al., 2010; Smith et al., 2017). Within the extracranial conduit arteries, Smith and colleagues (2017) showed that moderate intensity cycling was capable of driving significant elevations in shear rate. These findings are important as they were the first to demonstrate that conduit arteries associated with the cerebrovasculature, such as the internal carotid arteries (ICA), are exposed to exercise-invoked shear stress (Smith et al., 2017). Given that the ICA give rise to the middle cerebral arteries (MCA) (Chandra et al., 2017), it is reasonable to postulate that exercise-induced elevations in shear rate extend from the extracranial arteries into the intracranial vascular structures. To date however, no study has been able to definitively demonstrate the capacity of acute exercise to stimulate shear stress within the intracranial vasculature and for that reason, further research is required to ascertain the role that shear stress plays in increasing NO bioavailability within the brain.

Chronic stimulation (i.e. repeated daily across many days, weeks or years) of exercise-induced shear stress and NO on the other hand is associated with structural vascular adaptations such as vessel remodelling (Nierbauer and Cooke, 1996; Tinken et al., 2010); whereby, frequent elevations in blood flow and NO bioavailability result in a permanently enlarged vessel diameter to enable larger volumes of blood to flow to the exercising muscle (Korshunov, 2007). Structural vascular adaptations that occur as a result of chronic shear stress have also been shown to enhance the functional capabilities of the vessel by restoring and/or improving endothelial reactivity (Green et al., 2004; Goto et al., 2007; Thijssen et al., 2009). Given that endothelial dysfunction and arterial stiffening accompany ageing and sedentarism (Bolduc et al., 2013); exercise-invoked improvements in vascular reactivity and endothelial function through regular stimulation of shear stress could play a key role in reversing cerebrovascular disease. However, the long-term benefits of exercise-induced elevations in NO bioavailability have only been shown in the peripheral vasculature (Nierbauer and Cooke, 1996; Green et al., 2004; Goto et al., 2007; Thijssen et al., 2009; Tinken et al., 2010) and therefore, it

cannot be assumed without further study that frequent and chronic stimulation of shear stress occurs within the brain. That being said, it is known that shear rate is increased in the cerebrovasculature in response to the acute completion of exercise (Smith et al., 2017) and subsequently, shear stress stimulation could have a pertinent role to play in triggering beneficial cerebrovascular adaptations following adherence to long term physical activity. On the other hand, cerebrovascular-derived shear stress may not be prerequisite for NO to elicit its positive effects within the brain in response to acute or chronic exercise, as evidence suggests that NO can be transported along the vascular tree within plasma and red blood cells (Rassaf et al., 2004). It is therefore possible, that peripherally-derived NO could evoke positive vascular adaptation within the brain following exercise's completion, despite being produced from a site(s) remote from the cerebrovasculature.

2.1.2 The acute and chronic effects of exercise-induced cyclic strain

Another potential molecular mechanism suggested to be a key mediator of positive cerebrovascular adaptation is cyclic strain (Bolduc et al., 2013); an alternative mechanism to shear stress that is capable of increasing NO bioavailability through the activation of the NO transduction pathway (Green et al., 2017). Rather than stimulating NO release by increasing the frictional forces that are exerted upon a vessel's inner wall; cyclic strain up-regulates NO in response to the mechanical loading of a vessel wall (Lu and Kassab, 2011) which can be driven by exercise-induced elevations in pulse pressure (Laughlin et al., 2008; Green et al., 2017). Activation of the signalling cascade begins when the endothelium's conventional shape is altered (Lu and Kassab, 2011). This in turn activates KLF2 and the phosphorylation of Akt which increases eNOS and NO expression (Bolduc et al., 2013) and possibly VEGF production, based on evidence that suggests NO and VEGF are stimulated by very similar signalling cascades (Iemitsu et al., 2006; dela Paz et al., 2011; Bolduc et al., 2013). Within the current literature, acute stimulation of cyclic strain and NO has been shown to induce vessel vasodilation (Lu and Kassab, 2011) whilst chronic stimulation of the molecular cascade is associated with vessel remodelling (Nguten et al., 2011) and enhanced vascular reactivity following frequent long-term production of NO (Green et al., 2004; Thijssen et al., 2009). The studies that have demonstrated exercise-invoked cyclic strain's positive effects on the vasculature have only done so in the peripheral arteries however, and for this reason, further research is required to confidently outline cyclic strain as a molecular mechanism capable of inducing beneficial adaptation within the brain's vasculature.

2.1.3 A summary of exercise-induced shear stress and cyclic strain

As alluded to throughout this chapter, shear stress and cyclic strain are thought to be the drivers of exercise-induced cerebrovascular adaptation and whilst their respective transduction pathways and signalling cascades are well-understood, they have been predominantly studied in animal models (Bolduc et al., 2013), *in vitro* (Bolduc et al., 2013) and within the peripheral vasculature of humans (Tinken et al., 2010; Green et al., 2017). Consequently, our ability to take the findings from these studies and apply them to the cerebrovasculature is limited. Smith and colleagues' (2017) have demonstrated that acute, moderate intensity cycling can elevate shear rate within the cerebral conduit arteries (Smith et al., 2017) which has exciting implications for the role exercise-invoked shear stress could play in driving positive structural and functional adaptations within the brain's vasculature. Nevertheless, further study is necessary to gain a greater understanding into the influence exercise plays in stimulating shear stress and cyclic strain within the intracranial vasculature (Lucas et al., 2015; Smith et al., 2017) so that the optimal exercise strategy capable of maximally stimulating neurovascular signalling factor release can determined. Currently, our capacity to investigate exercise's effect on shear stress and cyclic strain within the brain is limited due to our reliance upon non-invasive neuroimaging techniques (Voss et al., 2013). For that reason, representative biomarkers of the aforementioned transduction pathways (NO, nitrite (NO_2), nitrate (NO_3) and VEGF) should be quantified in response to exercise completed at varying intensities, frequencies and durations, so that the exercise strategy capable of maximally increasing neurovascular signalling factor production is established. In doing so, healthcare professionals will be able to prescribe exercise regimens that can act as a preventative strategy in the fight against neurodegeneration or as a treatment for existing cases of poor brain health.

2.2 Exercise-induced up-regulation of vascular endothelial growth factor

Increasing VEGF expression has been outlined as a possible strategy to slow the deterioration of cerebrovascular health within sedentary and aging populations (Morland et al., 2017) through its ability to stimulate angiogenesis (Prior et al., 2003; Prior et al., 2004; Wood et al., 2006; Voss et al., 2013; Cotman et al., 2007; Morland et al., 2017). Angiogenesis is a complex process that constructs new blood vessels (van Praag et al., 2005; van der Borght et al., 2009) and could subsequently, combat age-related decreases in cerebral perfusion (Farkas et al., 2007; De Silva and Faraci, 2016) which often predispose an individual to developing Alzheimer's disease (Heyn et al., 2004; Weuve et al., 2004; Podewils et al., 2005; Larson et al., 2006) or dementia (Larson et al., 2006). Within human models however, exercise-invoked elevations in VEGF are yet to be quantified in the cerebrovasculature due to our inability to implement invasive techniques such as brain biopsies

(Voss et al., 2013). Whilst this is the case, an abundance of evidence exists demonstrating exercise's capacity to increase VEGF within the periphery (Adams et al., 2004; Rehmen et al., 2004; Morici et al., 2005; Sandri et al., 2005; Park et al., 2010; Sandri et al., 2011; Wahl et al., 2011; Wahl et al., 2014; Izzicupo et al., 2017) and given that VEGF can freely permeate across the blood brain barrier and enter the brain (Fabel et al., 2003; Lange et al., 2016; Morland et al., 2017), it is likely that exercise-induced elevations in peripheral VEGF translate to VEGF up-regulation within the cerebrovasculature of humans. Furthermore, shear stress, a known stimulator of the VEGF transduction pathway (Egginton et al., 1998; Prior et al., 2004; Suhr et al., 2007; dela Paz et al., 2011) has been quantified within the extracranial arteries (Smith et al., 2017), implying that VEGF could be up-regulated within the brain's vasculature. Exercise-driven increases in shear stress and VEGF are yet to be reported within the cerebrovasculature however, and accordingly, further investigation is needed.

In addition to research that is required to demonstrate exercise-induced VEGF up-regulation within the context of the brain in human models, a greater understanding of the specific molecular mechanisms responsible for exercise-induced VEGF production is required so that exercise interventions can be employed that maximally stimulate VEGF release from skeletal muscle cells (Gustafsson et al., 1999, 2001; Kraus et al., 2004), blood platelets (Mohle et al., 1997*),* adipocytes (Izzicupo et al., 2017), vascular endothelial cells and muscle endothelial cells (Prior et al., 2004). Currently within the literature, exercise-invoked shear stress (Egginton et al., 1998; Prior et al., 2004; Morici et al., 2005; Suhr et al., 2007; dela Paz et al., 2011; Wahl et al., 2011; Wahl et al., 2014) and hypoxia (Fukumura et al., 2001; Suhr et al., 2007; Wahl et al., 2011; Wahl et al., 2014) have been outlined as the primary mechanisms that mediate VEGF production. Cyclic strain may also yield VEGF up-regulating capabilities as it has been shown within animal models that the stretching of a rodent's Achilles tendon can increase VEGF production (Peterson et al., 2004), implying that a sufficient stretching stimulus that alters the endothelium's conventional shape is capable of activating the VEGF transduction pathway. However, there is currently no data to support this assertion within humans. In contrast, Suhr et al. (2007) have demonstrated within human subjects that shear stress is the most potent stimulant of VEGF release in peripheral circulation compared to hypoxia alone. It was specifically shown in Suhr and colleagues' (2007) experiment that exposing an exercising participant to increased levels of shear stress through the application of external vibrations yielded a significantly greater elevation in VEGF in comparison to the completion of the same exercise session in hypoxic conditions (Suhr et al., 2007). Based on these findings, it seems that elevating shear stress is more important for the increased production of VEGF during exercise than exercising in oxygen deficient environments (Suhr et al., 2007). However, several studies have

invoked exercise-induced shear stress and have failed to up-regulate VEGF (Gu et al., 2004; Rehmen et al., 2004; Wood et al., 2006; Adams et al., 2008; Danzig et al., 2010; Ogawa et al., 2010; Schlager et al., 2011; Beck et al., 2012; Voss et al., 2013). It therefore seems, that other factors aside from shear stress and hypoxia influence VEGF up-regulation during exercise and for this reason, it is important to consider the effects of these additional factors as they are likely to influence the optimal exercise strategy that can maximally increase circulating levels of VEGF within the periphery and therefore, the brain (Fabel et al., 2003; Lange et al., 2016; Morland et al., 2017).

2.2.1 Exercise intensities influence upon vascular endothelial growth factor production

The first of these factors pertains to the intensity of exercise completed. Despite claims that moderate intensity exercise produces the best outcomes for cerebrovascular health (Larson et al., 2006), the completion of high intensity exercise has consistently been shown to elevate VEGF concentrations within the peripheral vasculature of humans (Morici et al., 2005; Suhr et al., 2007; Wahl et al., 2011; Wahl et al., 2014) and is consequently likely to increase VEGF production within the cerebrovasculature (Fabel et al., 2003; Lange et al., 2016; Morland et al., 2017). In contrast, the implementation of low to moderate intensity exercise protocols have been shown to elicit heterogenous VEGF responses (Gu et al., 2004; Rehmen et al., 2004; Adams et al., 2008; Danzig et al., 2010; Ogawa et al., 2010; Park et al., 2010; Schalager et al., 2011; Beck et al., 2012; Voss et al., 2013). Izzicupo et al. (2017) proposed that the failure of low to moderate intensity exercise protocols to significantly elevate circulating VEGF was caused by the inability of lower exercising workloads, such as traditional walking (Voss et al., 2013; Izzicupo et al., 2017), to sufficiently stimulate shear stress and the accompanying VEGF transduction pathway. Conversely, the completion of vigorous physical activity is thought to evoke the required level of shear stress stimulation that can significantly increase circulating VEGF (Izzicupo et al., 2017). Wahl et al. (2014) assessed the impact exercise intensity has on VEGF, where it was shown that the completion of two differing high intensity interval training (HIIT) protocols (i. four, 4-minute bouts at 90% peak power output interspersed with 3 minutes of active recovery; ii. four, 30-second all-out bouts interspersed with 7.5 minutes active recovery) significantly increased VEGF release by 8.7% and 8.4% respectively, in comparison to the completion of one hours cycling at 50% peak power output, which was associated with reductions in VEGF production. These findings imply that exercise intensity plays an important role in determining whether VEGF is elevated or decreased following physical activity's completion and highlights that employing high intensity exercise is more likely to up-regulate peripheral, and therefore cerebral VEGF levels (Fabel et al., 2003; Lange et al., 2016; Morland et al.,

2017) than exercise completed at lesser intensities (Voss et al., 2013; Wahl et al., 2014; Izzicupo et al., 2017).

2.2.2 Blood lactate, vascular endothelial growth factor and exercise

Wahl and colleagues (2014) have speculated that the accumulation of blood lactate during HIITs completion could also play an important, mediatory role in the production of VEGF. In animal models, the accumulation of endogenous or the administration of exogenous lactate has been demonstrated to increase circulating VEGF (Fukumura et al., 2001; Hunt et al., 2007; Lezi et al., 2013; Skriver et al., 2014; Morland et al., 2017). This was first reported by Hunt and colleagues (2007), who implanted Matrigel plugs containing a hydrolysable lactate polymer into the subcutaneous space of mice, observing a significant elevation in VEGF. Since then, exercise-invoked elevations in lactate accumulation following chronic adherence to high intensity exercise have revealed that endogenous production of lactate can also up-regulate VEGF (Morland et al., 2017). Morland and colleagues (2017) showed that the completion of ten, 4-minute HIIT bouts, 5-days a week, for 7 weeks significantly increased VEGF expression, as well as increasing capillary density within the sensorimotor cortex and hilus of the dentate gyrus in the brains of wild mice. In contrast, in hydroxy-carboxylic acid receptor 1 (HCAR1) knockout mice, which were unable to detect lactate accumulation within the exercising muscle, no increases in VEGF or enhanced cerebral vascularisation occurred. These data indicate that the accumulation and detection of lactate is critical to successfully up-regulating VEGF production within the brains of rodents (Morland et al., 2017).

Exercise driven increases in endogenous lactate production are yet to be highlighted as a key activator of the VEGF transduction pathway within human models. That being said, it is known that lactate plays an important role in human tumour development by stimulating VEGF and the associated angiogenic pathways that lead to increased tumour-vascularisation (Ferrara, 2005). Furthermore, strong associations have been described in human studies that have matched exercise-invoked increases in lactate to elevations in VEGF (Wahl et al., 2011; Skriver et al., 2014; Wahl et al., 2014) and therefore, lactate may have a regulatory role to play in the production of VEGF during the completion of vigorous physical activities like HIIT, in human models. This postulation is strengthened by the known propensity of high intensity exercise to increase lactate accumulation (Hill and Long, 1924; Bang, 1936; Hermansen and Saltin, 1967; Hermansen, 1971; Wahl et al., 2011; Skriver et al., 2014; Wahl et al., 2014; Morland et al., 2017) and elevate shear stress (Wahl et al., 2014), which implies that HIIT is likely to be the optimal exercise strategy to elevate circulating VEGF

levels in the periphery and sequentially, the cerebrovasculature via dissemination of VEGF across the BBB (Fabel et al., 2003; Lange et al., 2016; Morland et al., 2017).

2.2.3 Potential dangers of high intensity interval training for the cerebrovasculature

However, whilst high intensity exercise may optimally increase circulating VEGF levels (Morici et al., 2005; Suhr et al., 2007; Wahl et al., 2011; Wahl et al., 2014; Morland et al., 2017) and improve other important factors contributing to good health such as insulin sensitivity (Marcinko et al., 2015), maximal oxygen uptake (Milanovic et al., 2015), glucose control (Weston et al., 2014), cardiorespiratory fitness (Dias et al., 2017; Weston et al., 2014), markers of cardiovascular fitness (Milanovic et al., 2015) and brachial artery FMD (Ramos et al., 2015); limited research has been conducted into HIITs impact on the cerebrovasculature in humans and therefore, prior to its prescription as an alternative exercise strategy to moderate intensity continuous training (MICT), additional research is requisite to ensure it is safe to employ within the context of the brain.

Apprehension surrounding vigorous physical activity's completion and brain health resides in the rapid elevations in cerebral perfusion that accompany the completion of explosive, all-out HIIT protocols, that could expose the cerebrovasculature to hyperaemia (Lucas et al., 2010; Willie et al., 2014). If left unregulated, HIIT-induced perturbations in cerebral blood flow (CBF) could predispose an individual to a hyperperfusion injury such as cerebral infarction, haemorrhage or breakthrough of the BBB (Bailey et al., 2011; Lucas et al., 2015). To prevent such injuries from occurring, the brain is equipped with a protective mechanism termed cerebral autoregulation, which is activated in the face of perfusion pressure changes to regulate cerebral perfusion (Aaslid et al., 1989). However, it takes ~3 seconds (Aaslid et al., 1989; Lucas et al., 2015) for cerebral autoregulation to employ its protective effect upon the cerebrovasculature and consequently, at the onset of HIIT, the brain may be defenceless against dramatic increases in CBF.

Elderly and sedentary populations are among those believed to be most at risk of suffering a hyperperfusion-related injury due to the arterial stiffening that occurs in conjunction with longevity and physical inactivity (Bolduc et al., 2013). This is concerning given that the target population for this exercise intervention are also those that could be most vulnerable to the health risks associated with HIIT. It seems therefore, that all-out HIIT protocols may be inappropriate for ageing and sedentary populations to complete and for that reason, other exercise strategies that are more suitable for such groups need to be developed so that at-risk populations can benefit from exercise-induced stimulation of the mechanistic pathways that increase neurovascular signalling factor production and evoke positive cerebrovasculature adaptations.

Weston et al.'s (2014) 'clinical HIIT model', comprised of four, 4-minute exercise bouts completed at 85% HR_{max}, interspersed with 3-minute periods of active recovery, could be a suitable alternative exercise strategy to all-out HIIT for the brain. A meta-analysis of this model demonstrated that it evoked superior metabolic, cardiac and systemic benefits compared with MICT; whilst also being deemed a safe and appropriate exercise strategy for clinical populations suffering from conditions such as cardiometabolic disease (Weston et al., 2014). Furthermore, implementation of clinical HIIT has been shown increase circulating VEGF (Wahl et al., 2014) as well as, the enjoyment of exercise in comparison to traditional training methods (Weston et al., 2014); a factor that plays a critical role in maintaining long-term adherence to physical activity (Deforche et al., 2011). Taken together, it seems possible that Weston and colleagues' clinical HIIT guidelines could offer a suitable alternative training approach to MICT and all-out HIIT in ageing, sedentary or clinical populations with brain health in mind. To confirm this however, research is required that affirms the safety of the clinical HIIT model on the brain, as there is currently limited research that has examined its effects upon the cerebrovasculature (Tsukamoto et al., 2019).

2.3 Cerebral blood flow velocity and $P_{ET}CO_2$ in response to exercise, with a focus on exercise intensity

As outlined above, all-out and clinical HIIT's effects on the cerebrovasculature are for the most part unknown. Consequently, it is undetermined whether HIIT can be employed as a safe and effective exercise intervention in comparison to MICT for the brain, whilst it is also uncertain whether HIIT can increase shear stress and cyclic strain within the cerebrovasculature to evoke neurovascular signalling factor release (Bolduc et al., 2013; Lucas et al., 2015; Barha et al., 2016; Smith et al., 2017). No studies to date have directly addressed this question. Although the safety of HIIT within the context of the brain can be advised, as can the likelihood of HIIT to increase shear stress and cyclic strain within the cerebrovasculature, through the examination of studies that investigated high intensity exercise's and HIIT's impact on intracranial artery blood flow velocity (also termed cerebral perfusion or cerebral blood flow velocity (CBFv)); a measure that is related to brain blood flow and known to be associated with exercise-invoked elevations in shear rate within the extracranial vessels (Smith et al., 2017).

Exercise's ability to significantly increase cerebral perfusion from resting values has been shown during the completion of running, rowing, cycling and single legged extension exercise (Jorgensen et al., 1992; Hellstrom et al., 1993; Moraine et al., 1993; Hellstrom et al., 1996; Potts et al., 1997; Gonzalez-Alonso et al., 2004; Ogoh et al., 2004; Fisher et al., 2013; Lyngeraa et al., 2013; Brugniaux et al., 2014; Hiura et al., 2014; Faull et al., 2015; Smith et al., 2016; Smith et al., 2017). As cycling has

been most frequently assessed for its impact on cerebral perfusion, (Hellstrom et al., 1993; Moraine et al., 1993; Hellstrom et al., 1996; Gonzalez-Alonso et al., 2004; Ogoh et al., 2004; Fisher et al., 2013; Brugniaux et al., 2014; Hiura et al., 2014; Smith et al., 2016; Smith et al., 2017; Curtelin et al., 2017; Tsukamoto et al., 2019) it will therefore be the focus of this literature review chapter.

The findings from studies that have assessed cycling's influence on CBFv with transcranial Doppler (TCD) ultrasound across a range of exercise intensities, have revealed that moderate intensity exercise (~65% VO_2max, ~60% HR_{max}) elicits the largest elevation in blood velocity from resting values, whilst exceeding this workload causes blood velocity to decrease towards baseline levels (Hellstrom et al., 1993; Moraine et al., 1993; Hellstrom et al., 1996; Gonzalez-Alonso et al., 2004; Ogoh et al., 2004; Fisher et al., 2013; Brugniaux et al., 2014; Smith et al., 2016; Curtelin et al., 2017; Tsukamoto et al., 2019). Based on these findings, it seems that the completion of moderate intensity cycling exercise is likely to produce the greatest stimulus for shear stress and therefore, NO production (Bolduc et al., 2013; Green et al., 2017; Smith et al., 2017) and possibly VEGF (dela Paz et al., 2011); whilst completing cycling at workloads that are greater than moderate intensity exercise (~65% VO_2max, ~60 HR_{max}) could reduce the stimulation of shear stress and its associated molecular pathways.

The underlying mechanism accounting for the 'inverted U' relationship (Tymko et al., 2018) has been attributed to the vasoconstrictive effects hypocapnia exerts upon the cerebrovasculature at workloads exceeding ~65% VO_2max and ~60% HR_{max} (Hellstrom et al., 1996). Hellstrom and colleagues (1996) termed this phenomenon hyperventilation-induced hypocapnia and stated that cycling at intensities greater than 65% VO_2max and ~60% HR_{max} causes the respiratory rate to increase, which reduces the carbon dioxide content within the arteries and results in vessel constriction and a reduction in intracranial blood flow. Since the first reports of hyperventilation-induced hypocapnia (Hellstrom et al., 1996), several studies have reported the regulatory impact that carbon dioxide has on CBFv across a range of workloads through the assessment of the partial pressure of arterial carbon dioxide ($PaCO_2$) (Hellstrom et al., 1996; Fisher et al., 2013) and the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) (Moraine et al., 1993; Brugniaux et al., 2014). It has been shown in these studies that cerebral perfusion and $PaCO_2$/$P_{ET}CO_2$ increase simultaneously to workloads equating to ~ 65% VO_2max/~ 60 HR_{max}, before falling towards resting values (Moraine et al., 1993; Hellstrom et al., 1996; Fisher et al., 2013; Brugniaux et al., 2014).

The regulatory effects hyperventilation-induced hypocapnia exert during high intensity cycling exercise could have negative implications for shear rate within the cerebrovasculature, as hypocapnia-driven vasoconstriction could reduce cerebral perfusion and sequentially decrease the

shear stress stimulus that is required for neurovascular signalling factor production; thereby, limiting the stimulus for positive vascular adaptation within the brain. This postulation is supported by the findings of two papers that have examined HIIT's effects on cerebral perfusion during cycling. Curtelin and colleagues' (2017) showed that an all-out 30-second sprint induced significant reductions in CBFv in conjunction with decreased $P_{ET}CO_2$ compared to baseline values; whilst, Tsukamoto et al. (2019) reported decreased cerebral perfusion and $P_{ET}CO_2$ levels throughout the completion of four, 4-minute HIIT bouts at 80-90% Watt maximum (W_{max}) interspersed with 3-minute bouts of cycling at 50-60% W_{max}. These studies failed to report any adverse effects associated with HIIT's completion for the brain, implying that both clinical and all-out HIIT may be safe exercise strategies for the cerebrovasculature; but they also failed to show any significant elevations in cerebral perfusion indicating that HIIT may not be the optimal exercise intervention to employ with significantly elevating shear stress within the cerebrovasculature in mind (Curtelin et al., 2017; Tsukamoto et al., 2019). That being said, no direct or indirect markers of shear stress were quantified during HIITs completion and therefore, HIIT's ability to up-regulate neurovascular signalling factors such as NO and VEGF in the cerebrovasculature cannot be ruled out. To gain a greater insight into the influence that HIIT has on the aforementioned transduction pathways, a study that quantifies CBFv and representative biomarkers of the shear stress and cyclic strain signalling cascades (NO, NO_2, NO_3 and VEGF) should be conducted, to determine whether clinical or all-out HIIT has a role to play in improving cerebrovascular health.

2.4 Rationale

It has been described throughout this literature review that exercise-induced shear stress and cyclic strain are the likely underlying molecular mechanisms capable of driving beneficial vascular adaptations within the brain (Bolduc et al., 2013; Lucas et al., 2015) through the up-regulation of neurovascular signalling factors, NO and VEGF. Whilst it is not yet certain whether peripherally-derived VEGF and NO enter the brain via diffusion and transport mechanisms respectively (Fabel et al., 2003; Rassaf et al., 2004; Lange et al., 2016; Morland et at., 2017), or cerebrovascular-derived VEGF and NO drive the primary stimulus for positive intracranial and extracranial adaptation; it is certain that exercise is capable of up-regulating the required neurovascular signalling factors for beneficial vascular change (Nierbauer and Cooke, 1996; Adams et al., 2004; Green et al., 2004; Rehmen et al., 2004; Morici et al., 2005; Sandri et al., 2005; Thijssen et al., 2009; Park et al., 2010; Tinken et al., 2010; Lu and Kassan, 2011; Sandri et al., 2011; Wahl et al., 2011; Wahl et al., 2014; Green et al., 2017; Izzicupo et al., 2017; Morland et al., 2017; Smith et al., 2017). To enable exercise to be implemented as a strategy that can maximally up-regulate NO and VEGF within the brain,

however, the optimal exercise intervention that is capable of stimulating the transduction pathways responsible for NO and VEGF's production must be determined.

The findings of studies that have examined exercise's effects on cerebral perfusion across a range of intensities may be able to inform optimal workload, as it is known that elevations in blood velocity are linked with an elevated shear stress stimulus within the cerebrovasculature (Smith et al., 2017) and therefore, NO (Bolduc et al., 2013; Green et al., 2017) and possibly VEGF (Suhr et al., 2007; dela Paz et al., 2011; Green et al., 2017). Moderate intensity cycling has been consistently shown to evoke the largest increase in cerebral perfusion from baseline levels (Hellstrom et al., 1993; Moraine et al., 1993; Hellstrom et al., 1996; Gonzalez-Alonso et al., 2004; Ogoh et al., 2004; Fisher et al., 2013; Brugniaux et al., 2014; Smith et al., 2016) and could therefore be capable of eliciting the greatest shear stress response within the cerebrovasculature. Smith and colleagues' (2017) findings support this idea, having demonstrated that moderate intensity cycling can significantly increase shear stress within the cerebral conduit arteries in conjunction with elevations in CBFv. Conversely, studies that have investigated the completion of clinical and all-out HIIT models on cerebral perfusion have reported significant reductions in CBFv attributed to the regulatory effects that hyperventilation-induced hypocapnia exert upon the brain during high intensity exercise (Curtelin et al., 2017; Tsukamoto et al., 2019) and for that reason, it seems that vigorous physical activity may not provide the optimal shear stress and cyclic strain stimulus.

No study to date, however, has been able to definitively quantify shear rate or cyclic strain within the intracranial arteries during exercise, and therefore, it is not currently possible to accurately advise the most beneficial exercise strategy that can maximally increase stimulation of NO and VEGF within the brain. To improve our understanding of this complex area, research needs to be performed that quantifies cerebral perfusion and $P_{ET}CO_2$ in conjunction with the assessment of biomarkers that are representative of the shear stress and cyclic strain signalling cascades (NO, NO_2, NO_3 and VEGF) across a variety of exercise protocols. In doing so, the optimal exercise intervention that yields the capacity to maximally elevate neurovascular signalling factors from baseline quantities will be determined, with an increased understanding of the regulatory mechanisms that could limit shear stress and cyclic strain within the cerebrovasculature. With this knowledge, our awareness of how exercise can be employed as a preventative strategy in the fight against neurodegeneration and as a treatment in existing cases of poor brain health will be strengthened; an area that requires immediate attention based on the increasing prevalence of debilitating neurodegenerative conditions such as Alzheimer's disease and dementia (Prince et al., 2014).

2.5 Aims

The aim of this study was to complete the first formal comparison between moderate intensity exercise (30 minutes of exercise at 65% VO_2max), clinical HIIT (4 x 4-minute at 85% HR_{max}) and all-out HIIT (4 x 30-second sprints) on their effects on CBFv, $P_{ET}CO_2$, VEGF and derivatives of NO, total NO_2/NO_3; to inform the optimal exercise workload that is capable of maximally elevating representative biomarkers of the shear stress and cyclic strain signalling cascades from resting values.

2.6 Hypothesis

It is hypothesised, that moderate intensity cycling exercise will induce significantly larger elevations in CBF (as indexed by TCD measures of blood velocity in the MCA) and $P_{ET}CO_2$ than clinical or all-out HIIT and subsequently increase shear rate and NO bioavailability (as indexed by total NO_2/NO_3 quantities) to a greater degree than either HIIT protocol. It is also hypothesised that clinical and all-out HIIT will evoke a significantly larger increase in VEGF production in comparison to moderate intensity exercise based upon evidence that suggests vigorous physical activity is a more potent stimulator of VEGF release than exercise at lower intensities (Wahl et al., 2014; Izzicupo et al., 2017).

3. Methods

3.1 Participants

Eight physically active male (6) and female (2) (age 26 ± 7 years, height 176 ± 9 cm, weight 71 ± 11 kg, relative VO_2max 51 ± 12 ml/min/kg) affiliates of the University of Birmingham gave written informed consent to participate in the current study, which was approved by the ethical committee at the University of Birmingham (ERN_17-1750) (see Appendix for documentation confirming ethical approval). The study was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants were confirmed to be healthy and physically active following the completion of the General Health Questionnaire and Physical Activity Questionnaire (The General Practice Physical Activity Questionnaire, 2006). All participants were informed of the study's purpose in writing and the possible risks of taking part, before providing written consent to participate. Female participants could participate at any point during their menstrual cycle and all participants were instructed to consume the same amount and composition of food prior to each laboratory visit at the School of Sport, Exercise and Rehabilitation Sciences. Laboratory visits were scheduled at the same time of day to help participants replicate the timing of the pre-exercise diet and limit potential diurnal effects for outcome measures. Food intake was recorded before the first session and reproduced before the remaining exercise sessions via diet recall. Caffeine and alcohol consumption were prohibited 6 and 24 hours, respectively, prior to arrival at the laboratory, and the completion of vigorous exercise 24 hours before each visit was also prohibited. Participants were advised to consume 0.5 litres of water in the 4 hours preceding each exercise session, with a further 0.25 litres of water being ingested 15 minutes prior to exercise initiation, to ensure adequate hydration status (Rodriguez et al., 2009).

3.2 Study Protocol

A repeated-measures, randomised crossover design was employed in this study that required each participant to attend a total of four separate laboratory visits. The first visit involved the collection of informed consent and completion of a maximal exertion test to determine a participant's maximum oxygen capacity (i.e., VO_2max); the findings from this test were used to set the target exercise intensities in the subsequent sessions. The remaining three visits were then completed in a randomised order, comprising of: 1) 30 minutes of cycling at 65% VO_2max; 2) a HIIT session based upon Weston et al.'s (2014) clinical HIIT guidelines (4 x 4-minute bouts at 85% HR_{max} interspersed with 3 minutes of active recovery), and 3) an all-out HIIT protocol (4 x 30-second sprints separated by 4.5 minutes of active recovery). All exercise sessions were completed at least 48 hours apart. All exercise sessions were preceded by a 5-minute warm-up that aimed to achieve a ratings of

perceived exertion (RPE) of 11 (Borg, 1982), confirmed by the participant in the final minute of the warm-up, and were succeeded by 3 minutes of active recovery and 15 minutes of seated passive recovery (see Figure 1 for schematics of each protocol).

Upon arrival to the laboratory, the participant's willingness to continue in the study was confirmed. A urine sample was then collected to assess hydration status and Rodriguez et al.'s (2009) hydration guidelines were referred to if action was necessary. Following this, the participant rested supine on a bed while a cannula was inserted into the antecubital vein to enable the extraction of multiple blood samples during the visit. Once cannulated, 5 minutes of supine rest was completed prior to the first blood sample being extracted, which was followed by the fitting of a TCD headset to assess cerebral blood velocity, and a leak-free face mask to quantify respiratory gases and volume (Vyntus^{TM} CPX metabolic cart). Once the equipment was fitted, 3 minutes of seated CBF (see figure 2 for an example of baseline middle cerebral artery velocity (MCAv)) and respiratory gas resting measurements were obtained. Participants were then seated on the cycle ergometer and the exercise protocol began.

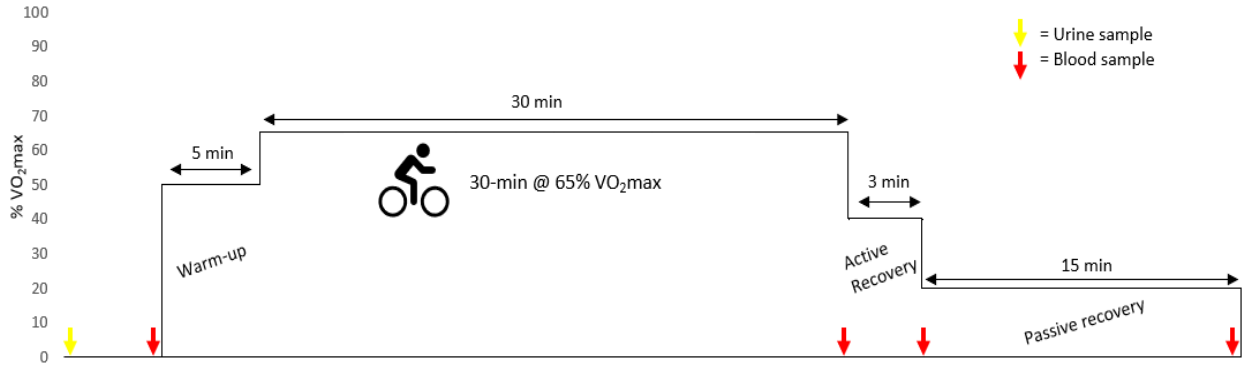

Figure 1A. The moderate intensity protocol completed by each participant in this study, consisting of 30 minutes of cycling at an exercise intensity of 65% VO_2max.

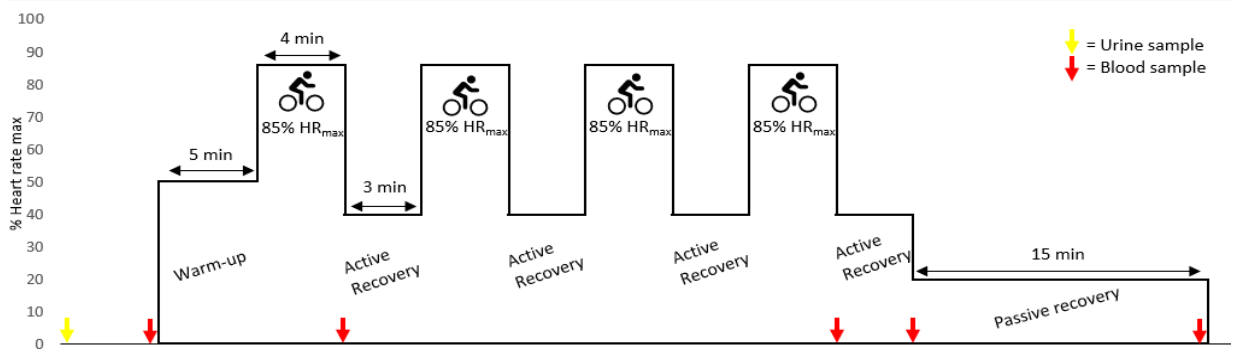

Figure 1B. The clinical HIIT protocol based upon Weston et al.'s (2014) HIIT guidelines, comprising of four 4-minute exercise bouts at 85% HR_{max} interspersed with 3 minutes of active recovery completed by each participant in this study.

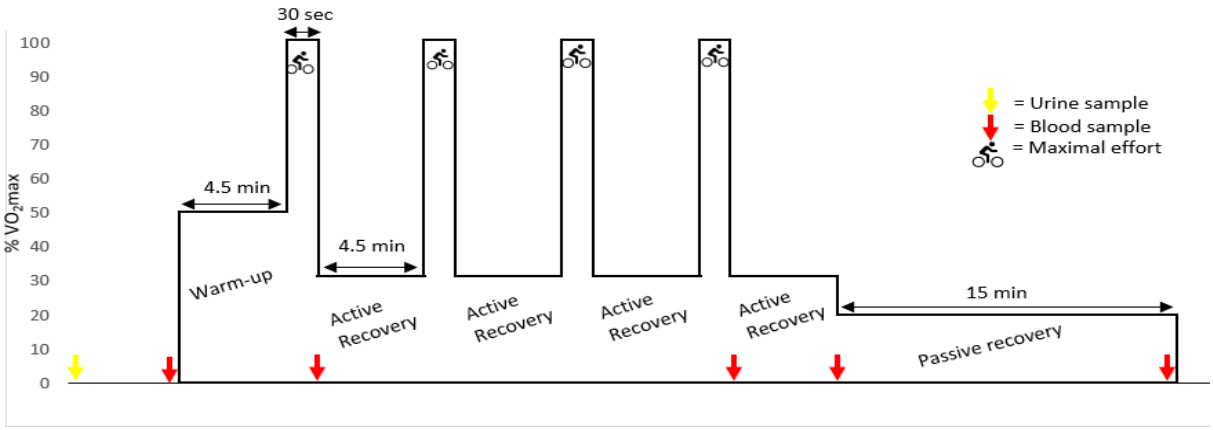

Figure 1C. The all-out HIIT protocol completed by each participant in this study, formed of four 30-second sprints completed at maximal effort, separated by 4.5 minutes of active recovery.

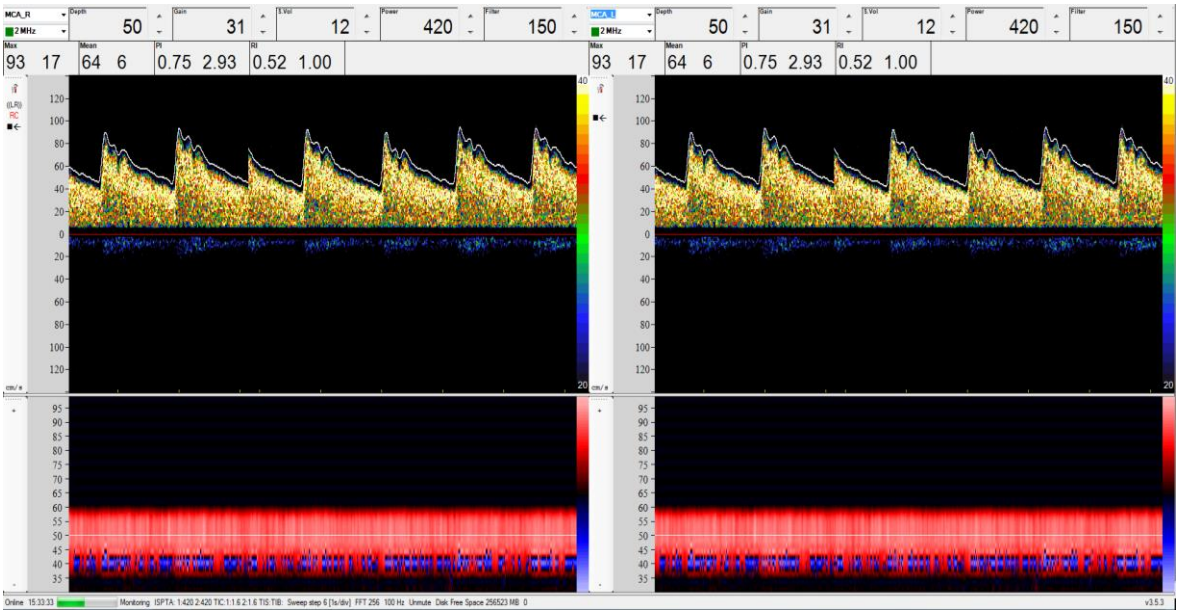

Figure 2. A screenshot of a typical middle cerebral artery velocity trace at baseline, acquired via TCD ultrasound.

3.3 Measurements

3.3.1 Height, weight and urine osmolality

Pre-exercise height and weight was measured with a Standometer (Seca, Germany) and electronic scales (Champ II, Ohaus, USA) respectively. Hydration status was measured from a urine sample provided by the participant upon arrival to the laboratory via an osmometer (Osmocheck, Vitech Scientific Ltd, UK).

3.3.2 Cerebral blood flow velocity, cardiorespiratory variables and perceived exertion

MCAv (an index of CBF) was measured using TCD ultrasound (TCD, Multi-Dop X, DWL, Germany). Ultrasound probes (2MHz) were placed above the zygomatic arch on the left and right side of the head to measure bilateral MCAv. Probes were prepared with ultrasound gel and held in place with a headset (DiaMon®, DWL). Search and identification procedures were performed in accordance with established guidelines (Willie et al., 2011), with settings kept constant between sessions. Beat-by-beat measurements were recorded via an analogue to digital converter (Powerlab 8/30, ADInstruments Ltd, New Zealand) and displayed in real time and stored for offline analysis using Lab Chart software (Labchart 7, ADInstruments Ltd). Oxygen consumption (VO_2) and respiratory (ventilation rate, ventilation volume, partial pressure of end-tidal CO_2) variables were measured using an indirect calorimetry system (VyntusTM CPX, Carefusion, Germany), and the data recorded via SentrysuiteTM software (Version 2.19, Carefusion, Germany). Calorimetry data were collected via a leak-free face mask (V2 series, Hans Rudolph Incorporated, USA). Resting and exercise heart rate values were obtained from the TCD ultrasound probes. During the last minute of each stage of the VO_2max tests, 65% VO_2max steady state and HIIT protocols, participants rated perceived exertion (RPE) via the 15-point Borg scale (Borg, 1982). All exercise sessions were completed on a cycle ergometer (Sport Excalibur, Lode, The Netherlands).

3.3.3 Nitrite, nitrate and VEGF

Venous blood samples were collected for the determination of VEGF and total NO_2/NO_3 before (resting supine), during and following each exercise session. As outlined in figure 1A-C, during exercise samples were obtained after the first and fourth HIIT bouts completed for the clinical and all-out HIIT

protocols as well as, at the end of 30-minutes of cycling. Post-exercise samples were obtained immediately after 3-minutes of active recovery and after 15-minutes of passive seated recovery (see figure 1A-C). Samples were drawn via a 22-gauge cannula (BD Venflon, India) inserted into an antecubital vein in the forearm to enable easy extraction of 12mL venous blood samples. These samples were immediately separated into two lithium heparin treated vacutainers (6mL in each) to prevent coagulation and promptly centrifuged (Heraeus Multifuge, Thermo Fisher Scientific, Germany) at 5000 revolutions per minute (*xg*) for 5 minutes at 4°C degrees. Plasma was then extracted and placed into 500 μ/L aliquots prior to freezing at -80°C for later analysis. A commercially available total NO_2/NO_3 immunoassay kit (R and D Systems, Minneapolis, USA) was used to assess the total NO_2/NO_3 concentration within plasma as a marker of nitric oxide, whilst VEGF was measured using a human VEGF Quantikine enzyme-linked immunosorbent assay (ELISA) kit (R and D Systems, Minneapolis, USA).

3.4 Data Analysis

Resting measures of total NO_2/NO_3 and VEGF were obtained after 5 minutes of rest in the supine position. Mean MCAv, $P_{ET}CO_2$, oxygen consumption (VO_2) and heart rate were averaged from the final 30 seconds of quiet seated rest (which was preceded by at least 10 minutes of sitting for equipment instrumentation and Doppler signal optimisation). Exercising measures of total NO_2/NO_3 (as a precursor of NO bioavailability) and VEGF were taken after 30 minutes of cycling at 65% VO_2max and after the first and fourth HIIT bouts in each respective HIIT protocol. Exercising measures of MCAv, $P_{ET}CO_2$, VO_2 and heart rate were averaged over the final 30 seconds for each clinical HIIT bout and the steady state cycle at 65% VO_2max, whilst measures of the same variables were over the final 15 seconds of each 30-second sprint in the all-out HIIT protocol. All post exercise measures of MCAv, $P_{ET}CO_2$, VO_2 and heart rate were obtained in the final minute of 15 minutes of passive recovery; this occurred at the same time as the final blood sample was taken. The MCAv measurements were obtained from the Doppler probe that provided the cleanest signal throughout the whole exercise session, which varied between participants but remained constant within participants across their four visits.

3.5 Statistical Analysis

A power calculation was not performed prior to the completion of the current study and for that reason, observed power (*P*) and effect size (shown as partial eta squared (np^2)) are reported throughout the findings for all statistically significant outcome variables (see section 4). The strength of effect sizes was interpreted as weak ($np^2 \leq 0.40$), moderate ($np^2 = 0.40 - 0.79$) and large ($np^2 \geq 0.80$) based upon Cohen's (1992) recommendations. Statistical significance was set at $p<0.05$.

Statistical analysis was performed using SPSS Statistics (v.24, IBM, Chicago, USA). A series of two-way repeated measures ANOVA was used to examine the within-and-between-modality differences in the dependent variables of interest, with post-hoc pairwise comparisons (Bonferroni-corrected) used to identify main and interaction effects. Specifically, HR, MCAv, $P_{ET}CO_2$, total NO_2/NO_3 and VEGF obtained during the 65% VO_2max, clinical HIIT and all-out HIIT protocols were compared via a 3 (condition) x 3 (time) two-way ANOVA at baseline, the end of exercise (4^{th} interval bout for HIIT protocols and final 30 seconds of 65% VO_2max) and after 15 minutes of passive recovery. All variables were shown to have a normal distribution by the Shapiro-Wilk test and Greenhouse-Geisser corrections were used throughout. Data are presented as mean ± standard deviation (SD).

4. Results:

Eight participants completed all four of the exercise protocols in this study, however, only six participants' data were analysed and included in the two-way ANOVA that assessed the effects of 65% VO_2max, clinical HIIT and all-out HIIT on VEGF production. This occurred due to abnormal baseline VEGF values that were two standard deviations above the mean for two different participants, in the 65% VO_2max and clinical HIIT exercise conditions. For the remaining analysis, all eight data sets were included.

4.1 Physical Characteristics

Table 1. Physical characteristics of the eight participants that took part in this study. Data are mean ± SD.

Variable	n=8
Age (years)	26 ± 7
Height (cm)	176 ± 9
Weight (kg)	71 ± 11
Relative VO_2max (ml/min/kg)	51 ± 12
Nitrite/Nitrate (µmol/l)	110 ± 25
VEGF (pg/ml)	48 ± 10

VO_2max, maximal oxygen consumption; VEGF, vascular endothelial growth factor.

4.2 Heart rate responses to 65% VO_2max, clinical HIIT and all-out HIIT exercise conditions

Table 2. Heart rate (bpm) at baseline, end of exercise and 15 minutes into passive recovery in eight participants. Data are mean ± SD. * indicates differences from 65% VO_2max (<0.05), ‡ indicates differences from clinical HIIT (<0.05).

Condition	Baseline	End of exercise	Recovery
65% VO_2max	60 ± 11	143 ± 15	73 ± 15
Clinical HIIT	59 ± 14	165 ± 11 *	87 ± 18 *
All-out HIIT	61 ± 12	169 ± 9 *	103 ± 15 *‡

There was a significant interaction effect between heart rate at the end of exercise and in recovery ($p<0.01$) with a minimum effect size (np^2) of 0.86 and an observed power (*P*) of 0.98. Post hoc analysis revealed that there were no significant differences between baseline heart rate in the three exercise protocols ($p = 1.0$, $np^2 = 0.094$, $P = 0.08$); however, at the end of exercise, the completion of 65% VO_2max exercise elicited a significantly lower heart rate than clinical HIIT ($p<0.01$, $np^2 = 0.86$, $P = 0.99$) and all-out HIIT ($p<0.01$, $np^2 = 0.86$, $P = 0.99$) (see Table 2). It was also found that heart rate

was significantly lower in recovery in the 65% VO_2max condition compared to both clinical ($p<0.01$, $np^2 = 0.95$, $P = 1$) and all-out HIIT ($p<0.01$, $np^2 = 0.95$, $P = 1$), and that heart rate remained higher following all-out HIIT compared to clinical HIIT ($p<0.01$, $np^2 = 0.95$, $P = 1$).

4.3 Cerebral blood velocity and $P_{ET}CO_2$ responses to 65% VO_2max, clinical HIIT and all-out HIIT exercise conditions

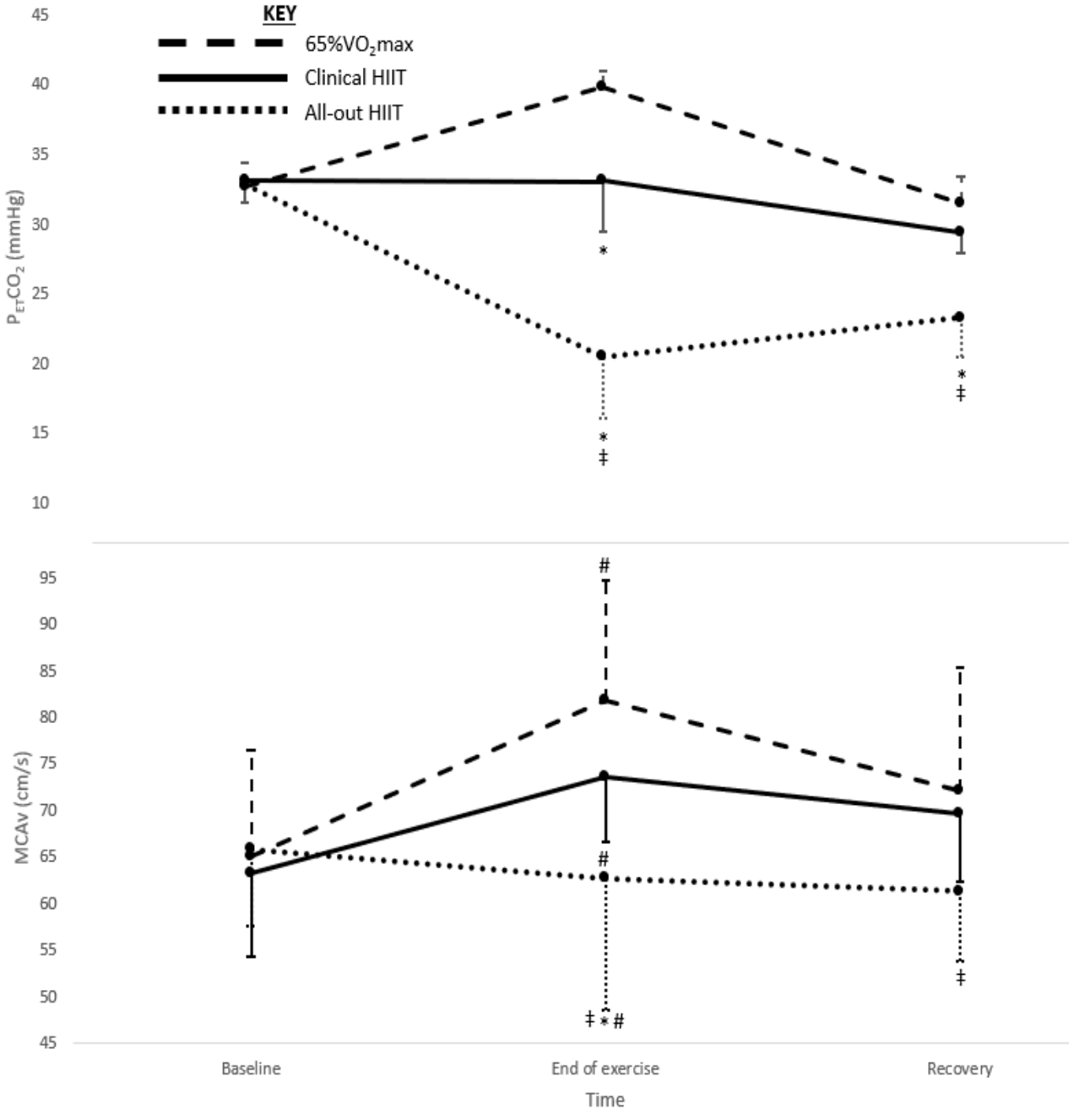

Figure 3. Absolute MCAv and $P_{ET}CO_2$ data at baseline, end of exercise and 15 minutes into passive recovery. Data are mean ± SD for 8 participants. * indicates differences from 65% VO_2max ($p<0.05$), ‡ indicates differences from clinical HIIT ($p<0.05$), # indicates differences from baseline ($p<0.05$).

$P_{ET}CO_2$

A significant interaction effect was also found for $P_{ET}CO_2$ between exercising conditions ($p<0.01$) at the end of exercise and in recovery, with a minimum effect size of 0.84 (np^2) and observed power (*P*) of 0.98 (figure 3). Post hoc analysis revealed that at the end of exercise, 65% VO_2max elicited an elevation in $P_{ET}CO_2$ that was significantly higher than both clinical HIIT ($p<0.01$, $np^2 = .97$, $P = 1.0$) and all-out HIIT ($p<0.01$, $np^2 = 0.97$, $P = 1.0$). Additionally, at the end of exercise, all-out HIIT induced a lower $P_{ET}CO_2$ response compared to clinical HIIT ($p<0.01$, $np^2 = 0.97$, $P = 1.0$) that also continued into recovery ($p<0.01$, $np^2 = 0.84$, $P = 0.98$). Likewise, all-out HIIT was significantly lower than 65% VO_2max in recovery ($p<0.01$, $np^2 = 0.84$, $P = 0.98$).

MCAv

A significant interaction effect for MCAv was revealed between exercising conditions at the end of exercise and in recovery ($p<0.01$), with a minimum effect size of 0.69 and observed power (*P*) of 0.7 (figure 3). A significant main effect for intensity was also found between all exercising conditions (p 0.01) with an effect size (np^2) of 0.79 and observed power (*P*) of 0.7. Post hoc analysis revealed that baseline MCAv data did not significantly differ between exercise protocols ($p = 0.27$, $np^2 = 0.37$, $P = 0.24$), but at the end of exercise, it was shown that MCAv had significantly changed from resting values in all exercise conditions; with clinical HIIT and 65% VO_2max significantly increasing above baseline values whilst all-out HIIT was significantly reduced ($p = 0.01$, np2 = 0.79, $P = 0.9$). Additionally, it was found that the 65% VO_2max and clinical HIIT exercise conditions induced a significantly larger elevation in MCAv compared to the all-out HIIT protocol ($p = 0.03$, $np^2 = 0.71$, $P = 0.75$). Following 15-minutes of passive recovery, MCAv in the all-out HIIT condition remained lower than during the clinical HIIT condition ($p=0.017$, $np^2 = 0.69$, $P = 0.7$).

4.4 Nitrite and nitrate responses to 65% VO_2max, clinical HIIT and all-out HIIT exercise conditions

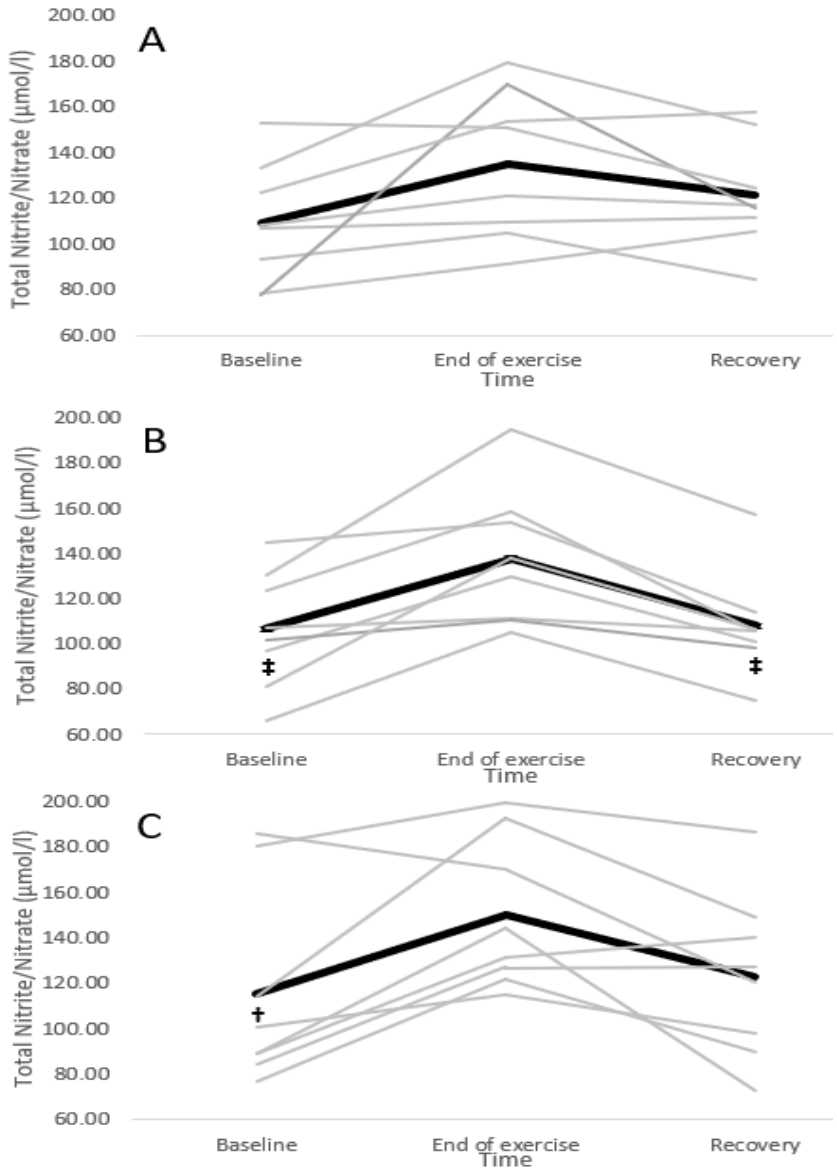

Figure 4. Total NO_2/NO_3 quantities at baseline, the end of exercise and 15 minutes into passive recovery for; (A) 65% VO_2max steady state; (B) clinical HIIT, and (C) all-out HIIT protocols. Grey lines indicate data for 8 participants; the black line indicates mean. ‡ indicates differences from exercise during the clinical HIIT protocol ($p<0.05$), † indicates differences from exercise during the all-out protocol ($p<0.05$).

A significant main effect for intensity was found for total NO_2/NO_3 quantities at rest, at the end of exercise and in recovery ($p<0.01$), with a minimum effect size (np^2) of 0.75 and an observed power (*P*) of 0.99 (see figure 4). Post hoc analysis revealed that total NO_2/NO_3 levels at rest were significantly lower than levels at the end of exercise for clinical and all-out HIIT ($p<0.01$, np2 = 0.9, *P*

= 0.99) protocols. Similarly, following clinical HIIT's completion, total NO_2/NO_3 production in recovery was significantly lower in comparison to levels at the end of exercise (p<0.01, np2 = 0.9, *P* = 0.99).

4.5 Vascular endothelial growth factor responses within blood plasma to 65% VO_2max, clinical HIIT and all-out HIIT exercise conditions

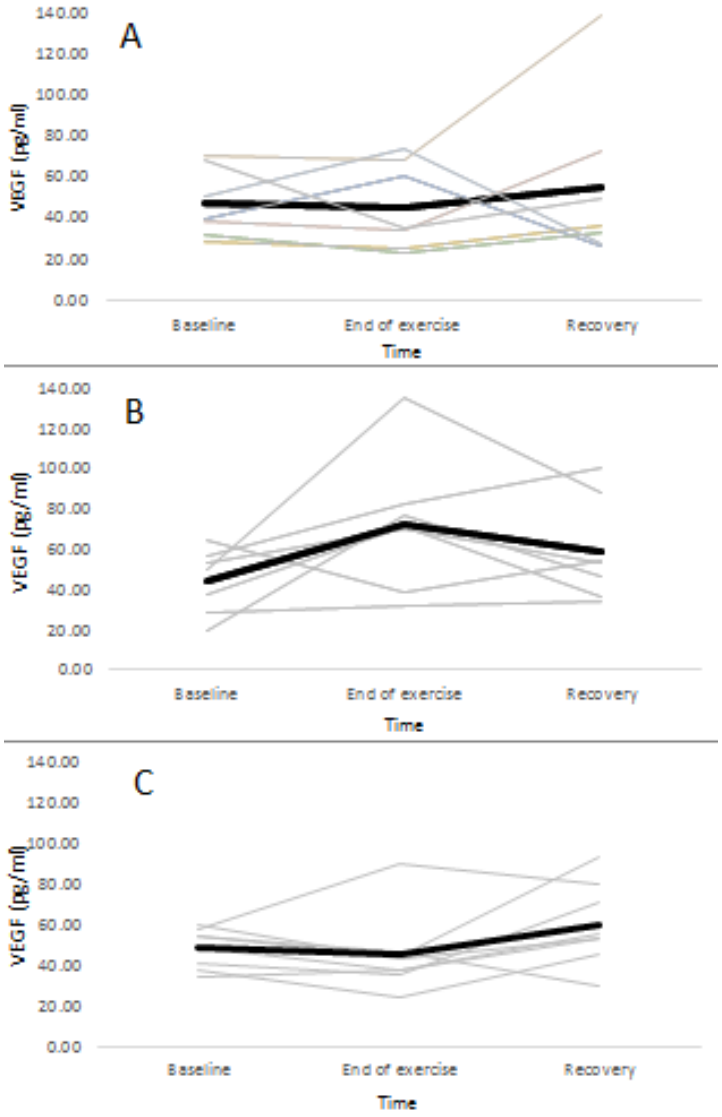

Figure 5. VEGF plasma concentrations at baseline, the end of exercise and 15 minutes into passive recovery for; (A) 65% VO_2max steady state exercise; (B) clinical HIIT and; (C) all-out HIIT. Grey lines indicate data for 6 participants; the black line indicates mean.

No significant main effects (*time*: p = 0.92, np^2 = 0.46, *P* = 0.48; *condition*: p = 0.22, np^2 = 0.28, *P* = 0.24) or interaction effects (p = 0.08, np^2 = 0.38, *P* = 0.5) were revealed for VEGF within or between exercising conditions. On average, VEGF concentrations increased from baseline measures following

the completion of clinical HIIT exercise, however this change did not reach statistical significance ($p = 0.08$, $np^2 = 0.67$, $P = 0.39$).

5. Discussion:

The purpose of this study was to assess the impact that moderate intensity exercise (65% VO_2max), clinical HIIT and all-out HIIT had on MCAv (an index of cerebral perfusion), $P_{ET}CO_2$, VEGF concentrations and derivatives of NO, NO_2 and NO_3. The main findings of the study were: (a) 30 minutes of moderate intensity exercise at 65% VO_2max and the clinical HIIT protocol elicited a significantly higher MCAv and $P_{ET}CO_2$ response than all-out HIIT; (b) clinical and all-out HIIT were associated with significant elevations in total NO_2/NO_3 concentrations in comparison to baseline measurements, whereas, moderate intensity exercise failed to significantly increase total NO_2/NO_3 production; (c) moderate intensity exercise, clinical HIIT and all-out HIIT did not measurably increase circulating VEGF. Taken together, these findings imply that the acute completion of all-out HIIT or Weston and colleagues' clinical HIIT guidelines are most likely to expose the cerebrovasculature to the favourable effects associated with NO up-regulation, and therefore, present the extracranial and intracranial vessels with the stimulus required for positive vascular and endothelial adaptations.

5.1 Cerebral blood flow velocity and respiratory responses to 65% VO_2max, clinical HIIT and all-out HIIT exercise conditions

The effects that moderate intensity cycling, clinical HIIT and all-out HIIT have on MCAv and $P_{ET}CO_2$ have previously been reported within the literature (Hellstrom et al., 1993; Moraine et al., 1993; Hellstrom et al., 1996; Gonzalez-Alonso et al., 2004; Ogoh et al., 2004; Fisher et al., 2013; Brugniaux et al., 2014; Smith et al., 2016; Curtelin et al., 2017; Tsukamoto et al., 2019), however, the current study was the first to formally compare the impact of these exercise protocols on MCAv and $P_{ET}CO_2$ within the same participant cohort. Findings indicate that moderate intensity exercise and clinical HIITs completion significantly elevate cerebral perfusion and $P_{ET}CO_2$ in comparison to all-out HIIT (figure 3). Based on the assumption that such changes in MCAv translate to elevated levels of shear stress within the cerebrovasculature (Smith et al., 2017), this study's findings imply that steady state cycling and Weston et al.'s (2014) clinical HIIT model are likely to drive a greater stimulus for shear stress-induced adaptation than all-out exercise.

These findings, for the most part, conform with the existing literature that have previously reported the capacity of moderate intensity cycling to evoke the largest changes in MCAv and $P_{ET}CO_2$ from resting values (Hellstrom et al., 1993; Moraine et al., 1993; Hellstrom et al., 1996; Gonzalez-Alonso et al., 2004; Ogoh et al., 2004; Fisher et al., 2013; Brugniaux et al., 2014; Smith et al., 2016) as well

as, the significant reductions in MCAv that are driven by hyperventilation-induced hypocapnia in response to the completion of all-out HIIT (Curtelin et al., 2017). However, the implementation of clinical HIIT in the current study elicited significantly elevated MCAv values which differed to the response recently reported by Tsukamoto et al. (2019), who demonstrated no changes in MCAv from baseline values following clinical HIIT's completion. The reason for the conflicting findings is not entirely clear and warrants further investigation; although one possible explanation accounting for the different MCAv responses to clinical HIIT resides in the increased workload (50-60% W_{max}) that Tsukamoto and colleagues' (2019) participants were required to complete in the 3-minute 'recovery' bouts, which may have led to the earlier onset of hyperventilation-induced hypocapnia and subsequent reductions in MCAv (Hellstrom et al., 1996).

The effects of hyperventilation-induced hypocapnia were also observed in the present study during the completion of all-out HIIT, whereby the completion of four, 30-second all-out sprints led to significant reductions in MCAv that dropped below baseline measurements in conjunction with significant decreases in $P_{ET}CO_2$. Based on the association between MCAv and $P_{ET}CO_2$ it is therefore highly likely that the failure of the MCAv response to increase above baseline values was governed by the regulatory influence arterial carbon dioxide concentration ($PaCO_2$) exert on the cerebrovasculature during exercise at intensities exceeding 65% VO_2max (Moraine et al., 1993; Hellstrom et al., 1996; Fisher et al., 2013; Brugniaux et al., 2014; Smith et al., 2016; Curtelin et al., 2017). Interestingly however, similar levels of hypocapnia were observed during clinical and all-out HIIT, yet the decreased $P_{ET}CO_2$ values produced during clinical HIITs completion did not induce significant reductions in MCAv compared to the significant decrease in MCAv during all-out HIIT and consequently, these findings indicate that exercise intensity plays a key role in determining the MCAv response to exercise.

The impact that the exercise-induced changes in MCAv had on the cerebrovasculature in the current study are unknown as it is still uncertain whether increased cerebral perfusion translates to increased intracranial shear stress (Smith et al., 2017). However, as mentioned above, it is feasible to associate increases in MCAv with the elevated stimulation of shear stress and cyclic strain within the brain's vasculature, based on Smith et al.'s (2017) experiment that demonstrated increases in extracranial shear rate in conjunction with elevated MCAv. Given that extracranial arteries like the ICA give rise to intracranial vessels such as the MCA (Chandra et al., 2017), the transduction pathways of shear stress and cyclic strain are likely to be stimulated within the brain's vasculature, resulting in the elevated production of neurovascular signalling factors, NO (Bolduc et al., 2013; Lucas et al., 2015) and VEGF (Egginton et al., 1998; Suhr et al., 2007; dela Paz et al., 2011; Wahl et

al., 2011; Wahl et al., 2014; Izzicupo et al., 2017). Based on the MCAv findings of the present study, it seems that the completion of moderate intensity exercise and clinical HIIT may have stimulated a greater release of neurovascular signalling factors through activation of the shear stress and cyclic strain signalling cascades. It remains to be determined however, whether these acute responses translate to beneficial chronic adaptations. A long-term training study is therefore required to be carried out, that examines how the repeated completion of steady state exercise at 65% VO_2max, clinical HIIT and all-out HIIT impact the cerebrovascular and brain function.

5.2 Nitrite and nitrate responses to 65% VO_3max, clinical HIIT and all-out HIIT exercise conditions

Total NO_2/NO_3 were assessed in this study to provide a measure of NO; a key neurovascular signalling factor up-regulated in response to exercise-induced shear stress (Bolduc et al., 2013; Green et al., 2017) and cyclic strain stimulation (Laughlin et al., 2008; Lu and Kassab, 2011; Green et al., 2017). NO could not be directly quantified in the current study due to its highly reactive nature and extremely short half-life in biological samples (Bryan and Grisham, 2007). For that reason, validated precursors of NO: NO_2 and NO_3, were assessed in response to exercise to provide a valuable insight into NO metabolism (Piknova and Schecter, 2011) within the context of the brain. Due to our inability to quantify NO_2 and NO_3 directly from the cerebrovasculature (Voss et al., 2013) however, the aforementioned neurovascular signalling factor derivatives had to be assessed as indicators of the shear stress and cyclic strain transduction pathways from the periphery. Whilst this may appear invalid, it is known that NO can travel along the vascular tree in plasma and red blood cells (Rassaf et al., 2004) and consequently, NO_2 and NO_3 concentrations within the periphery are likely to reflect NO_2 and NO_3 quantities within the cerebrovasculature. Furthermore, exercise-induced elevations in shear rate have previously been measured within the cerebral conduit arteries (Smith et al., 2017), and extensive literature has reported shear stress and cyclic strain as the mediating mechanisms of cerebrovascular adaptation *in vitro* and in animal models (see review by Bolduc et al., 2013). Therefore, it seems reasonable to assume that elevations in NO bioavailability, or NO_2 and NO_3 concentrations, observed within the periphery also extend to the cerebrovasculature.

The current study found that clinical and all-out HIIT were associated with significant elevations in total NO_2/NO_3 in comparison to baseline concentrations, whereas the completion of moderate intensity exercise failed to increase total NO_2/NO_3 levels relative to resting values. Based on these findings, it seems that clinical and all-out HIIT may provide greater activation of the shear stress and cyclic strain molecular pathways than moderate intensity exercise, indicating that acute HIITs

completion could be more beneficial for the cerebrovasculature. Additionally, these findings imply that MCAv may not be a valid indirect measurement of shear rate; given that moderate intensity cycling induced the largest increase in MCAv from rest, yet failed to elicit an increase in total NO_2/NO_3; and all-out HIIT induced a reduction in MCAv values, but was associated with increased total NO_2/NO_3 concentrations. An alternative explanation for the non-linear relationship observed between MCAv and total NO_2/NO_3 up-regulation during the completion HIIT compared to 30 minutes of cycling at 65% VO_2max however, could reside in the haemodynamic forces the endothelium was exposed during the different exercise protocols.

As previously mentioned, HIIT was associated with hyperventilation-induced hypocapnia in this study, and thus, was likely to be accompanied by the constriction of major cerebral blood vessels (Hellstrom et al., 1996; Curtelin et al., 2017; Tskuamoto et al., 2019). Pairing HIIT-induced hypocapnia and vessel constriction with the large perturbations in cerebral perfusion that are commonly associated with HIITs completion (Lucas et al., 2015), could have exposed the endothelium to greater amounts of circumferential stretch and frictional force; both of which are known to be prerequisite in stimulating the molecular pathways of cyclic strain and shear stress respectively (Nierbauer and Cooke, 1996; Lu and Kassab, 2011; Green et al., 2017). This would account for the HIIT-related elevations in NO_2 and NO_3 as well as, the non-significant increases in NO_2 and NO_3 bioavailability following moderate intensity cycling. That being said, this explanation is speculative and whilst this is the first study to examine the impact moderate intensity exercise, clinical HIIT and all-out HIIT have on representative biomarkers of the shear stress and cyclic strain transduction pathways, additional investigations must be conducted to further our understanding pertaining to HIITs capacity to up-regulate precursors of NO, NO_2 and NO_3.

5.3 Vascular endothelial growth factor responses to 65% VO_2max, clinical HIIT and all-out HIIT exercise conditions

An abundance of literature has accumulated demonstrating exercises capacity to increase the concentration of VEGF within peripheral circulation (Adams et al., 2004; Rehmen et al., 2004; Sandri et al., 2005; Morici et al., 2005; Suhr et al., 2007; Park et al., 2010; Sandri et al., 2011; Wahl et al., 2011; Wahl et al., 2014; Izzicupo et al., 2017). Additionally, studies have outlined that the completion of vigorous physical activity is an exercise strategy that can optimally elevate VEGF release (Morici et al., 2005; Suhr et al., 2007; Morland et al., 2017) in comparison to low or moderate intensity exercise (Wahl et al., 2011; Wahl et al., 2014). Despite the implementation of high intensity exercise protocols within the current study however, no elevations in VEGF were observed following clinical or all-out HIIT's completion. Thus, none of the exercise protocols

administered within this study significantly up-regulated VEGF. This was an unexpected finding, given that Wahl and colleagues (2014) have previously implemented near-identical HIIT protocols (1. 4 x 4 minutes at 90% peak power output; 2. 4 x 30-second sprints) and demonstrated significant increases in VEGF production associated with their completion. Furthermore, HIIT's inability to elicit significant VEGF release within this study, was especially surprising, given that the markers of shear stress and cyclic strain used here (NO_2 and NO_3) were significantly elevated following both HIIT conditions and shear stress is reported as a known and potent stimulus capable of increasing VEGF release in humans (Egginton et al., 1998; Suhr et al., 2007; dela Paz et al., 2011; Wahl et al., 2011; Wahl et al., 2014; Izzicupo et al., 2017).

There are several possible explanations accounting for the failure of high intensity exercise to increase VEGF release within this study. Firstly, however, it must be outlined that the non-significant VEGF elevation associated with the completion of moderate intensity exercise in the current study was expected, based upon several studies findings that failed to up-regulate VEGF concentrations through implementation of physical activity at moderate workloads (Gu et al., 2004; Wood et al., 2006; Adams et al., 2008; Danzig et al., 2010; Ogawa et al., 2010; Beck et al., 2011; Schlager et al., 2011; Wahl et al., 2011; Voss et al., 2013; Wahl et al., 2014). One possible explanation accounting for this finding in the current study, resides in the re-uptake of VEGF within the peripheral circulation following the completion of low or moderate intensity exercise (Wahl et al., 2014) that prevented VEGF increasing significantly above resting levels. An alternative explanation given by Gu and colleagues' (2004) following the failure of high volume, low intensity running to increase VEGF expression, was attributed to the large increase in VEGF binding proteins released during moderate workloads, in conjunction with increased affinity of VEGF to attach to VEGF binding proteins resulting in lower circulating VEGF levels. This reasoning would permit for VEGF release within the skeletal muscle, which has been extensively reported by Gustafsson et al. (1999, 2001), but account for the decrease in circulating VEGF observed in this study and others (Gu et al., 2004; Wood et al., 2006; Adams et al., 2008; Danzig et al., 2010; Ogawa et al., 2010; Beck et al., 2011; Schlager et al., 2011; Wahl et al., 2011; Voss et al., 2013; Wahl et al., 2014). However, it is only possible to speculate the mechanism that induced a non-significant VEGF response to moderate intensity exercise in this study, as the quantification of binding proteins or the vasculatures re-uptake of VEGF, did not take place. Future studies should be carried out that quantify VEGF's re-uptake, to establish the reasons why moderate intensity exercise fails to increase the pro-angiogenic cytokine.

As previously mentioned, the inability of HIIT protocols to significantly elevate circulating VEGF levels was a surprising finding in the current study. A likely explanation accounting for this result resides in the time points that blood was extracted from participants following completion of the clinical and

all-out HIIT protocols. As displayed in figure 1A-C, blood sampling occurred immediately after the fourth HIIT bout in the clinical and all-out high intensity exercise protocols and at the end of 30 minutes moderate intensity cycling. Accordingly, the quantity of neurovascular signalling factor present in the blood at the end of exercise was analysed and reported in this study. However, VEGF produced and released from arterial vascular endothelial cells, the body's predominant source of exercise-induced VEGF release (dela Paz et al., 2011), may not have had adequate time to circulate around the body or cross the blood brain barrier (Fabel et al., 2003; Lange et al., 2016; Morland et al., 2017) and accumulate within venous circulation prior to the extraction of the blood sample. Consequently, this may account for the non-significant elevation in VEGF release during clinical HIIT and the unchanged VEGF response to all-out HIIT in this study. In contrast, Wahl and colleagues' (2014) extracted blood samples from participants following a post-HIIT, low intensity (45% peak power output) cycle for 10 minutes. This cool down period may have aided the transportation of VEGF from its arterial sources to the venous circulation, as well as, providing additional time for VEGF to accumulate within the veins at the time of the blood draw. Taken together, it therefore seems possible that the HIIT protocols implemented in the current study could have significantly elevated VEGF release, however, due to the time course of blood sampling, the accumulation of VEGF within the venous circulation did not occur and consequently, no significant elevations in VEGF were detected.

On the other hand, a series of other factors could have interfered with the exercise-induced VEGF responses observed in the current study, including the participants' aerobic capacity (Kraus et al., 2004); which has previously been shown to influence an individual's VEGF response to physical activities completion. It is thought that possessing superior fitness levels increases an individual's susceptibility to increase VEGF release during and upon cessation of exercise (Morici et al., 2005; Izzicupo et al., 2017). Given that the removal of two highly trained participants relative VO_2max data causes the mean maximal oxygen consumption value to drop from 51 ml/min/kg to 43 ml/min/kg, it is possible that the reduced fitness levels of the remaining subjects were associated with reduced VEGF release and this could have therefore, contributed to the failure of exercise to increase VEGF production. Large inter-individual variations in VEGF responses to exercise, previously documented throughout the literature (Kraus et al., 2004; Wahl et al., 2011; Wahl et al., 2014), could have also impacted the VEGF responses to exercise in the current study. For example, due to the low sample size here, a large decrease or increase in exercise-invoked VEGF could skew the mean VEGF values of the group. Presentation of the individual VEGF data produced in this study (displayed in figure 5) demonstrates that there is considerable disparity between VEGF responses associated with exercises completion in all conditions. To counteract this issue in future studies, larger sample sizes should be

used, to ensure that any abnormal exercise-induced VEGF responses do not affect the magnitude of the exercise intervention.

All things considered however; it is entirely possible that the current study's exercise protocols simply did not up-regulate VEGF. This is feasible considering the number of studies that have failed to increase VEGF expression in response to an exercise bout (Gu et al., 2004; Adams et al., 2008; Ogawa et al., 2010; Beck et al., 2011; Schlager et al., 2011; Voss et al., 2013). Nonetheless, given that Wahl and colleagues' (2014) have demonstrated that the completion of four, 4-minute HIIT bouts and four, 30-second all-out sprints significantly elevate circulating VEGF, it is likely that the findings produced in the present study were more likely caused by the time course of blood sampling, rather than failure of the exercise protocols to increase VEGF release.

5.4 Perspectives

The findings of the present study indicate that the acute completion of Weston and colleagues' (2014) clinical HIIT guidelines or all-out HIIT may produce the best outcomes for the cerebrovasculature through the up-regulation of NO-precursors, NO_2 and NO_3. Whilst all-out HIIT's implementation has demonstrated its capacity to increase NO_2/NO_3 and therefore, NO bioavailability in the current study (Bryan and Grisham, 2007; Piknova and Schecter, 2011), safety concerns regarding its completion within sedentary and elderly populations have rendered it unsuitable for widespread prescription (Wahl et al., 2014; Lucas et al., 2015). For that reason, the more conservative clinical HIIT model may offer a more suitable exercise strategy for vulnerable populations to take part in, given that it is already recommended for clinical groups with cardiometabolic disease (Weston et al., 2014). Combined with the superior metabolic, cardiac and systemic benefits it is known to provide the body in comparison to MICT, Weston et al.'s (2014) clinical HIIT model could accordingly be the optimal exercise intervention to prescribe as a preventative strategy in the fight against neurodegeneration and as a treatment in existing cases of poor brain.

Prior to its widespread advocation however, further study must be conducted into the effects clinical HIIT exerts upon the cerebrovasculature in elderly and physically inactive groups, as the current study has only demonstrated clinical HIIT model's propensity to elevate NO_2 and NO_3 release in young, physically fit populations. Consequently, it is still undetermined whether these findings extend to clinical, older or sedentary populations. In addition to this, it must be determined whether chronic adherence to the aforementioned guidelines exerts beneficial change in brain structure and function, as to date, no study has investigated the influence chronic HIIT-based exercise has on the

cerebrovasculature or representative biomarkers of the shear stress and cyclic strain transduction pathways.

5.5 Limitations

5.5.1 Sample size

As alluded to previously, the small number of participants that completed this study is a limitation, as it increases the likelihood of a type II error that could skew the results and lead to the acquisition of unclear findings. In the current study, this could have included over-estimating the magnitude of an exercise-induced response upon neurovascular signalling factors, NO_2 and NO_3; thus, compromising the power of the findings and undermining their reliability. To circumvent this limitation and demonstrate that the current study's findings were as a result of the independent variables impact on the dependent variables, effect size and observed power have been reported throughout the findings. Here, it has been demonstrated that the smallest effect size reported for exercise's effects on HR, $P_{ET}CO_2$ and total NO_2/NO_3 was 0.84, whilst the minimum observed power value was 0.95. Within the literature, an effect size of 0.84 is considered to be large (Cohen, 1992), whilst an observed power >0.8 implies that the null hypothesis can be confidently rejected. For these reasons, it is likely that the small sample implemented in the current study had a limited effect on the HR, $P_{ET}CO_2$ and total NO_2/NO_3 observations reported herein, and therefore the null hypothesis can be rejected.

However, the interaction effect observed for MCAv was reported to have an observed power of 0.7, which indicates that this particular finding may not be reliable. Ideally, more participants would have been tested in the current study to avoid the acquisition of insufficiently powered findings, but due to time restraints this was not possible. To avoid this in future research, larger numbers of participants should complete the experiment. In addition to this, the sample size implemented in future studies should be more representative of the target population of the research, as in the current study, all the participants were physically fit and active which limits our ability to apply the findings to clinical, older and sedentary communities.

5.5.2 The use of transcranial Doppler ultrasound as a measure of cerebral blood flow

The use of TCD ultrasound to assess the CBF response to exercise in this study also has limitations. It is known that TCD ultrasound is only a valid measure of CBF providing that the diameter of the insonated vessel remains constant. Recent studies have demonstrated, that the diameter of intra-cranial arteries, such as the MCA, are subject to change in response to exercise and elevated $PaCO_2$ (Coverdale et al., 2014; Verbree et al., 2014; Coverdale et al., 2015; Coverdale et al., 2017; Verbree

et al., 2017), and thus, the reliability of TCD ultrasounds' use in the present study is questionable. That being said, obtaining whole-body, ecologically valid exercising CBF data are not possible via other approaches that address TCD-velocity based limitations (e.g., PET or ASL MRI) (Tymko et al., 2018). Additionally, established guidelines by Willie and colleagues' (2011) were followed throughout to ensure that blood velocity values were as similar between exercising conditions as possible.

5.5.3 The use of peripheral venous blood samples as a reflective measure of changes observed in neurovascular signalling factor concentration in the cerebrovasculature

The use of peripheral venous blood samples to reveal changes in neurovascular signalling factor content within the cerebrovasculature was a limitation of the current study, as it cannot be stated with absolute confidence that the quantification of total NO_2/NO_3 and VEGF from a peripheral source, reflected total NO_2/NO_3 and VEGF quantities within the intracranial and extracranial arteries. Whilst this is acknowledged as a weakness of the present study, as mentioned previously, it is known that VEGF is able to permeate across the BBB (Fabel et al., 2003; Lange et al., 2016; Morland et al., 2017) and therefore circulating levels of VEGF quantified from the periphery may well have been reflective of the VEGF levels within the cerebrovasculature. Similarly, the ability of NO to be transported along the vascular tree within the plasma or red blood cells (Rassaf et al., 2004) has been documented, and for that reason quantifying peripheral-NO bioavailability via assessment of NO_2 and NO_3 will in part be reflective of the NO levels (Bryan and Grisham, 2007; Piknova and Schecter, 2011) within the cerebrovasculature. To circumvent this limitation in the future and reduce speculation surrounding the findings, venous blood samples should be obtained from the internal jugular vein to provide an increasingly valid measure of neurovascular signalling factor changes occurring within the cerebrovasculature during or following exercise's completion.

5.5.4 The use of partial pressure of end-tidal carbon dioxide as an index of partial pressure of arterial carbon dioxide during exercise

A final limitation of this study resides in the use of $P_{ET}CO_2$ as an index of $PaCO_2$ during exercise. At rest, it has been shown that $P_{ET}CO_2$ accurately represents $PaCO_2$ (Williams and Babb, 1997), however during exercise, $P_{ET}CO_2$ has been shown to overestimate $PaCO_2$ (Williams and Babb, 1997; Benallal and Busso, 2000). More recent exercise-based studies have measured both $P_{ET}CO_2$ and $PaCO_2$ in response to moderate intensity cycling (Tymko et al., 2018) and all-out HIIT (Curtelin et al., 2017) and reported similar increases in $P_{ET}CO_2$ and $PaCO_2$ during all-out cycling HIIT (Curtelin et al., 2017). Therefore, it is likely that $P_{ET}CO_2$ reliably reflected the $PaCO_2$ in the current study.

In the current study, the $P_{ET}CO_2$ values obtained at rest were seen to be unusually low (~33 mmHg). However, values similar to these data have previously been reported within the literature (Jorgensen et al., 1992), suggesting that this was not an abnormal finding. To determine if $P_{ET}CO_2$ is a valid and reflective measure of arterial carbon dioxide levels in future studies, $PaCO_2$ could be quantified in conjunction with $P_{ET}CO_2$. Alternatively, carbon dioxide levels could be determined from capillary blood samples obtained from the ear lobe at rest and during exercise, as it is known that assessing blood gas quantities from capillary-derived blood samples is a reliable indicator of arterial concentrations of carbon dioxide (Zavorsky et al., 2007).

5.5.5 Menstrual cycle phase and cerebral blood flow velocity

Female participants were able to participate in the current study at any point during their menstrual cycle due to time restraints. Accordingly, the known effects that the menstrual cycle exerts on CBFv (Brackley et al., 1999) could not be controlled for. Within the literature, it has been outlined that MCAv is significantly higher during the luteal phase of the menstrual cycle in comparison to the follicular phase (Brackley et al., 1999) and subsequently, failing to control for these changes in the current study reduces the confidence with which we can state that the significant MCAv findings were induced by exercise alone. However, the sample in the current study was predominantly male and therefore, it is unlikely that the effects of the menstrual cycle significantly altered the measured cerebral perfusion responses to exercise. Nonetheless, in future research that investigates blood velocity responses to exercise that includes female participants; the menstrual cycle should be controlled for.

5.6 Alternative Approaches

5.6.1 Alternative methods for measuring vascular endothelial growth factor

VEGF was assessed in the current study from plasma using a commercially available ELISA kit (R and D Systems, Minneapolis, USA), as a means of informing the optimal exercise strategy that is capable of up-regulating the neurovascular signalling factor from baseline quantities. As outlined above, no significant exercise-induced elevations in VEGF were reported in the present study. This was surprising based upon the findings of previous experiments that have reported significant elevations in VEGF in response to exercise using the same ELISA kit (Adams et al., 2004; Gu et al., 2004; Kraus et al., 2004; Rehmen et al., 2004; Sandri et al., 2005; Wood et al., 2006; Suhr et al., 2007; Adams et al., 2008; Danzig et al., 2010; Ogawa et al., 2010; Beck et al., 2012; Sandri et al., 2011; Wahl et al., 2011; Wahl et al., 2014; Izzicupo et al., 2017). The methodology employed in the current study to quantify VEGF has therefore been questioned; as has the sensitivity of the commercially available ELISA kits.

Alternative approaches for VEGF quantification should therefore be considered in future research examining exercise's effects on VEGF, such as capture-antibody immobilised macro-porous poly(ethylene) glycol diacrylate (PEGDA) hydrogel microspheres as it is known that PEGDA hydrogel microspheres can reliably and consistently measure VEGF from serum at accuracy levels that exceed those associated with the implementation of ELISA kits (Al-Ameen and Ghosh, 2013). To determine whether this approach's accuracy and reliability extends to VEGF quantification in response to exercise in human models, VEGF could be quantified with ELISA kit and PEGDA hydrogel microspheres in future studies to compare methodologies. That being said, the failure of the current study to increase VEGF concentrations within the periphery may also be related to the timing the blood samples were extracted (and measured within this thesis) and for that reason using an ELISA kit to assess VEGF levels may still be a valid approach to assess the efficacy of difference exercise strategies to induce VEGF release.

5.6.2 Alternative methods for quantifying nitric oxide, nitrite and nitrate

The current study assessed total NO_2 /NO_3 concentrations as a representative marker of NO, based upon the knowledge that NO_2 and NO_3 are both known precursors of NO and their quantification can provide an invaluable insight into NO metabolism (Piknova and Schecter, 2011). A commercially available total NO_2/NO_3 immunoassay kit (R and D Systems, Minneapolis, USA) was employed to determine NO_2 and NO_3 concentrations in response to exercise, via the greiss reagent reaction. Greiss is known to be a simple, low-cost and repeatable test that determines the NO_2 content within a biological sample following a reaction with sulfanilic acid to produce a water soluble azo-dye that has its NO_2 concentration determined via chemiluminscence (Bryan and Grisham, 2007; Tsikas et al., 2007; Csonka et al., 2015). Whilst the greiss reagent reaction was once considered to be the gold standard methodology for quantifying NO_2 from a biological sample (Tsikas et al., 2007), technological advancements have produced alternative techniques that are capable of quantifying NO as well as, NO-derivatives (NO_2 and NO_3) without interfering with thiols, proteins and plasma constituents (Berkels et al., 2001). The most promising of these emerging techniques uses specialised electrochemical electrodes to directly quantify NO or its precursors (NO_2 and NO_3) from human blood samples (Berkels et al., 2001).The major advantages associated with this strategy is that it enables NO content to be determined in real-time, without any chemical reduction steps (as required in greiss) that could lead to sample contamination (Csonka et al., 2015). In addition, systems that use ion-specific electrodes such as the Straight Arrow system have been shown to be capable of detecting NO quantities as low as <3nmol/l (Berkels et al., 2001). Consequently, in future studies aiming to establish the optimal exercise strategy for increasing NO bioavailability, a system

that uses electrochemical electrodes to quantify NO should be implemented to reduce the risk of human error and contamination, whilst acquiring increasingly accurate results.

5.6.3 Alternative methods for assessing cerebral blood flow

TCD ultrasound was implemented in the current study to index CBF through insonation of blood velocity in the MCA. The limitations associated with this methodology are outlined in section 5.2.2 and state that TCD may not be a valid measure of CBF as the diameter of intracranial vessels, such as the MCA, may not remain constant during exercise (Coverdale et al., 2014; Verbree et al., 2014; Coverdale et al., 2015; Coverdale et al., 2017; Verbree et al., 2017). Consequently, alternative methodologies that are capable of measuring cerebral perfusion during exercise should be considered for their use in future studies that aim to examine cerebral blood flow throughout the completion of dynamic exercise.

Hiura et al. (2014) demonstrated that oxygen-15-labelled water and positron emission tomography (PET) imaging could be used to assess the impact that cycling had on regional and global cerebral blood flow. However, this methodology did not enable continuous monitoring of cerebral perfusion during exercise's completion and subsequently, would not be appropriate to document the effects that short bouts of exercise such as all-out HIIT for example, have on cerebral blood flow. Similarly, whilst magnetic resonance imaging (MRI) has previously been used to assess cerebral blood flow through the assessment of blood oxygenation level dependent (BOLD) signals (Tymko et al., 2018), acquisition of a MRI requires an individual to lay supine and motionless and subsequently, could not be used to assess cerebral blood flow during dynamic exercise (Tykmo et al., 2018).

The implementation of Duplex ultrasound in conjunction with TCD ultrasound may however, provide a valid and appropriate methodology of assessing both extracranial blood flow and intracranial blood flow velocity during dynamic exercise's completion. Furthermore, using Duplex ultrasound in conjunction with TCD ultrasound would also enable real-time measurements of FMD to be made, which as outlined above is known to be a physiological phenomenon driven by shear stress and the up-regulation of NO bioavailability (Smith et al., 2016; Smith et al., 2017). Accordingly, future studies that plan to investigate the responses of the extracranial and intracranial cerebrovasculature to dynamic exercise, should employ a combined approach formed of Duplex and TCD ultrasound, to enable CBF and MCAv to be quantified in conjunction with markers of shear stress. That said, assessing flow with Duplex Doppler during high intensity exercise is challenging, and limited to semi-recumbent cycling.

6. Conclusion

In conclusion, this study has demonstrated that the acute completion of all-out HIIT or Weston and colleagues' (2014) clinical HIIT guidelines are capable of significantly elevating precursors of NO, total NO_2/NO_3 in comparison to resting values, in contrast to the completion of moderate intensity exercise that failed to up-regulate total NO_2/NO_3. Based on this, it seems that clinical and all-out HIIT are more likely to provide the cerebrovasculature with the stimulus required for positive vascular adaptation within the brain and therefore, could be implemented in the future as preventative strategies in the fight against neurodegeneration or as a treatment in existing cases of poor brain health. Prior to this however, further investigation is required to be conducted into the effects HIIT exerts on the cerebrovasculature of clinical, older and populations, to affirm that vigorous physical activity is appropriate for such groups to complete whilst also confirming that the beneficial effects associated with clinical and all-out HIIT extend to the aforementioned groups.

Appendix

Dear Dr Samuel J.E. Lucas & Dr Rebekah Lucas

Re: "Investigating the interaction between exercise mode and exercise intensity on brain vascular health."
Application for Ethical Review ERN_17-1570

Thank you for your application for ethical review for the above project, which was reviewed by the Science, Technology, Engineering and Mathematics Ethical Review Committee.

On behalf of the Committee, I confirm that this study now has full ethical approval.

I would like to remind you that any substantive changes to the nature of the study as described in the Application for Ethical Review, and/or any adverse events occurring during the study should be promptly bought to the Committee's attention by the Principal Investigator and may necessitate further ethical review.

Please also ensure that the relevant requirements within the University's Code of Practice for Research and the information and guidance provided on the University's ethics webpages (available at https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Links-and-Resources.aspx) are adhered to and referred to in any future applications for ethical review. It is now a requirement on the revised application form (https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-Forms.aspx) to confirm that this guidance has been consulted and is understood, and that it has been taken into account when completing your application for ethical review.

Please be aware that whilst Health and Safety (H&S) issues may be considered during the ethical review process, you are still required to follow the University's guidance on H&S and to ensure that H&S risk assessments have been carried out as appropriate. For further information about this, please contact your School H&S representative or the University's H&S Unit at healthandsafety@contacts.bham.ac.uk.

Kind regards,

Ms Sam Waldron
Deputy Research Ethics Officer
Research Support Group
C Block Dome (room 132)
Aston Webb Building
University of Birmingham
Edgbaston B15 2TT
Tel: 0121 414 8101
Email: s.m.waldron@bham.ac.uk

List of References

Aaslid, R., Lindegaard, K.F., Sorteberg, W., et al. (1989) Cerebral autoregulation dynamics in humans. **Stroke,** 20 (1): 45-52.

Adams, V., Lenk, K., Linke, A., et al. (2004) Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. **Arteriosclerosis, Thrombosis, and Vascular Biology,** 24(4): 684-90.

Adams, V., Linke, A., Breuckmann, F., et al. (2008) Circulating progenitor cells decrease immediately after marathon race in advanced-age marathon runners. **European Journal of Cardiovascular Prevention & Rehabilitation,** 15 (5): 602-607.

Ainslie, P.N., Cotter, J.D., George, K.P., et al. (2008) Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. **The Journal of Physiology,** 586 (16): 4005-4010.

Al-Ameen, M.A., and Ghosh, G., (2013) Sensitive quantification of vascular endothelial growth factor (VEGF) using porosity induced hydrogel microspheres. **Biosensors and Bioelectronics,** 49: 105-110.

Bailey, D.M., Evans, K.A., McEneny, J., et al. (2011) Exercise-induced oxidative–nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood–brain barrier leakage. **Experimental Physiology,** 96 (11): 1196-1207.

Bang, O. (1936) The lactate content of the blood during and after muscular exercise in man. **Skandinavisches Archiv Für Physiologie,** 74 (S10): 51-82.

Barha, C.K., Galea, L.A., Nagamatsu, L.S., et al. (2017) Personalising exercise recommendations for brain health: considerations and future directions. **British Journal of Sports Medicine,** 51 (8): 636-639.

Bates, J.N. (1992) Nitric oxide measurement by chemiluminescence detection. **Neuroprotocols,** 1 (2): 141-149.

Beck, E.B., Erbs, S., Möbius-Winkler, S., et al. (2012) Exercise training restores the endothelial response to vascular growth factors in patients with stable coronary artery disease. **European Journal of Preventive Cardiology,** 19 (3): 412-418.

Berkels, R., Purol-Schnabel, S., Roesen, R. (2001) A new method to measure nitrate/nitrite with a NO-sensitive electrode. **Journal of Applied Physiology,** 90 (6): 317–320.

Benallal, H. and Busso, T. (2000) Analysis of end-tidal and arterial PCO2 gradients using a breathing model. **European Journal of Applied Physiology,** 83 (4-5): 402-408.

Bishop, C.C., Powell, S., Rutt, D., et al. (1986) Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. **Stroke,** 17 (5): 913-915.

Blagosklonny, M.V. (2010) Why human lifespan is rapidly increasing: solving "longevity riddle" with "revealed-slow-aging" hypothesis. **Aging,** 2 (4): 177-182.

Bolduc, V., Thorin-Trescases, N. and Thorin, E. (2013) Endothelium-dependent control of cerebrovascular functions through age: exercise for healthy cerebrovascular aging. **American Journal of Physiology - Heart and Circulatory Physiology,** 305 (5): H620-33.

Borg, G.A. (1982) Psychophysical bases of perceived exertion. **Med Sci Sports Exercise,** 14 (5): 377-381.

Brackley, K.J., Ramsay, M.M., Broughton Pipkin, F, et al. (1999) The effect of the menstrual cycle on human cerebral blood flow: studies using Doppler ultrasound. **Ultrasound in Obstetrics and Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology,** 14 (1): 52-57.

Brown, A.D., McMorris, C.A., Longman, R.S., et al. (2010) Effects of cardiorespiratory fitness and cerebral blood flow on cognitive outcomes in older women. **Neurobiology of Aging,** 31 (12): 2047-2057.

Brugniaux, J.V., Marley, C.J., Hodson, D.A., et al. (2014) Acute exercise stress reveals cerebrovascular benefits associated with moderate gains in cardiorespiratory fitness. **Journal of Cerebral Blood Flow & Metabolism,** 34 (12): 1873-1876.

Bryan, N.S. and Grisham, M.B., (2007) Methods to detect nitric oxide and its metabolites in biological samples**. Free Radical Biology and Medicine,** 43 (5): 645-657.

Buchner, D.M. (2007) Exercise slows functional decline in nursing home residents with Alzheimer's disease. **Australian Journal of Physiotherapy,** 53 (3): 204.

Burley, C.V., Bailey, D.M., Marley, C.J., et al. (2016) Brain train to combat brain drain; focus on exercise strategies that optimize neuroprotection. **Experimental Physiology,** 101 (9): 1178-1184.

Burns, D.M. and MacDonald, S.G. (1971) Physics for Biology and Pre-Medical Students. **American Journal of Physics,** 39 (9): 1108-1109.

Chandra, A., Li, W.A., Stone, C.R., Geng, X., et al. (2017) The Cerebral Circulation and Cerebrovascular Disease: Anatomy. **Brain Circulation,** 3 (2): 45.

Churchill, J.D., Galvez, R., Colcombe, S., et al. (2002) Exercise, experience and the aging brain. **Neurobiology of Aging,** 23 (5): 941-955.

Cohen, J., (1992) Quantitative methods in psychology: A power primer. **Psychological Bulletin,** 112 (1) 155-159.

Colcombe, S.J., Erickson, K.I., Raz, N., et al. (2003) Aerobic fitness reduces brain tissue loss in aging humans. **The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,** 58 (2): M176-M180.

Colcombe, S. and Kramer, A.F. (2003) Fitness effects on the cognitive function of older adults: a meta-analytic study. **Psychological Science,** 14 (2): 125-130.

Colcombe, S.J., Erickson, K.I., Scalf, P.E., et al. (2006) Aerobic exercise training increases brain volume in aging humans. **The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences,** 61 (11): 1166-1170.

Cotman, C.W., Berchtold, N.C. and Christie, L. (2007) Exercise builds brain health: key roles of growth factor cascades and inflammation. **Trends in Neurosciences,** 30 (9): 464-472.

Coverdale, N.S., Badrov, M.B. and Shoemaker, J.K. (2017) Impact of age on cerebrovascular dilation versus reactivity to hypercapnia. **Journal of Cerebral Blood Flow & Metabolism,** 37 (1): 344-355.

Coverdale, N.S., Gati, J.S., Opalevych, O., et al. (2014) Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. **Journal of Applied Physiology,** 117 (10): 1090-1096.

Coverdale, N.S., Lalande, S., Perrotta, A., et al. (2015) Heterogeneous patterns of vasoreactivity in the middle cerebral and internal carotid arteries. **American Journal of Physiology - Heart and Circulatory Physiology,** 308 (9): H1030-H1038.

Csonka, C., Páli, T., Bencsik, P., et al. (2015) Measurement of NO in biological samples. **British Journal of Pharmacology,** 172 (6): 1620-1632.

Curtelin, D., Morales-Alamo, D., Torres-Peralta, R., et al. (2017) Cerebral blood flow, frontal lobe oxygenation and intra-arterial blood pressure during sprint exercise in normoxia and severe acute hypoxia in humans. **Journal of Cerebral Blood Flow & Metabolism,** 0271678X17691986.

Danzig, V., Mikova, B., Kuchynka, P., et al. (2010) Levels of circulating biomarkers at rest and after exercise in coronary artery disease patients. **Physiological Research,** 59 (3):.

De Silva, T.M. and Faraci, F.M. (2016) Microvascular dysfunction and cognitive impairment. **Cellular and Molecular Neurobiology,** 36 (2): 241-258.

Deforche, B., Haerens, L. and De Bourdeaudhuij, I. (2011) How to make overweight children exercise and follow the recommendations. **International Journal of Paediatric Obesity,** 6 (S1): 35-41.

dela Paz, N.G., Walshe, T.E., Leach, L.L., et al. (2012) Role of shear-stress-induced VEGF expression in endothelial cell survival. **Journal of Cell Science,** 125 (Pt 4): 831-843.

Department of Health (2006) The General Practice Physical Activity Questionnaire.

Dias, K.A., Ingul, C.B., Tjønna, A.E., et al. (2018) Effect of high-intensity interval training on fitness, fat mass and cardiometabolic biomarkers in children with obesity: a randomised controlled trial. **Sports Medicine,** 48 (3): 733-746.

Digital, N. (2018) Recorded Dementia Diagnoses January 2018.

Ding, D., Lawson, K.D., Kolbe-Alexander, T.L., et al. (2016) The economic burden of physical inactivity: a global analysis of major non-communicable diseases. **The Lancet,** 388 (10051): 1311-1324.

Egginton, S., Hudlicka, O., Brown, M., et al. (1998) Capillary growth in relation to blood flow and performance in overloaded rat skeletal muscle. **Journal of Applied Physiology,** 85 (6): 2025-2032.

Erickson, K.I., Voss, M.W., Prakash, R.S., et al. (2011) Exercise training increases size of hippocampus and improves memory. **Proceedings of the National Academy of Sciences of the United States of America,** 108 (7): 3017-3022.

Fabel, K., Tam, B., et al. (2003) VEGF is necessary for exercise-induced adult hippocampal neurogenesis. **European Journal of Neuroscience,** 18 (10): 2803-2812.

Faraci, F.M. and Heistad, D.D. (1998) Regulation of the cerebral circulation: role of endothelium and potassium channels. **Physiological Reviews,** 78 (1): 53-97.

Farkas, E., Luiten, P.G. and Bari, F. (2007) Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. **Brain Research Reviews,** 54 (1): 162-180.

Fukumura, D., Xu, L., Chen, Y., et al. (2001) Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. **Cancer Research,** 61 (16): 6020-6024.

Goto, C., Nishioka, K., Umemura, T., et al. (2007) Acute moderate-intensity exercise induces vasodilation through an increase in nitric oxide bioavailiability in humans. **American Journal of Hypertension,** 20 (8): 825-830.

Green, D.J., Hopman, M.T., Padilla, J., et al. (2017) Vascular adaptation to exercise in humans: role of hemodynamic stimuli. **Physiological Reviews,** 97 (2): 495-528.

Green, D.J., Maiorana, A., O'driscoll, G., et al. (2004) Effect of exercise training on endothelium-derived nitric oxide function in humans. **The Journal of Physiology,** 561 (1): 1-25.

Green, D., Cheetham, C., Reed, C., et al. (2002) Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry. **Journal of Applied Physiology,** 93 (1): 361-368.

Gu, J., Gadonski, G., Wang, J., et al. (2004) Exercise increases endostatin in circulation of healthy volunteers. **BMC Physiology,** 4 (1): 2.

Gustafsson, T., Bodin, K., Sylven, C., et al. (2001) Increased expression of VEGF following exercise training in patients with heart failure. **European Journal of Clinical Investigation,** 31 (4): 362-366.

Gustafsson, T., Puntschart, A., Kaijser, L., et al. (1999) Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. **American Journal of Physiology - Heart and Circulatory Physiology,** 276 (2): H679-H685.

Hallal, P.C., Andersen, L.B., Bull, F.C., et al. (2012) Global physical activity levels: surveillance progress, pitfalls, and prospects. **The Lancet,** 380 (9838): 247-257.

Hamer, M. and Chida, Y. (2009) Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence. **Psychological Medicine,** 39 (1): 3-11.

Hellström, G. and Wahlgren, N.G. (1993) Physical exercise increases middle cerebral artery blood flow velocity. **Neurosurgical Review,** 16 (2): 151-156.

Hellstrom, G., Fischer-Colbrie, W., Wahlgren, N.G., et al. (1996) Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. **Journal of Applied Physiology,** 81 (1): 413-418.

Hermansen, L. and Saltin, B. (1967) Blood lactate concentration during exercise at acute exposure to altitude. **Exercise at Altitude. New York, NY: Excerpta Medica Foundation,** 48-53.

Hermansen, L. (1971) "Lactate production during exercise" In **Muscle Metabolism during Exercise** Springer. pp. 401-407.

Heyn, P., Abreu, B.C. and Ottenbacher, K.J. (2004) The effects of exercise training on elderly persons with cognitive impairment and dementia: A meta-analysis1. **Archives of Physical Medicine and Rehabilitation,** 85 (10): 1694-1704.

Hiura, M., Nariai, T., Ishii, K., et al. (2014) Changes in cerebral blood flow during steady-state cycling exercise: a study using oxygen-15-labeled water with PET. **Journal of Cerebral Blood Flow & Metabolism,** 34 (3): 389-396.

Hötting, K. and Röder, B. (2013) Beneficial effects of physical exercise on neuroplasticity and cognition. **Neuroscience & Biobehavioural Reviews,** 37 (9): 2243-2257.

Hunt, T.K., Aslam, R.S., Beckert, S., et al. (2007) Aerobically derived lactate stimulates revascularization and tissue repair via redox mechanisms. **Antioxidants & Redox Signalling,** 9 (8): 1115-1124.

Hyodo, I., Doi, T., Endo, H., et al. (1998) Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. **European Journal of Cancer,** 34 (13): 2041-2045.

Iemitsu, M., Maeda, S., Jesmin, S., et al. (2006) Exercise training improves aging-induced downregulation of VEGF angiogenic signalling cascade in hearts. **American Journal of Physiology - Heart and Circulatory Physiology,** 291 (3): H1290-H1298.

Isaacs, K.R., Anderson, B.J., Alcantara, A.A., et al. (1992) Exercise and the brain: angiogenesis in the adult rat cerebellum after vigorous physical activity and motor skill learning. **Journal of Cerebral Blood Flow & Metabolism,** 12 (1): 110-119.

Izzicupo, P., D'Amico, M., Di Blasio, A., et al. (2017) Nordic walking increases circulating VEGF more than traditional walking training in postmenopause. **Climacteric,** 20 (6): 533-539.

Jelkmann, W., (2001). Pitfalls in the measurement of circulating vascular endothelial growth factor. **Clinical Chemistry,** 47(4): 617-623.

Jervell, O. (1928) Investigation of the concentration of lactic acid in blood and urine under physiologic and pathologic conditions. **Nationaltrykkeriet**.

Killgore, W.D., Olson, E.A. and Weber, M. (2013) Physical exercise habits correlate with gray matter volume of the hippocampus in healthy adult humans. **Scientific Reports,** 3 3457.

Korshunov, V.A., Schwartz, S.M. and Berk, B.C. (2007) Vascular remodeling: hemodynamic and biochemical mechanisms underlying Glagov's phenomenon. **Arteriosclerosis, Thrombosis, and Vascular Biology,** 27 (8): 1722-1728.

Kramer, A.F., Hahn, S., Cohen, N.J., et al. (1999) Ageing, fitness and neurocognitive function. **Nature,** 400 (6743): 418-419.

Kraus, R.M., Stallings III, H.W., Yeager, R.C., et al. (2004) Circulating plasma VEGF response to exercise in sedentary and endurance-trained men. **Journal of Applied Physiology,** 96 (4): 1445-1450.

Lange, C., Storkebaum, E., De Almodóvar, C.R., et al. (2016). Vascular endothelial growth factor: a neurovascular target in neurological diseases. **Nature Reviews Neurology,** 12(8): 439.

Larson, E.B., Wang, L., Bowen, J.D., et al. (2006) Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. **Annuals of Internal Medicine,** 144 (2): 73-81.

Laughlin, M.H., Newcomer, S.C. and Bender, S.B. (2008) Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. **Journal of Applied Physiology,** 104 (3): 588-600.

Lautenschlager, N.T., Cox, K.L., Flicker, L., et al. (2008) Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. **Journal of American Medical Association,** 300 (9): 1027-1037.

Lee, I., Shiroma, E.J., Lobelo, F., et al. (2012) Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. **The Lancet,** 380 (9838): 219-229.

Lee, C.D., Folsom, A.R. and Blair, S.N. (2003) Physical activity and stroke risk: a meta-analysis. **Stroke,** 34 (10): 2475-2481.

Lee, I.M., Hennekens, C.H., Berger, K., et al. (1999) Exercise and risk of stroke in male physicians. **Stroke,** 30 (1): 1-6.

Lezi, E., Lu, J., Selfridge, J.E., et al. (2013) Lactate administration reproduces specific brain and liver exercise-related changes. **Journal of Neurochemistry,** 127 (1): 91.

Lu, D. and Kassab, G.S. (2011) Role of shear stress and stretch in vascular mechanobiology. **Journal of the Royal Society, Interface,** 8 (63): 1379-1385.

Lucas, S.J., Cotter, J.D., Brassard, P., et al. (2015) High-intensity interval exercise and cerebrovascular health: curiosity, cause, and consequence. **Journal of Cerebral Blood Flow & Metabolism,** 35 (6): 902-911.

Lucas, S.J., Tzeng, Y.C., Galvin, S.D., et al. (2010) Influence of changes in blood pressure on cerebral perfusion and oxygenation. **Hypertension,** 55 (3): 698-705.

Lyngeraa, T., Pedersen, L.M., Mantoni, T., et al. (2013) Middle cerebral artery blood velocity during running. **Scandinavian Journal of Medicine & Science in Sports,** 23 (1): e32-e37.

Manson, J.E., Greenland, P., LaCroix, A.Z., et al. (2002) Walking compared with vigorous exercise for the prevention of cardiovascular events in women. **New England Journal of Medicine,** 347 (10): 716-725.

Marcinko, K., Sikkema, S.R., Samaan, M.C., et al. (2015) High intensity interval training improves liver and adipose tissue insulin sensitivity. **Molecular Metabolism,** 4 (12): 903-915.

Middleton, L.E. and Yaffe, K. (2009) Promising strategies for the prevention of dementia. **Archives of Neurology,** 66 (10): 1210-1215.

Milanović, Z., Sporiš, G. and Weston, M. (2015) Effectiveness of high-intensity interval training (HIT) and continuous endurance training for VO 2max improvements: a systematic review and meta-analysis of controlled trials. **Sports Medicine,** 45 (10): 1469-1481.

Möhle, R., Green, D., Moore, M.A., Nachman, R.L. and Rafii, S. (1997) Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. **Proceedings of the National Academy of Sciences** (94(2)): pp.663-668.

Morici, G., Zangla, D., Santoro, A., et al. (2005) Supramaximal exercise mobilizes hematopoietic progenitors and reticulocytes in athletes. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,** 289 (5): R1496-R1503.

Morland, C., Andersson, K.A., Haugen, Ø.P., et al. (2017) Exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1. **Nature Communications,** 8 15557.

Murray, C.J. and Lopez, A.D. (2013) Measuring the global burden of disease. **New England Journal of Medicine,** 369 (5): 448-457. .

Niebauer, J. and Cooke, J.P. (1996) Cardiovascular effects of exercise: role of endothelial shear stress. **Journal of the American College of Cardiology,** 28 (7): 1652-1660.

Ogawa, K., Sanada, K., Machida, S., et al. (2010) Resistance exercise training-induced muscle hypertrophy was associated with reduction of inflammatory markers in elderly women. **Mediators of Inflammation,** 2010 171023.

Ogoh, S., Fadel, P.J., Zhang, R., et al. (2005) Middle cerebral artery flow velocity and pulse pressure during dynamic exercise in humans. **American Journal of Physiology - Heart and Circulatory Physiology,** 288 (4): H1526-H1531.

Ogoh, S. and Ainslie, P.N. (2009) Cerebral blood flow during exercise: mechanisms of regulation. **Journal of Applied Physiology,** 107 (5): 1370-1380.

Padilla, J., Simmons, G.H., Bender, S.B., et al. (2011) Vascular effects of exercise: endothelial adaptations beyond active muscle beds. **Physiology (Bethesda, Md.),** 26 (3): 132-145.

Park, J., Nakamura, Y., Kwon, Y., et al. (2010) The effect of combined exercise training on carotid artery structure and function, and vascular endothelial growth factor (VEGF) in obese older women. **Japanese Journal of Physical Fitness and Sports Medicine,** 59 (5): 495-504.

Petersen, W., Varoga, D., Zantop, T., et al. (2004) Cyclic strain influences the expression of the vascular endothelial growth factor (VEGF) and the hypoxia inducible factor 1 alpha (HIF-1α) in tendon fibroblasts. **Journal of Orthopaedic Research,** 22 (4): 847-853.

Phillips, C., Baktir, M.A., Srivatsan, M., et al. (2014) Neuroprotective effects of physical activity on the brain: a closer look at trophic factor signaling. **Frontiers in Cellular Neuroscience,** 8 170.

Piknova, B. and Schechter, A.N., (2011) Measurement of nitrite in blood samples using the ferricyanide-based hemoglobin oxidation assay. **Nitric Oxide,** :39-56.

Podewils, L.J., Guallar, E., Kuller, L.H., et al. (2005) Physical activity, APOE genotype, and dementia risk: findings from the Cardiovascular Health Cognition Study. **American Journal of Epidemiology,** 161 (7): 639-651.

Prince, M., Knapp, M., Guerchet, M., et al. (2014) Dementia UK: update. **Alzheimer's Society**.

Prior, B.M., Lloyd, P.G., Yang, H., et al. (2003) Exercise-induced vascular remodelling. **Exercise and Sport Sciences Reviews,** 31 (1): 26-33.

Prior, B.M., Yang, H. and Terjung, R.L. (2004) What makes vessels grow with exercise training? **Journal of Applied Physiology,** 97 (3): 1119-1128.

Querido, J.S. and Sheel, A.W. (2007) Regulation of cerebral blood flow during exercise. **Sports Medicine,** 37 (9): 765-782.

Ramos, J.S., Dalleck, L.C., Tjonna, A.E., et al. (2015) The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. **Sports Medicine,** 45 (5): 679-692.

Rassaf, T., Feelisch, M., and Kelm, M., (2004) Circulating NO pool: assessment of nitrite and nitroso species in blood and tissues. **Free Radical Biology and Medicine,** 36 (4): 413-422.

Rehman, J., Li, J., Parvathaneni, L., et al. (2004) Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. **Journal of the American College of Cardiology,** 43 (12): 2314-2318.

Rodriguez, N.R., DiMarco, N.M. and Langley, S. (2009) Nutrition and athletic performance. **Medicine and Science in Sports and Exercise,** 41 (3): 709-731.

Sacco, R.L., Gan, R., Boden-Albala, B., et al. (1998) Leisure-time physical activity and ischemic stroke risk: the Northern Manhattan Stroke Study. **Stroke,** 29 (2): 380-387.

Sandri, M., Adams, V., Gielen, S., et al. (2005) Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes: results of 3 randomized studies. **Circulation,** 111 (25): 3391-3399.

Sandri, M., Beck, E.B., Adams, V., et al. (2011) Maximal exercise, limb ischemia, and endothelial progenitor cells. **European Journal of Cardiovascular Prevention & Rehabilitation,** 18 (1): 55-64.

Schlager, O., Giurgea, A., Schuhfried, O., et al. (2011) Exercise training increases endothelial progenitor cells and decreases asymmetric dimethylarginine in peripheral arterial disease: a randomized controlled trial. **Atherosclerosis,** 217 (1): 240-248.

Skriver, K., Roig, M., Lundbye-Jensen, J., et al. (2014) Acute exercise improves motor memory: exploring potential biomarkers. **Neurobiology of Learning and Memory,** 116 46-58.

Smith, J.C., Nielson, K.A., Woodard, J.L., et al. (2014) Physical activity reduces hippocampal atrophy in elders at genetic risk for Alzheimer's disease. **Frontiers in Aging Neuroscience,** 6 61.

Smith, K., MacLeod, D., Willie, C., et al. (2014) Influence of high altitude on cerebral blood flow and fuel utilization during exercise and recovery. **The Journal of Physiology,** 592 (24): 5507-5527.

Smith, K.J. and Ainslie, P.N. (2017) Regulation of cerebral blood flow and metabolism during exercise. **Experimental Physiology,** 102 (11): 1356-1371.

Suhr, F., Brixius, K., de Marées, M., et al. (2007) Effects of short-term vibration and hypoxia during high-intensity cycling exercise on circulating levels of angiogenic regulators in humans. **Journal of Applied Physiology,** 103 (2): 474-483.

Thijssen, D.H., Dawson, E.A., Tinken, T.M., et al. (2009) Retrograde flow and shear rate acutely impair endothelial function in humans. **Hypertension (Dallas, Tex.: 1979),** 53 (6): 986-992.

Thompson, P.D., Buchner, D., Piña, I.L., et al. (2003) Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. **Arteriosclerosis, Thrombosis, and Vascular Biology,** 23 (8): e42-e49.

Thompson, P.D., Buchner, D., Piña, I.L., et al. (2003) Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). **Circulation,** 107 (24): 3109-3116.

Tiecks, F.P., Lam, A.M., Aaslid, R., et al. (1995) Comparison of static and dynamic cerebral autoregulation measurements. **Stroke,** 26 (6): 1014-1019.

Tinken, T.M., Thijssen, D.H., Hopkins, N., et al. (2010) Shear stress mediates endothelial adaptations to exercise training in humans. **Hypertension (Dallas, Tex.: 1979),** 55 (2): 312-318.

Tsikas, D., (2007) Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. **Journal of Chromatography,** 851 (1-2): 51-70.

Tymko, M.M., Ainslie, P.N. and Smith, K.J. (2018) Evaluating the methods used for measuring cerebral blood flow at rest and during exercise in humans. **European Journal of Applied Physiology,** 1-12.

Van der Borght, K., Kóbor-Nyakas, D.É., Klauke, K., et al. (2009) Physical exercise leads to rapid adaptations in hippocampal vasculature: temporal dynamics and relationship to cell proliferation and neurogenesis. **Hippocampus,** 19 (10): 928-936.

Van Praag, H., Shubert, T., Zhao, C., et al. (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. **The Journal of Neuroscience: the Official Journal of the Society for Neuroscience,** 25 (38): 8680-8685.

Vaynman, S. and Gomez-Pinilla, F. (2006) Revenge of the “sit”: how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. **Journal of Neuroscience Research,** 84 (4): 699-715.

Vaynman, S. and Gomez-Pinilla, F. (2006) Revenge of the “sit”: how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. **Journal of Neuroscience Research,** 84 (4): 699-715.

Verbree, J., Bronzwaer, A., van Buchem, M.A., et al. (2017) Middle cerebral artery diameter changes during rhythmic handgrip exercise in humans. **Journal of Cerebral Blood Flow & Metabolism,** 37 (8): 2921-2927.

Verbree, J., Bronzwaer, A.G., Ghariq, E., et al. (2014) Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. **Journal of Applied Physiology,** 117 (10): 1084-1089.

Voss, M.W., Vivar, C., Kramer, A.F., et al. (2013) Bridging animal and human models of exercise-induced brain plasticity. **Trends in Cognitive Sciences,** 17 (10): 525-544.

Wahl, P., Jansen, F., Achtzehn, S., et al. (2014) Effects of high intensity training and high volume training on endothelial microparticles and angiogenic growth factors. **PLoS One,** 9 (4): e96024.

Wahl, P., Zinner, C., Achtzehn, S., et al. (2011) Effects of acid–base balance and high or low intensity exercise on VEGF and bFGF. **European Journal of Applied Physiology,** 111 (7): 1405-1413.

Wang, Y., Chang, J., Li, Y., et al. (2004) Shear stress and VEGF activate IKK via the Flk-1/Cbl/Akt signaling pathway. **American Journal of Physiology - Heart and Circulatory Physiology,** 286 (2): H685-H692.

Warburton, D.E., Nicol, C.W. and Bredin, S.S. (2006) Health benefits of physical activity: the evidence. **CMAJ: Canadian Medical Association Journal = Journal de l'Association Medicale Canadienne,** 174 (6): 801-809.

Weston, K.S., Wisloff, U. and Coombes, J.S. (2014) High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. **British Journal of Sports Medicine,** 48 (16): 1227-1234.

Weuve, J., Kang, J.H., Manson, J.E., et al. (2004) Physical activity, including walking, and cognitive function in older women. **Journal of American Medical Association,** 292 (12): 1454-1461.

WHO (2012) Prevalence of Insufficient Physical Activity. World Health Organization: Geneva, 2012.

Williams, J.S. and Babb, T.G. (1997) Differences between estimates and measured PaCO2 during rest and exercise in older subjects. **Journal of Applied Physiology,** 83 (1): 312-316.

Wood, R.E., Sanderson, B.E., Askew, C.D., et al. (2006) Effect of training on the response of plasma vascular endothelial growth factor to exercise in patients with peripheral arterial disease. **Clinical Science,** 111 (6): 401-409.

Zavorsky, G.S., Cao, J., Mayo, N.E., Gabbay, R. and Murias, J.M., (2007) Arterial versus capillary blood gases: a meta-analysis. **Respiratory Physiology & Neurobiology,** 155 (3): 268-279.

Zschucke, E., Gaudlitz, K. and Ströhle, A. (2013) Exercise and physical activity in mental disorders: clinical and experimental evidence. **Journal of Preventive Medicine and Public Health,** 46 (Suppl 1): S12-S21.