CHARACTERISING CEREBRAL HAEMODYNAMIC OSCILLATIONS DURING RUNNING

by

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ABSTRACT

Running involves repetitive foot-strikes that induces a 'beat phenomenon', identified as a summation of two pressure waves that result in periodic pulse pressure oscillations. To date, only one study has examined this phenomenon to investigate how it affects cerebral blood flow (CBF) responses, reporting that blood pressure oscillations are reflected in CBF (as indexed by middle cerebral artery blood velocity, MCAv). Based on this work, we tested the hypothesis that altering the heel-strike force would impact the amplitude, frequency and duration of MCAv oscillations.

Ten participants completed four exercise sessions: a treadmill maximal oxygen consumption test (i.e. VO_{2peak}), followed by a randomised crossover design of three submaximal exercise sessions: (i) gradient running protocol; (ii) step frequency and foot-strike running protocol, and (iii) 30-minute cycling 65% VO_{2peak} protocol.

Oscillations were present during each running protocol with a variety of oscillatory patterns occurring between participants and between different gradients, running styles and step frequencies. No statistical difference in the oscillatory pattern occurred between submaximal treadmill running protocols ($p>.05$), while no MCAv oscillations were present during cycling.

Data collectively demonstrated that altering the heel-strike force during running affects the oscillatory pattern in MCAv, albeit not statistically supported from this small sample size.

ACKNOWLEDGEMENTS

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Ach	Acetylcholine
BP	Blood pressure
BPM	Beats per minute
CBF	Cerebral blood flow
CLS	Cardio-locomotor synchronization
CNS	Central nervous system
CO_2	Carbon dioxide
cO_2Hb	Prefrontal cortical oxyhaemoglobin
COM	Centre of mass
dBP	Diastole blood pressure
dMCAv	Diastole middle cerebral artery blood velocity
ECA	External carotid artery
eNOS	Endothelial nitric oxide synthase
FMD	Flow-mediated dilation
H+	Hydrogen
HR	Heart rate
HRmax	Maximal heart rate
ICA	Internal carotid artery
IMP	Intramuscular pressure
MAP	Mean arterial pressure
MCAv	Middle cerebral artery blood velocity
NIRS	Near-infrared spectroscopy
NO	Nitric oxide

O_2	Oxygen
PCO_2	Partial pressure of carbon dioxide
$PETCO_2$	Partial pressure of end-tidal carbon dioxide
PPA	Pulse pressure amplitude
RPE	Rate of perceived exertion
RPM	Revolutions per minute
sBP	Systole blood pressure
sMCAv	Systole middle cerebral artery blood velocity
TCD	Transcranial Doppler
ThCOx	Cerebral oxygenation threshold
THI	Tissue haemoglobin index
THI_{leg}	Tissue haemoglobin index of the rectus femoris
THI_{head}	Tissue haemoglobin index of the prefrontal cortex
TOI	Tissue oxygenation index
TOI_{head}	Tissue oxygen index of the prefrontal cortex
TOI_{leg}	Tissue oxygen index of the rectus femoris
VO_2	Oxygen consumption
VO_{2peak}	Peak oxygen consumption

INTRODUCTION

Exercise is known to improve cerebral blood flow (CBF; Querido and Sheel, 2007), benefiting brain health (Burley et al. 2016). Currently, optimal exercise parameters and underlying mechanisms to enhance cerebrovasculature remains underdetermined. The majority of research on CBF profiles during exercise has been based on cycling, a non-weight bearing modality (Olmedillas *et al.* 2012). These studies indicate that moderate intensity exercise ($\sim65\%VO_{2peak}$) is optimal for brain health due to eliciting the maximal increase in CBF (Brugniaux *et al.* 2014). However, running and rowing have both been shown to produce an alternative CBF profile across a range of intensities (Faull *et al.* 2014; Lyngeraa *et al.* 2013; Pott *et al.* 1997), which suggests that universal recommendations across exercise modalities for CBF responses may be incorrect.

In comparison to cycling-based research, the treadmill as a modality has been neglected when exploring the influence of exercise intensity on CBF and partial pressure of end-tidal carbon dioxide ($PETCO_2$) responses. Running has been specifically associated with a 'beat phenomenon', which has been identified as a summation of two pressure waves (Palatini *et al.* 1989). The mechanical impact of the repetitive heel strikes during running gait shocks blood vessels, thus generating a wave that combines with arterial pressure waves (Palatini *et al.* 1989). Together they induce periodic pulse pressure oscillations within arterial blood pressure (BP) that are transmitted into CBF and change with exercise intensity (Lyngeraa *et al.* 2013). Previously, an increase in the oscillation wavelength duration was observed to increase with an increasing running speed (Palatini *et al.* 1989 and Lyngeraa *et al.* 2013). However, the effect running gradient has on the oscillatory pattern, has not

been explored. Interestingly, investigations of running kinematics have shown that uphill running induces a forefoot striking pattern that potentially reduces the amount of force penetrating through the heel, while downhill running induces a rear-foot striking pattern that augments the heel-strike force (reviewed in Vernillo *et al.* 2017). These kinematic-observations of running gait are likely to impact the heel-strike force associated changes in BP fluctuations and thus CBF. Therefore, the first aim of this study is to explore the use of a stepwise gradient change of the VO_{2peak} test, to investigate the impact of the increasing intensity (i.e. gradient) on MCAv oscillations (i.e. oscillation duration and frequency). The second aim is to compare CBF profiles during uphill and downhill running. The third aim is to explore how running styles impact the MCAv oscillatory pattern. Specifically, toe-weighted and heel-weighted running which mimics uphill and downhill running.

Step frequency is also known to have an influence over the BP oscillatory pattern. Palatini *et al.* (1989) and Lyngeraa *et al.* (2013) observed that the CBF oscillation wavelength duration increased as the difference between the step frequency and heart rate (HR) decreased. Additionally, Lyngeraa *et al.* (2013) found that CBF oscillations temporarily disappeared with a change in treadmill belt speed when running rhythm was interrupted, which then returned once participants found their running rhythm again. These observations indicate that there may be a direct association between individuals running rhythm, step frequency and the development of oscillations. Nevertheless, the relationship between step frequency and CBF oscillation frequency during running has not been examined. Therefore, the final aim of this study will be to explore the influence step frequency has on MCAv oscillations.

The primary purpose of the present study is to examine how changing the heel strike force and pattern during running impacts CBF and pulse pressure oscillations. It is hypothesised that downhill running will increase the heel-strike force, elevating the amplitude of MCAv pulse pressure oscillations with a reduction in the wavelength duration compared to uphill running. The findings of this study will improve the understanding of how to optimise exercise parameters for improved brain health.

LITERATURE REVIEW

The purpose of this review is to extensively evaluate and critique literature investigating the impact that exercise has on brain health. The review will be broken down into eight sections that will each discuss available evidence that contributes to the understanding of how exercise improves cerebrovascular health. An essential component of understanding the influences exercise has on CBF, is the knowledge of the regulators that mediate CBF. After a brief overview of how exercise affects brain health, the following section will explore the different regulators and their influence on CBF. The next question that may be postulated, is how does exercise elicit positive effects within the cerebrovasculature? Therefore, evidence of possible underlying mechanisms within the brain that induce beneficial cerebrovasculature adaptations will be explored. There is currently minimal literature that has directly examined the underlying mechanisms of cerebrovasculature adaptations in humans. The majority of evidence in humans stems from exercise interventions examining effects within the peripheral vasculature. Of note, limited research exists given the difficulties associated with direct invasive studies on humans. Accordingly, this research will be briefly reviewed, including the context of how it might translate to the cerebrovasculature. Exercise is a broad term which includes a range of different exercise parameters such as mode and intensity. Consequently, the subsequent section will explore the influence that different exercise modalities and intensities may have on CBF. Running is a common mode of exercise, involving repetitive foot-strikes that have been observed to induce pulse pressure oscillations both within arterial BP and CBF. Currently, limited literature exists that examines the effects of different exercise modalities on CBF other than cycling. Therefore, running

locomotion will be examined including the influence that gradient has on the kinematics of this exercise modality and how this may potentially have an impact on CBF. Penultimately, evidence of entrainment between HR and running cadence will be examined including speculation of how this may influence CBF. Finally, a summary will conclude all the aforementioned sections and evidence presented.

Current Understanding of the Positive Benefits of Exercise on Cerebral Health

A plethora of studies have indicated that regular exercise is associated with positive cerebrovasculature health. CBF is essential for brain function with precise control over nutrient supply and removal of biproducts (Smith and Ainsle, 2017). Exercise exerts beneficial effects on the cerebral circulation, which is associated with reduced cerebrovascular events such as stroke (Padilla *et al.* 2011). Regular exercise engenders beneficial effects on cerebrovasculature health in clinical and non-clinical populations (Lautenschlager *et al.* 2012). Despite such known benefits, a third of the global population neglect the minimum physical activity requirements to sustain optimum health (Halla *et al.* 2012). Indeed, physical inactivity is ranked within the top 10 risk factors for poor health sustainability (Lucas *et al.* 2015) and contributes to ~6-10% deaths from non-communicable disease (Halla *et al.* 2012).

Sedentary behaviour and ageing both increase the risk of developing a cerebrovascular and/or neurodegenerative disease (Deary *et al.* 2009). As a result of improved healthcare, the United Kingdom has an increased percentage of an ageing population. Due to prevalent age-related health issues, cognitive decline and pathological brain disorders such as Alzheimer's and cerebral infarction have become increasingly evident. Epidemiological studies found ~30% of individuals with Alzheimer's were related to risk factors such as physical inactivity and poor

cardiovascular health (Norton *et al.* 2014). An array of stressors has been shown to induce positive health-related physiological adaptations within the brain (Burley *et al.* 2016). This includes physical activity, which improves cognition (Kramer *et al.* 1999). Specifically, acute exercise causes episodic increases in CBF and through repeated exposure (i.e. regular exercise) has been associated with counteracting the natural decline in cerebral tissue density (Colcombe *et al.* 2003), aiding the maintenance of brain volume (Colcombe *et al.* 2006) and cognitive function (Lautenschlager *et al.* 2012; Davenport *et al.* 2012). Therefore, physical activity has been prescribed to patients at risk of dementia to improve cognitive capacity as results have shown improvements in memory tasks, and grey matter hippocampal volume (Erickson *et al.* 2011 and Killgore *et al.* 2013). Overall, improving cerebrovasculature health, enhances a healthier longevity and well-being for individuals that will ultimately reduce the economic burden on health care services (Lucas *et al.* 2015). Nevertheless, while it is clear regular physical activity and habitual exercise offset natural ageing and disease-related impairments in brain health, what the optimal exercise is for mediating these effects are is uncertain. This uncertainty is in part related to the complex regulation of CBF, especially during exercise (Smith and Ainslie, 2017).

Regulation of Cerebral Blood Flow

This section will explore the regulation of CBF during acute exercise, taking an integrative physiological perspective due to the array of mediators involved (Ainslie and Ogoh, 2009). The main regulators of CBF are believed to be neural activity, metabolic demands, BP, cerebral autoregulation, cardiac output, and partial pressure of carbon dioxide (PCO_2) (Lucas *et al.* 2015; Willie *et al.* 2014). A multitude of

mechanisms are considered to be responsible for influencing cerebrovascular resistance and thus blood flow, which include myogenic, neurogenic, metabolic and endothelial factors (Brassard *et al*. 2017; Tzeng and Ainslie, 2014). Nonetheless, it remains unclear how these regulators govern changes in CBF during exercise in humans.

The regulators of CBF work efficiently to continuously maintain CBF homeostasis. The vascular resistance of the large arteries, as well as parenchymal arterioles within the brain, aid CBF regulation (Faraci and Heistad, 1990). The large arteries have a crucial role in mediating downstream microvascular pressure changes in response to increases in systemic arterial pressure to maintain CBF. Investigation of pressure gradients inside the cerebral circulation show that large extracranial vessels and intracranial pial vessels contribute to ~50% of cerebral vascular resistance (Faraci and Heistad, 1990). This is in contrast to the peripheral vasculature where small arterioles are predominantly the sites of vascular resistance. Segmental vascular resistance within the cerebrovasculature is an essential protective mechanism, mediating pressure gradients and ensuring metabolic demands are met (Cipolla, 2009). Additionally, cerebral autoregulation is vital for reducing CBF deviation in response to changes in BP by modulating cerebrovascular resistance (Aaslid et al. 1989).

Cerebral metabolism relates to the exchange of oxygen (O_2), glucose, and lactate between arterial and venous circulations in the brain. The brain has a substantial metabolic demand that requires ~20% of total oxygen consumption and ~15% of the total cardiac output, albeit only comprising 2-3% of total body mass (Willie *et al*. 2011). However, the brain has a small capacity to store energy thus requiring a

constant supply of nutrients for optimal function. Exercise increases both energy

demand and neuronal activity, thus CBF increases to provide adequate perfusion to

the cerebrovasculature (Davenport *et al*. 2012; Lucas *et al*. 2015). A compensatory

uptake of lactate, glucose and oxygen will occur if CBF fails to meet the metabolic

demands (Smith and Ainsle, 2017). Thus, evidencing the large energy demand of

the brain and the tight precision of CBF that is required from the regulators,

especially during exercise.

The contraction of the heart induces variations in cardiac output that impacts the

cerebral circulation through changes in pressure and flow. However, these two

factors should be considered separately. Fluctuations in pressure propagate

throughout the brain instantaneously, whereas with flow, pulsations are directly

dependant on the location and require displacement of fluid from one compartment to

another (Wagshul *et al*. 2011). Two haemodynamic principles explain how cardiac

output governs CBF regulation; 1) perfusion is dependent on BP and the vasculature

resistance of an organ, and 2) the metabolic rate determines the distribution of

cardiac output within an organ (Smith and Ainslie, 2017). Therefore, the cardiac

cycle has considerable influence over BP and is important to take this into

consideration when examining CBF regulation during exercise.

Cerebral vasculature is extremely sensitive to changes in PCO_2 (Smith and Ainsle,

2017). This has been demonstrated during investigations by inhaling varying

amounts of carbon dioxide (CO_2). The inhalation of 5% CO_2 has been found to

increase CBF by ~50% whereas 7% induces an increase of ~100% (Kety and

Schmidt, 1948). CO_2 diffuses across the blood brain barrier to form hydrogen (H+)

and bicarbonate, which influences the resistance and diameter of vessels (Querido

and Sheel, 2007). CO_2 has opposing effects on CBF depending on whether there is a high or low level. Exercising above the anaerobic threshold results in anaerobic glycolysis occurring, producing H+ ions which buffers into PCO_2 simulating a hyperventilation response, attenuating PCO_2 levels (Myers and Ashley, 1997). This results in hypocapnia (Hellstörm *et al.* 1996), leading to vasoconstriction which ultimately lowers CBF. Therefore, current research indicates that moderate intensity exercise (~65%VO_{2peak}) is optimal for brain health due to eliciting the maximal increase in CBF (Brugniaux *et al.* 2014; Moraine *et al.* 1993). This is explained in greater detail below.

Cerebral integration during exercise of active limbs influences CBF (Pott *et al.* 1997), as cortical regions representing the involved muscles will receive an increase in blood flow (Lyngeraa *et al.* 2013). Additionally, motor neuron activity increases with exercise intensity. This would imply that CBF increases with exercise intensity (Moraine *et al.* 1993; Smith *et al.* 2012). However, an array of studies has demonstrated a decrease in CBF beyond the anaerobic threshold (e.g. Brugniaux *et al.* 2014). Research has suggested central nervous system (CNS) motor output is related to O_2 availability. Therefore, a reduction in CBF may correlate with a decline in O_2 delivery, which potentially decreases CNS motor efficiency causing a detrimental impact on an individual's exercise capacity (Subudhi *et al.* 2007). This remains a controversial topic as under normoxia conditions, cerebral oxygenation levels remain above resting values even during strenuous exercise (Subudhi *et al.* 2007, 2011). Additionally, this decline in CBF potentially impacting cerebral oxygenation is because of the powerful influence PCO_2 has on vasculature, as mentioned above.

Overall, there are many factors which influence CBF regulation during acute exercise. The contribution and combination of these regulators may be affected by the different exercise parameters, such as exercise mode and intensity.

Mechanisms of Cerebrovasculature Adaptations

This section will discuss the potential underlying mechanisms that have been suggested to induce cerebrovasculature adaptations. Research has demonstrated exercise-induced haemodynamic forces (i.e. cyclic and shear stress) occur within peripheral vasculature. Shear stress is a frictional force acting on the walls of a vessel, which elevated with increased blood velocity (Topper and Gimbrone, 1999). Whereas cyclic strain is the mechanical deformation of the vessel, caused by the pressure of blood acting on the endothelium (Papaioannou and Stefanadis, 2005). Chronic exercise training is known to elicit vascular conditioning, due to the direct effects of these haemodynamic forces on the vascular wall within arteries (Padilla *et al*. 2014). However, evidence remains limited regarding these underlying mechanisms within the cerebrovasculature.

Exposure of haemodynamic forces within the peripheral vasculature are known to induce vascular adaptations through the release of nitric oxide (NO), in a dose-related manner (Laughlin *et al*. 2008; Lu and Kassab, 2011). NO is a potent vasodilator, crucial for vessel calibre regulations and is an essential component involved in eliciting vascular adaptations (Lu and Kassab, 2011). Numerous studies have explored the impact shear stress has within the peripheral vasculature during human intervention studies that involved repetitive exercise bouts, causing the release of vasoactive substances (Rakobowchuk *et al*. 2008; Green *et al*. 2002). The release of these chemical agents is dependent on the blood flow characteristics and

the corresponding cellular events within the periphery (Papaioannou and Stefanadis, 2005), that potentially transcend into cerebral circulation. Additionally, NO bioavailability is believed to be essential for optimal CBF regulation and cerebrovascular function (Lucas *et al.* 2015). However, to date most human-based studies have only assessed shear stress in the brachial and popliteal arteries.

The relationship between blood flow and shear stress is not parallel, for example if the diameter of the artery increases then shear stress would not change (Padilla *et al.* 2011). This knowledge is required when examining CBF and the potential underlying mechanisms that induce cerebrovasculature adaptations. However, due to difficulties of direct measurements on humans, no studies have clarified this relationship within cerebral circulation *per se*.

Cyclic strain is another mechanism which potentially elicits cerebrovascular adaptations (Birukov, 2009). It improves regulation of endothelial permeability by enhancing greater efficiency of nutrients through increase of endothelial NO synthase activity, which enables more effective cell respiration (Awolesi *et al.* 1995; Tang *et al.* 2016). Chronic exposure to cyclic strain aids greater compliance of the endothelium, through remodelling and proliferation of endothelial cells. This enables the vessel to change diameter more efficiently, enhancing vascular tone (Schad *et al.* 2011). Research is required to explore whether cyclic strain occurs within human cerebral circulation. However, there is limited research examining human cerebral circulation due to the complications of direct measurements. Therefore, it remains unknown whether cyclic strain occurs within the cerebrovasculature.

In summary, haemodynamic forces (i.e. cyclic strain and shear stress) have been evidenced to increase during exercise inducing peripheral vasculature adaptations.

This increase of the haemodynamic forces are potentially the underlying mechanisms that induce cerebrovasculature adaptations during exercise. Therefore, an assumption can be made that the modality and intensity of exercise which induces the greatest increase of CBF, would also produce the greatest stimulus for shear stress and cyclic strain. Overall, the examination of exercise interventions on cerebrovasculature health should be twofold. Firstly, to discover the direct influence that different exercise parameters have on CBF and then to investigate whether these haemodynamic forces occur within the brain, thus leading to discovering the optimal exercise parameters to achieve maximum cerebrovasculature health.

Pulsatility

Exercise is known to generate an acute increase in pulsatility due to the contraction of skeletal muscles in conjunction with an increased HR (Babcock *et al.* 2015). Systolic pressure increases as diastolic pressure remains relatively constant, resulting in a greater pulse pressure across an exercise bout (Palatini *et al.* 1988). Pulse pressure is the difference in maximum systolic and diastolic arterial pressures (Ündar et al. 1999). An exercise-induced increase in pulsatility will elevate the haemodynamic forces within the vasculature, potentially increasing the stimulus for adaptation. Gosling's pulsatility equation is followed, as an index of vascular resistance and pulsatile flow (shown as $Pulsatility = \frac{systolic - diastolic}{velocity}$ (Levine *et al.* 1994). The change in pulse pressure can also be examined by calculating pulse pressure amplitude as shown in Lyngeraa *et al.* (2013) (PPA; sMCAv - dMCAv). Both equations enable analysis of the shape of pressure waveforms during exercise. Poiseuille's Law states that CBF is dependent on cerebral perfusion pressure, alongside the radius of blood vessels. Cerebral perfusion pressure is heavily

influenced by mean arterial pressure (MAP); therefore, any systemic pressure alterations will directly impact CBF (Hill and Gwinnutt, 2008). Following this law, CBF regulation is determined by BP, coupled with any resistance change, which is predominantly mediated by PCO_2. Poiseuille's law could therefore be applied to determine the shear rate within a vessel. This equation indicates that shear stress is directly proportional to blood flow rate, and inversely proportional to vessel diameter, assuming the vessel is straight and inelastic with laminar flow (Papaioannou and Stefanadis, 2005). Therefore, fluctuations in BP during exercise transcend into CBF. Different exercise parameters (i.e. mode and intensity) potentially induce different pulse pressure and shear rate profiles. Overall, research is required to establish what exercise parameters elicit the greatest stimulus on cerebrovasculature to induce adaptations.

The brain is encapsulated within the cranium, a fixed structure, and therefore pulsations from fluctuations in pressure and flow directly transfer into brain tissues and fluids (Wagshul *et al.* 2011). Cerebral vessels are compliant and adapt to exercise-induced increases in both pressure and flow, preventing an excessive accumulation of pressure. Elastic components of central arteries have an essential role by influencing the amplitude of pulsations in flow, which prevents microvascular damage in target organs from excess energy (Mitchell *et al.* 2011). Vessel compliance is the ratio of volume over pressure change. It is fundamental within the brain as high flow organs are extremely susceptible to haemodynamic pulsatility. A system with high compliance can withstand large increases in volume, resulting in a small pressure increase (Wagshul *et al.* 2011). In summary, different exercise parameters (i.e. mode and intensity) potentially induce unique pressure changes that

the brain is highly sensitive to. The systemic BP fluctuations transcend into the CBF, influencing the haemodynamic forces (i.e. shear stress and cyclic strain) within the cerebrovasculature. Therefore, an understanding of the properties of cerebral vessels is crucial when examining haemodynamic responses to different exercise parameters, which potentially improve cerebrovasculature health.

Peripheral Vascular Studies

There is limited research exploring human cerebral circulation during exercise due to difficulties with obtaining direct measures. Therefore, this section will explore several areas within peripheral vasculature, which are as follows: 1) exercise interventions which explore underlying mechanisms of vasculature adaptations; 2) whether different stimuli invoke different haemodynamic profiles, and 3) consider if this would induce stimuli-dependant adaptations. Table 1 summarises current exercise investigations that have examined peripheral vascular haemodynamics within a range of systemic vessels, following unique experimental protocols.

Exercise is known to improve endothelial function (Tinken *et al.* 2009) with chronic training inducing vascular conditioning through direct effects on the endothelium (Padilla *et al.* 2014). Research has shown the influence of exercise-induced haemodynamic forces (i.e. cyclic strain and shear stress) induce endothelial cell morphology, and increased production of vasoactive substances within peripheral vasculature, as mentioned in the above section (Awolesi *et al.* 1994). Evidence remains limited concerning these underlying mechanisms within the cerebrovasculature. Additionally, the results of these vascular adaptations have been inconsistent, in particular when involving different exercise parameters and muscle groups of healthy individuals (Green, 2009).

Literature has revealed a distinct time course in vascular adaptations. Tinken *et al.* (2009) demonstrated the initial functional endothelium response to exercise within localised vasculature. This investigation included three 30-minute interventions, designed to explore whether passive heating, alongside lower and upper limb exercise induce different shear stress profiles within the brachial artery (see table 1). Blood flow and shear stress increased at similar rates across all three interventions, resulting in an elevation of flow-mediated dilation (FMD). Interestingly, the magnitude and frequency of pulse pressure varied between each intervention. Therefore, demonstrating that shear stress patterns are modality dependant, inducing specific training vasculature adaptations. Thus, contributing to the growing body of evidence that training induces limb specific vasculature adaptations. Localised vasculature remodelling is known to occur after the initial functional response during long-term exercise training, targeting a specific limb (Tinken *et al.* 2008, see table 1). This adaptation occurs in response to the repeated exposure of elevated shear stress levels. Overall, it is important to consider that CBF regulation is unique and complex in contrast to systemic circulation. Therefore, specific investigations are required to explore the regulation of CBF during exercise in conjunction with the underlying mechanisms of exercise-induced cerebrovasculature adaptations.

The size of the active vascular tissue has been found to influence exercise-induced haemodynamic forces (Green *et al.* 2017). The mass of the exercising tissue determines whether arterial remodelling is restricted to the local vasculature or has a systemic effect (Green, 2009). Green *et al.* (1994) discovered from a 4-week unilateral hand grip intervention that only local modifications occurred within the vascular bed, with no impact on the contralateral limb and with only a marginal

increase in cardiac output and BP (Green *et al.* 1994; Green *et al.* 1996). In comparison, lower limb exercise (i.e. cycling) requires a greater elevation in blood flow to meet metabolic demands. Therefore, inducing a hyperaemic response thus increasing haemodynamic forces (Linke *et al.* 2001). Collectively, the mass of the exercising muscle influences the haemodynamic response and therefore the potential stimulus for inducing cerebrovasculature adaptations. Overall, various types of exercise modalities need to be considered when examining haemodynamic responses in the cerebral circulation during exercise. This research will help develop optimal exercise parameters to induce cerebrovasculature adaptations.

Exercise intensity is another key parameter which may influence vascular adaptations. Few peripheral vasculature investigations have explored the impact of different intensities on vascular function and structure within an exercise modality. Goto *et al.* (2003) observed during a 12-week cycling intervention, low intensity exercise appeared unable to induce a sufficient stimulus to engender vascular adaptations, while moderate intensity improved NO-induced endothelial function whilst alleviating oxidative stress, and high intensity training elicited oxidative stress and inflammation that inhibited the underlying functional vascular adaptions. Additionally, an increase in skeletal muscle's oxygen demand with strenuous exercise has been correlated with an elevation of free radical formation (Davies *et al.* 1982) and a decrease in antioxidants (Bergholm *et al.* 1999). Strenuous exercise has also been found to have a negative relationship with FMD. Birk *et al.* (2012) evidenced that FMD reduced considerably after high intensity cycling in comparison to lighter workloads. Together both these findings suggest intense exercise could impair endothelium-dependent vasodilation because of a reduction in NO

bioavailability, inducing a detrimental effect on the vasculature. Although, to date there is no evidence of an impaired endothelial function within healthy individuals from intense exercise. Moreover, investigations have found a correlation between intensity and production of NO (Marsumoto *et al.* 1994). One potential hypothesis is that high intensity exercise induces an elevation of oxidative stress inhibiting NO bioavailability. However, within healthy individuals the increase in NO production appears to override this issue (Goto *et al.* 2003). Further research is essential to examine the relationship between the NO bioavailability and intensity, with regards to peripheral vasculature health as well as establishing whether this relationship transcends into the cerebrovasculature.

An abundance of evidence has explored the impact of exercise on peripheral vasculature, verifying that episodic increases in haemodynamic forces are a stimulus for vascular adaptations. Overall, research is needed to explore whether these exercise-induced haemodynamic forces (i.e. shear stress and cyclic strain) occur within the cerebral circulation and then to examine if these haemodynamic responses are modality and intensity dependant. The answer to these questions will help discover the optimal exercise parameters to improve cerebrovasculature health.

Table 1: Investigations that have examined peripheral adaptive responses to exercise training

Authors	Participants	Vessel	Protocol	Findings
Hambrecht et al. 2003	35 male patients, 70 years old with Coronary Artery Disease	Internal mammary	Exercised 3 times a day: 10 minutes on row ergometer and 10 minutes on bicycle ergometer for 4 weeks	• ↑*in vivo* and vitro Ach responses • ↑ adenosine-mediated blood flows • ↑eNOS expression, mRNA, protein expression, shear stress related to phosphorylation
Green et al. 1996	8 Tennis players	Brachial artery	Forearm blood flow responses to a 5-minute ischemic stimulus and intrabrachial infusion of Ach, sodium nitroprusside, and NG-monomethyl-L-arginine in both arms	• ↑vasodilator capacity in dominant limb • ↑ Forearm volume, girth, and grip strength in the dominant limb • ↑reactive hyperemic response in the dominant limb • No differences between the arms in response to Ach, sodium nitroprusside or NG-monomethyl-L-arginine
Green et al. 1994	11 young participants in training group with 6 aged matched controls	Brachial artery	4-week hand grip training program: 4 x 30-minute sessions of left handgrip exercise per week	• ↑ peak dilator responses both in trained forearm • No improvement in untrained participants • No change in forearm blood flow in response to an endothelium-dependent vasodilator in the exercise or control participants
Clarkson et al. 1999	25 healthy young male military recruits	Brachial artery	10 weeks military training: daily 3-mile run with upper body strength and endurances exercises	• ↑ FMD
Zeppilli et al. 1995	15 elite cyclists, long-distance runners, volley-ball players 10 wheelchair basketball players 11 wheelchair distance runners and 20 sedentary controls	Aortic arch, left carotid, left subclavian artery, right pulmonary artery, abdominal aorta, mesenteric artery, superior and inferior vena cava	Measurement of vessel diameter *via* a two-dimensional echocardiography	• ↑ large artery size in endurance trained athletes • ↑ vessel size in able-bodied athletes, cyclists and long-distance runners compared to control • ↑ upper-body vessels and ↓ lower-body vessels within wheelchair athletes than control participants • ↑ abdominal aorta and inferior vena cava of wheelchair distance runners greater than wheelchair basketball players

Birk *et al.* 2012	10 young healthy males	Brachial artery	A 30 minute of cycle at 50, 70 and 8%HRmax	• Negligible change in brachial artery FMD after cycling at 50%HRmax • ↓ in FMD after cycling at 70%HRmax • ↓ in FMD after cycling at 80%HRmax
Dinenno *et al.* 2001	53 middle-aged sedentary and 55 endurance exercise-trained middle-aged men	Brachial and femoral artery	A 3-month walking to jogging intervention, exercising 5–7 days per week, 40–50 minutes per day, at 65–80% of HRmax.	• ↑resting diameter • ↑ tangential wall stress in endurance-trained men • ↑ femoral artery lumen diameter and ↓ intima media wall thickness in endurance leg-trained athletes in comparison to sedentary participants
Miyachi *et al.* 2001	10 young men	Ascending aorta and common femoral artery	6-week one-legged cycling at 80% VO_{2peak}, 40-minutes a day, 4 days a week. Then detraining for another 6 weeks	• No difference in baseline one-legged VO_{2peak} • No difference in cross-sectional area of the femoral artery and vein in trained and control legs at baseline • No change in cross-sectional area of the femoral artery or VO_{2peak} in control limb • ↑ cross-sectional area of ascending and abdominal aorta, femoral artery and femoral vein in trained limb • ↑16% VO_{2peak} in trained leg • Improvements returned to baseline values during detraining
Tinken *et al.* 2008	13 healthy males and 7 non-active male controls	Brachial and popliteal arteries	8-week cycling and treadmill running exercise training	• ↑ vascular function from training • Control group - no change in dilator capacity, brachial and popliteal artery FMD • ↑ brachial artery FMD from baseline to week 4 and ↓ towards baseline levels by week 8 • ↑ brachial artery dilator capacity through the intervention duration • ↑ popliteal artery FMD from baseline to week 6 and↓ by week 8 • ↑ popliteal dilator capacity from baseline at week 4 and 8

Tinken *et al.* 2009	10 healthy recreationally active males	Brachial artery	30-minute intervention consisting of bilateral forearm heating, recumbent leg cycling or bilateral handgrip exercise with unilateral cuff inflated (60mmhg)	• ↑ antegrade flow, shear rate, FMD in non-cuffed arm similar response in each intervention • ↓ antegrade shear rate in cuffed arm with no increase in FMD
Rakobowchu k *et al.* 2008	10 young females and 10 males	Popliteal artery and carotid artery distensibility	6-week exercise intervention of sprint interval training (6 x 30 seconds bouts, 3 days per week) or endurance training (40–60 minutes of cycling at 65%VO_{2peak}, 5 days a week)	• ↑ popliteal artery distensibility and endothelial function in both training interventions • ↑ ~10% in VO_{2peak} in both training interventions • Carotid artery distensibility did not change in either training intervention
Huonker *et al.* 2003	18 able-bodied elite tennis players, 34 able-bodied elite road cyclists, 26 athletes with paraplegia, 17 below-knee amputated athletes, and 30 able-bodied, untrained participants	Thoracic and abdominal aorta, subclavian artery, and common femoral artery	Cross-sectional study of central and peripheral arteries within athletes and in untrained participants	• ↑ diameter of 19% in subclavian artery of the dominant arm in comparison to the non-dominant arm of able-bodied tennis players. • ↑ diameter of femoral artery in able-bodied road cyclist athletes and within the intact limb of below-knee amputated athletes • ↓ diameter of 37% and 21% in femoral artery in athletes with paraplegia and of below-knee amputated athletes, respectively. With ↓ cross-sectional area of 57 and 31%, respectively, compared with controls. • ↑ diameter of 19% and cross-sectional area of 54% in subclavian artery compared to controls. • No modification in diameter of the thoracic and abdominal aorta between any participant group

(Key: ↑, increase; ↓, decrease; Ach, Acetylcholine; FMD, flow mediated dilation; eNOS, endothelial nitric oxide synthase; VO_{2peak}, maximal oxygen consumption and HRmax, maximal heart rate)

Impact of Exercise on Cerebral Blood Flow

This section covers research examining the influence exercise has on CBF. More specifically whether a modality and intensity relationship exists. No research to date has explored the influence of more than one modality on CBF within one study, thus preventing a direct comparison. A more complicated relationship between exercise intensity and CBF may exist than is presented within current literature, which is based on predominantly cycling studies (e.g. Brugniaux et al. 2014). Subsequently, current literature investigating the influence of cycling, rowing and running on CBF will be discussed with table 2 summarising relevant evidence. To note, all exercise studies discussed following this utilised a transcranial doppler (TCD) to measure MCAv, as an index of CBF. The TCD has a high temporal resolution and measures beat-to-beat changes in cerebral perfusion (Ogoh and Ainslie, 2009). Near-infrared spectroscopy has also been used simultaneously with a TCD in research to explore cerebral haemodynamics, as an index of haemoglobin volume to represent flow (as seen in Gonzales-Alonso et al. 2004 study; Boushel et al. 2001). A Vyntus metabolic cart is commonly used to measure respiratory variables to a high-level of accuracy and precision (Perez-Suarez et al. 2018) and enables breath-to-breath analysis (Vyaire Medical, 2019). The aforementioned measures are all non-invasive and enable continuous monitoring of parameters.

Cycling

Cycling is the most frequently used modality when investigating CBF profiles during exercise. This is understandable due to the simplicity the mode offers for lab organisation and recording measures. Cycling is a continuous exercise, with no rapid head movement and does not involve whole-body movement, or the influence of

body weight due to being seated. All these factors are likely to influence the CBF profile. Moreover, supine-cycling is often used to aid data collection (e.g. duplex ultrasound imaging the extracranial vessels; Sato *et al.* 2011).

There is a considerable body of literature based on cycling exercise that reports a bi-phasic profile for both MCAv and PETCO$_2$ across the full range of intensities (see figure 1). Specifically, both MCAv and PETCO$_2$ increase with intensity until the anaerobic threshold is reached, where values begin to decrease towards baseline levels (Hellstörm *et al.* 1996; Brugniaux *et al.* 2014; Moraine *et al.* 1993). The use of duplex ultrasound enables the blood flow through the carotid arteries to be imaged and therefore, the relationship between extracranial blood flow and CBF during exercise to be examined. Sato *et al.* (2011) reported regional differences between internal carotid artery (ICA) and vertebral artery blood flow during incremental cycling. Blood flow within the common carotid artery was reported to progressively increase. This resulted in an abrupt elevation in blood flow within the external carotid artery (ECA) during moderate-high intensity exercise, in contrast to a decrease ICA blood flow. Evidencing, blood flow within the ECA is selectively increased over ICA blood flow during strenuous exercise, which was hypothesized to be associated with thermoregulation (Sato *et al.* 2011). Furthermore, blood flow within the vertebral artery was found to increase during high intensity exercise compensating for the decline in ICA blood flow. To note, the ICA blood flow has been found to follow a similar pattern to MCAv in relation to intensity (Hellstörm *et al.* 1996). Overall, a coupling between PETCO$_2$ and MCAv is present during exercise with the influence of intensity determining the CBF profile. Overall, there are different regional blood flow profiles during exercise that are influenced by exercise intensity and PETCO$_2$.

Hyperventilation-induced hypocapnia is thought to cause the reduction of MCAv at high intensity exercise (Hellstörm *et al.* 1996). During high intensity, anaerobic exercise, the rate of lactate breakdown cannot match the rate of lactate synthesis. Lactate accumulation increases H+ ion concentration, resulting in metabolic acidosis (Stringer *et al.* 1994). Endogenous stores of bicarbonate buffer the elevation of H+ ions, which therefore increases the PCO_2 and that ultimately stimulates a hyperventilation response to offload CO_2 in order to maintain acid-base balance (Querido and Sheel, 2007). All these physiological processes underlie the concept that moderate intensity exercise (~65%VO_{2peak}) is optimal for brain health due to eliciting the maximal increase in CBF (Brugniaux *et al.* 2014). Indeed, moderate intensity exercise induces small elevations in PCO_2, which contributes to the exercise-induced increase in CBF (Myers and Ashley, 1997). In addition, MAP increases perfusion pressure alongside neural activation, all of which facilitate CBF elevation. However, this bi-phasic CBF profile (i.e. MCAv) is predominantly from cycling based studies, whereas differences between exercise modalities could exist. To note, posterior circulation has been shown to increase up to 80%VO_{2peak} during cycling, evidenced by the vertebral artery blood flow (Sato *et al.* 2011). Indeed, both running and rowing have been shown to produce an alternative CBF profile across a range of intensities (Faull *et al.* 2014; Lyngeraa *et al.* 2013; Pott *et al.* 1997). The examination of the prefrontal cortex haemodynamics during exercise using near-infrared spectroscopy (NIRS) has facilitated the measurements of haemoglobin volume in cerebral tissue, which allows the interpretation of cerebral haemodynamics dynamics including both O_2 delivery and O_2 utilisation. Additionally, NIRS enables the examination of the potential conflict between cerebral oxygen delivery and

maintenance of CBF during strenuous exercise, which could elicit hyperperfusion. This would augment structural damage, specifically to the blood brain barrier and the brain parenchyma, if the increase in CBF is not controlled. Curtelin *et al.* (2017) investigated cerebral haemodynamic activity during 30-second maximal cycling sprints in normoxia and hypoxia. This investigation demonstrated CBF followed a curvilinear pattern across the sprint in both the normoxia and hypoxia conditions. In normoxia CBF peaked within the first 7.5 s of a sprint, before coupling with $PETCO_2$ and declining towards baseline values. Frontal lobe oxygenation decreased by 10% that paralleled the progressive decline of CBF by 5% across the sprint during normoxia. These findings indicate that the prevention of hyperperfusion was prioritised over cerebral perfusion during normoxia. However, priority of O_2 delivery occurred in the hypoxic conditions despite a greater increase in perfusion pressure. The point where cerebral oxygenation starts to decline has been labelled the cerebral oxygenation threshold (ThCOx) (Bhambhani *et al.* 2007; Oussaidene *et al.* 2013; Rupp and Perrey, 2008; Subudhi *et al.* 2007, 2008). The reduction in cerebral oxygenation beyond the ThCOx follows a similar pattern as the reduction in CBF beyond the anaerobic threshold (González-Alonso *et al.* 2004; Vogiatzis *et al.* 2011). This reduction in cerebral oxygenation and CBF, is postulated to be due to exercise-induced hypocapnia, resulting in vasoconstriction as previously mentioned. Overall, hypocapnia induced by high intensity exercise induces a reduction in CBF and therefore cerebral oxygenation (Oussaidene *et al.* 2014; Bhambhani *et al.* 2007). The inverted-U pattern of CBF and $PETCO_2$ with intensity has been extensively documented within cycling-based exercise.

Exercise induces cerebrovascular stress through the augmentation of haemodynamic forces (i.e. shear stress and cyclic strain), endorsing cerebrovasculature adaptations. However, based on the current understanding of CBF profile to exercise intensity, which is from cycling based studies, exercising beyond moderate intensities may attenuate the stimulus for cerebrovasculature adaptations or even cause maladaptations. Further research is required to explore CBF profiles within a range of exercise modalities and intensities to discover the most beneficial exercise parameters for brain health, especially given the recent attention and promotion of high intensity exercise strategies for general health gains (Lucas *et al.* 2015). Currently, the underlying mechanisms instigating neuroprotective benefits from exercise, are yet to be clarified.
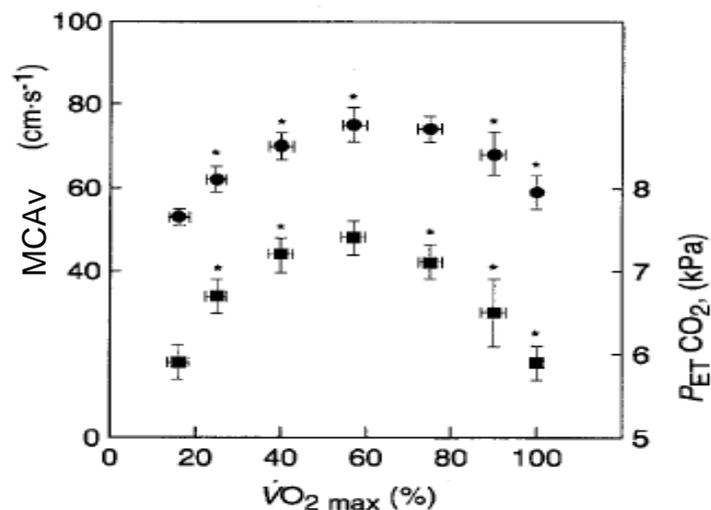
Figure 1: A graph illustrating the bi-phasic profile for both middle cerebral artery blood velocity (MCAv; circles) and partial pressure of end-tidal carbon dioxide ($PETCO_2$; squares) across a full range of intensities (VO_{2peak}). Figure adapted from Moraine et al. 1993.

Rowing

In contrast to cycling, rowing is a full body exercise with two distinct movement phases. Pott *et al.* (1997) and Faull *et al.* (2014) explored how rowing would impact

arterial and venous pressure, and ultimately CBF and cerebral metabolism (as indexed from MCAv). The findings from these studies illustrated a different CBF profile than the aforementioned cycling studies, indicating a modality dependant relationship. In particular, Faull *et al.* (2014) observed MCAv increased throughout submaximal and maximal intensities, despite the presence of hypocapnia, revealing a different CBF perfusion profile to that previously shown with cycling. Several explanations for this profile were presented, such as MCAv potentially becoming pressure passive with a Valsalva manoeuvre occurring at the catch, aiding the elevation in BP. Synchronisation between BP and CBF demonstrated that systemic pressure fluctuations are transmitted into the cerebrovasculature, whereas during cycling no correlation between these two variables was reported (Heckmann *et al.* 2000). Also, BP and central venous pressure fluctuations corresponded with changes in intrathoracic pressure relating to the different phases of rowing (Pott *et al.* 1997). The BP and intrathoracic pressure peaked at the catch phase, which is when force is applied against the oar. In summary, cerebral perfusion during rowing is evidently influenced by rapid fluctuations in perfusion pressure which is synchronised with strokes. Therefore, demonstrating the dependency of peripheral and cerebral vasculature haemodynamics on the exercise modality.

$PETCO_2$ was not measured within Pott *et al.* (1997) study. However, Faull *et al.* (2014) observed an attenuation in $PETCO_2$ reflecting hypocapnia, although recognised this may not replicate the same CO_2 stimulus within cerebral vessels. An elevated level of PCO_2 may occur within the cerebrovasculature, suggesting an uncoupling between $PETCO_2$ and cerebral PCO_2 and thus inducing vasodilation and contributing to the elevation in MCAv. Although extremely invasive measures would

be required to evidence this explanation and promotes the question, why would this elevation of PCO_2 occur during rowing and not in other modalities? Of note, it is recognised that rowing elicits a restriction on the ventilatory pattern, which does provide some rationale for this uncoupling. Importantly, one key implication of these rowing-based studies is that the consideration of exercise modality is essential when exploring the influence exercise elicits on a range of physiological factors which aid CBF regulation, rather than simply assuming all modalities induce the same CBF profile.

Running

Running as a modality has been used significantly less within research examining CBF, therefore published findings are limited. Palatini *et al.* (1989) completed early research examining the pattern of BP during track running compared to cycling. Invasive techniques were employed within this investigation including an arterial line and a sophisticated pressure transducer system. Palatini *et al.* (1989) discovered that the mechanical impact of the foot-strike shocked vessels during running gait, generating a pressure wave that combines with an arterial pressure wave, resulting in periodic pulse pressure oscillations. The combination of these waves produces a 'beat', therefore giving rise to the term 'beat phenomenon'. The mechanical pressure wave was subtracted from the radial artery recording, which produced a flat trace demonstrating the two pressure waves had equal frequencies. Oscillations within BP were present throughout the track run, and varied between participants, based on individual differences. Oscillations appeared sharp when individuals reached their maximal speed, coinciding with peak pulse pressure amplitude (PPA). As already mentioned above, Lyngeraa *et al.* (2013) demonstrated that these blood flow

oscillations are transferred to the cerebral circulation, with their wavelength

increasing as intensity increased (at least up to 75% of HR reserve). This information

indicates that an individual's heel-strike force may increase with speed, inducing

greater pressure fluctuations within the mechanical wave. This is in comparison to

moderate intensity running, where oscillations appeared rhythmical, potentially due to

individuals finding their running rhythm. Lyngeraa *et al.* (2013) observed that

oscillations disappeared with a change in belt speed and returned once individuals

found their running rhythm. This coincided with participants vocalising when they had

found their running rhythm. It was observed that step frequency, HR and oscillation

wavelength all increased with velocity. Palatini *et al.* (1989) reported no rhythmic

oscillations were recorded during cycling, although the HRs between modalities were

similar; thus demonstrating that fluctuations within BP are related to the movement

pattern of running, in particular the transfer of weight between the feet. Collectively,

Lyngeraa *et al.* (2013) proposes the repetitive foot-strikes during running induces

constant BP fluctuations which transcend into CBF, where cerebral autoregulation is

unable to counteract the pressure changes. Therefore, running may induce a greater

stimulus over cycling for cerebrovasculature adaptations, due to increased blood flow

pulsatility, eliciting an elevation in haemodynamic forces (i.e. shear stress and cyclic

strain).

Palatini *et al.* (1989) discovered an apparent relationship between HR and stride rate

and the generation of oscillations within pulse pressure. The difference in frequency

of these two rhythms was equivalent to the frequency of pulse pressure oscillations.

Therefore, when these two rates approached each other the oscillations periodically

disappeared. Palatini *et al.* (1989) observed at the onset of running HR gradually

increased approaching the step rate. A disappearance of pulse pressure oscillations occurred when these two rates were at equal frequencies and reappeared once HR increased above the step rate. Additionally, Lyngeraa *et al.* (2013) noted that indifferences between these two rates decreased with an increase in intensity, which coincided with an elongation in oscillation wavelength within BP and CBF. However, a disappearance in oscillations was not observed during this investigation, likely due to the lower exercise intensity reached. Therefore, the intensity may not have been strenuous enough for running cadence to match HR. Together, these findings indicate that the beat phenomenon has an intensity-dependant component. Further investigations need to examine the impact oscillations elicit on cerebrovasculature alongside the optimal oscillatory pattern for inducing adaptations, through manipulating the heel-strike force and step frequency.

In addition, Lyngeraa *et al.* (2013) reported a similar pattern of mean MCAv with running as previously reported with cycling, with MCAv peaking at a moderate intensity before declining at higher intensities. Unfortunately, Lyngeraa *et al.* 2013) did not measure respiratory variables, limiting the interpretation of the relationship between MCAv and $PETCO_2$ during running. However, the relationship of MCAv and $PETCO_2$ is speculated to be similar between these two exercise modes, consisting of an inverted-U profile with increasing intensity. The influence running exerts on cerebral haemodynamics beyond the anaerobic threshold still requires exploring. The maximum exercise intensity of Lyngeraa *et al.* (2013) investigation was only 75%HR reserve equivalent to 65%VO_{2peak} (Swain *et al.* 1994). Therefore, no current investigation has explored the impact of high intensity running on CBF and how this is related to changes in PCO_2. In summary, both running and rowing investigations

have demonstrated that CBF is modality dependent. However, the mean CBF profile has been predominantly reported during cycling. Further research is required to explore the effectiveness of various exercise parameters such as mode, intensity and duration. This knowledge may advance the current limited evidence of optimal parameters of exercise to improve brain health.

Table 2: Investigations that have examined cardiovascular and cerebrovascular responses during exercise

Authors	Participants	Modality	Exercise Protocol	Results
Brugniaux et al. 2014	12 active and 12 sedentary healthy young males	Cycling	Initial load 100w ↑ 25w per minute until volitional exhaustion	• ↑ MCAv and cO_2Hb in active group increased above the sedentary participants values • ↑ MCAv and $PETCO_2$ during low-to-moderate intensity • ↓ MCAv and $PETCO_2$ during high intensity • cO_2Hb in sedentary participants did not change • ↑ cO_2Hb in active participants with ↑ intensity
Heckman et al. 2000	18 women and 12 young males	Supine-cycling	3 x 3-minute bouts set at an intensity to ↑ BP by 10% and HR by ↑25% HR from baseline values	• ↑ sBP towards the end of the bouts • ↑ MCAv after 1-minute • ↑ PCO_2 in the last minute of exercise
Hellström et al. 1996	11 young healthy males	Supine-cycling	4 consecutive workloads (very light, light, moderate and maximal) intensity ↑ by 20%VO_{2peak} each 6th minute	• ↑ MCAv low-moderate intensity • ↓ MCAv high intensity • ↑ $PETCO_2$ low intensity • ↓ $PETCO_2$ moderate-high
Moraine et al. 1993	14 healthy young male participants	Cycling	Initial workload 50w ↑ 50w every 4 minutes until volatile exhaustion	• ↑ MCAv low-moderate intensity • ↓ MCAv high intensity • ↑ $PETCO_2$ low intensity • ↓ $PETCO_2$ moderate-high • ↑ MAP low-moderate • ↓ MAP high intensity
Curtelin et al. 2017	Study 1: 20 active young men Study 2: 11 active young men	Cycling	Study 1: All-out 30s sprint wingate test @ 80rpm Study 2: 3 incremental exercise tests: ↑ 20 and 30w per minute until volitional exhaustion 2 x 30-second wingate test in normoxia or hypoxia	Study 1: • ↓MCA by 10% • ↓ Frontal lobe cerebral oxygenation • Dissociation between MCA and $PETCO_2$ • ↓ Vastus lateralis tissue oxygenation Study 2: • ↑, ↓MCAv curvilinear pattern across sprints in both conditions • ↑ MCAv was 25% greater in hypoxia vs normoxia • Cerebral vascular conductance paralleled MCA index • ↑ Cerebral vascular conductance by 26% in hypoxia vs normoxia • ↓$PaCO_2$ in hypoxia vs normoxia
Tallon et al. 2019	8 children, 7-11 years	Cycling	HIIE: 6 x 1-minute sprints @ 90%Wmax MISS: 15-minute @ 44%Wmax	• HIIE HRmax: 82% • MISS HRmax: 69% • MISS: first 4-minutes ↑MCAv, 4+ minutes ↓MCAv • HIIE: 1-5th sprint MCA no change, 6th sprint ↓MCAv

Tsukamoto et al. 2019	9 young males	Cycling	4 x 4-minute HIIE bouts @ 80-90%Wmax	• ↑MAP, HR, SV and cardiac output • ↓ Total perihal resistance • Mean MCAv no change
Klein *et al*. 2019	11 young and 10 old males	Cycling	10-minute cycle @ 60%Wmax 10 x 1-minute @ 60%Wmax	• Greater ↑MCAv and ↑PETCO$_2$ during continuous cycling vs intervals in young males • Greater ↑MAP in old vs young males • ↑MCAv in continuous exercise for both age groups • ↑ absolute values for MCAv, PETCO$_2$ and HR in young vs old males • Mean %ΔMCAv, %ΔMAP and %ΔPETCO$_2$ all correlated in continuous exercise for young males
Pott *et al*. 1997	12 young-middle aged experienced rowers with 1 female	Rowing	10min row at ~75%VO$_{2peak}$	• ↑ HR, MAP, CVP, sBP, MCAv • Beat-to-beat variation within MCAv and BP • ↓ Arterial CO$_2$ tension and pH towards the end of the row
Faull *et al*. 2014	13 healthy young male athletes	Rowing	6-minute row at 60%HRR, 2-minute recovery, 3-minute row at 75%HRR, 30-second maximal sprint, 3-minute rest, 2,000m maximal effort row	• ↑ MCAv with intensity • ↑ PETCO$_2$ but ↓ during the sprint • MCAv oscillated with each rowing stroke
Palatini. *et al* 1989	6 normotensive and 17 borderline hypertensive, young-middle aged amateur runners	Cycling and track running	Cycling: initial workload 50w ↑ 20w per 2-minutes Running: warm-up ↑ speed to steady state, until volitional exhaustion	• Cycling: ↑ sBP, dBP minor change • No pulse pressure oscillations • Running: ↑ step frequency increase with ↑ speed • Pulse pressure oscillations present throughout duration • Continuous ↑ sBP with ↓ dBP oscillations • Rhythmic oscillations unrelated to respiration
Lyngeraa *et al*. 2013	15 young healthy participants	Running	3 x 5-minute bouts at 50%, 65%, and 75%HRR	• ↑ BP and MCAv till 65%HRR • ↓ BP and MCAv at 75%HRR • Rhythmic oscillations in BP linked to the difference between step rate and HR frequencies

(Key: ↑, increase; ↓, decrease; w, watts; m, meters; %Δ, percentage change; MCAv, middle cerebral artery velocity; HR, heart rate; BP; blood pressure; PETCO$_2$, end-tidal carbon dioxide; cO$_2$Hb, prefrontal cortical oxyhaemoglobin; pCO$_2$, partial pressure of carbon dioxide; MAP, mean arterial pressure; CVP, central venous pressure; sBP, systolic blood pressure; dBP, diastolic blood pressure, FMD, flow mediated dilation; VO$_2$peak, maximal oxygen consumption; FMD, flow mediated dilation; HRmax, maximal heart rate; HHR, heart rate reserve; SV, stroke volume; Wmax, maximal workload; PaCO$_2$, arterial partial pressure of carbon dioxide; HIIE, high-intensity interval exercise and MISS, moderate-intensity steady-state exercise)
* Note this is not an exhaustive list of cycling studies

Running Locomotion

Running is a unique exercise form in comparison to other exercise modalities due to the repetitive foot-strikes. Therefore, this section will examine the different phases of running on level ground, followed by kinematic adaptations that occur in gait, as a response to gradient and conceivably other influences such as footwear and running experience. In order to gain an understanding of how this modality, including running at different gradients, may influence BP and CBF.

A clear distinction exists between walking and running locomotion, other than velocity. Walking involves a phase of double support, where both feet are on the ground simultaneously during the stance phase. Whereas running contains an aerial phase at the start and end of the swing phase, where both feet are off the ground, often referred to as the flight phase (Novacheck, 1998). The analogy of a controlled fall and a swinging pendulum has been used to describe the locomotion of walking (Novacheck, 1998). In contrast, running has been compared to a pogo stick, which propels the centre of mass (COM) from the lowest point during the stance phase to peak height during the aerial phase (Alexander, 1992). An important component of running is the metabolic efficiency, which can be illustrated as the ratio of mechanical power output to the metabolic power (Farris and Sawicki, 2012). The intention is to achieve the upmost mechanical power output from the metabolic power. The efficiency of running is highly dependent on the individual, such as their fitness and running technique. Initially it is important to understand the different phases of running, displayed in figure 2.
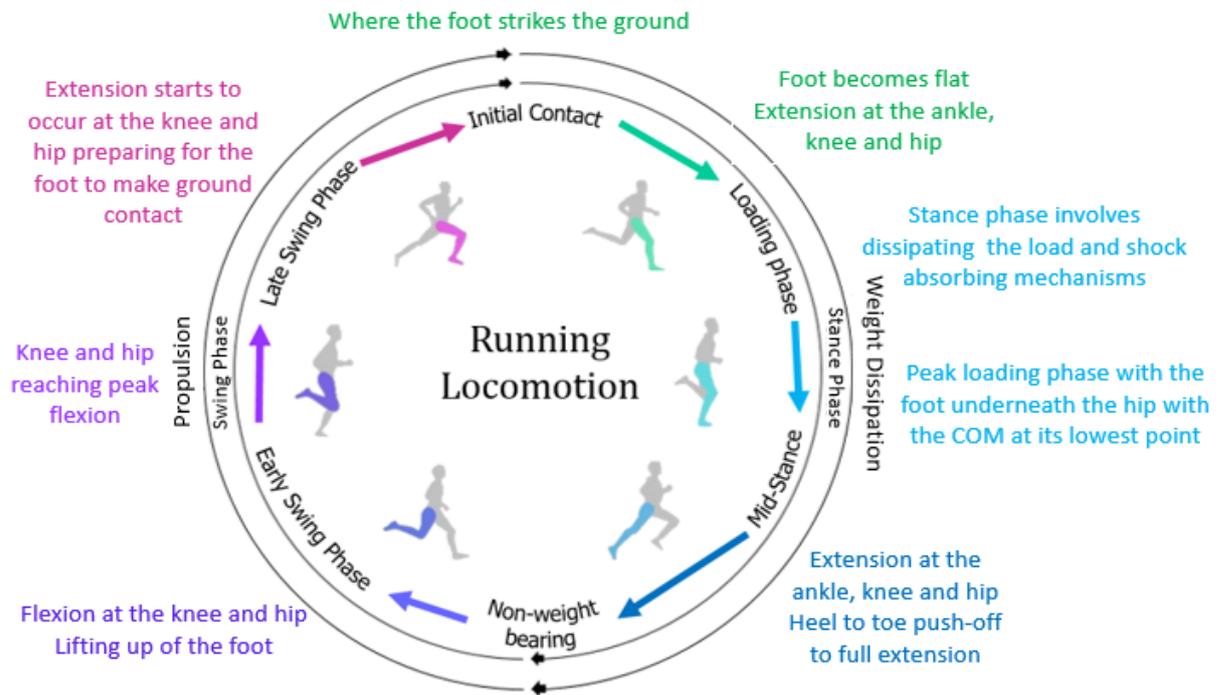
Figure 2: A schematic illustrating the phases of the running cycle.

Running economy is complex with a multitude of components involving the integration between metabolic, cardiorespiratory, biomechanical and neuromuscular factors (Barnes and Kilding, 2015; summarised in figure 3). An individual's running experience directly influences the biomechanics of the movement such as gait patterns, stride length and rate, which are fundamental components of efficient running, associated with the utilisation of muscular force into translocation (Anderson, 1996). The implication of short or long stride lengths on running economy will be explored subsequently. Overall, repeated training sessions lead to the adoption of a more economical running style (Cavanagh and Williams, 1982). Of note, no current research has examined how enhancing running economy impacts CBF and cerebrovasculature health.
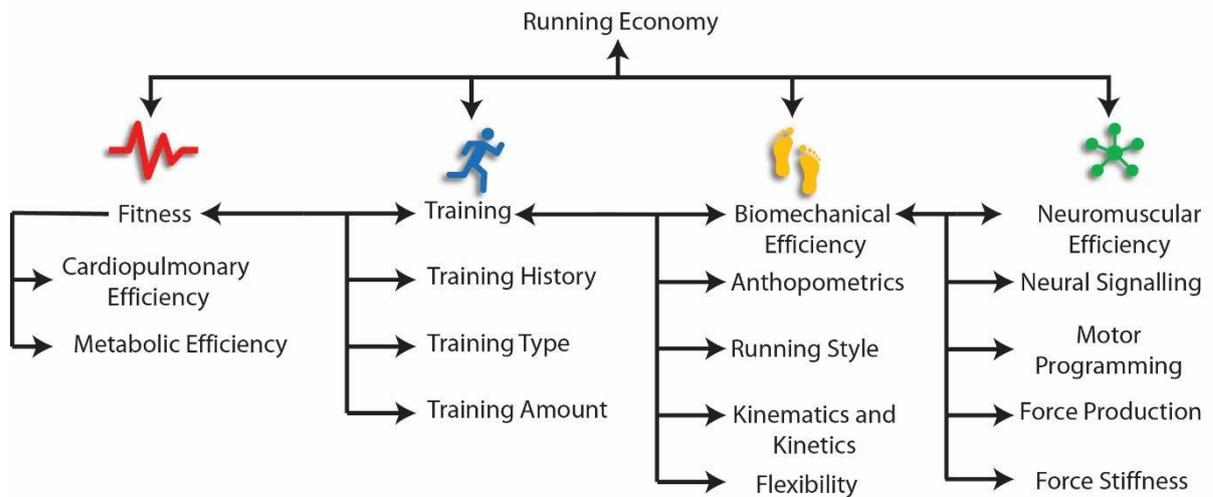
Figure 3: A schematic illustrating influential factors that impact running economy (adapted from Barnes and Kilding, 2015).

Interestingly, a U-shape relationship exists between step frequency and metabolic demands. Individuals have a natural preferred stride frequency, which is near their optimal stride frequency reducing the metabolic demand of running (Snyder and Farley, 2011). The mechanical power of muscles decreases with an increase metabolic cost, when step frequency goes beyond the optimal stride frequency and *vice versa*. The metabolic demand elevates with an increase in step frequency, to attempt to produce power over a shorter period (Snyder and Farley, 2011). Metabolic cost has been evidenced to increase linearly with an incline in gradient. In conjunction with an increase in lactate accumulation and a reduction in velocity in comparison to flat and downhill running. Thus, evidencing how running at different gradients will elicit differential influences over pulse pressure oscillatory patterns and CBF.

During uphill and downhill running, the biomechanics adapt to the gradient (Padulo *et al.* 2013). Grade-specific adjustments in running cadence (step frequency) ensure efficient running. Dunne (2012) uses the analogy of cycling uphill, changing into a low

gear while maintaining a high cadence. This is equal to adjusting step frequency by shortening or lengthening stride length. Again, this differs between novice and elite runners and directly impacts their running efficiency (Dunne, 2012). Eccentric loading occurs during downhill running. The COM is moving forward over the landing foot, parallel to the gradient (Eston *et al.* 1995). Amateur runners tend to lean backwards as they run, compensating for the decline in gradient. Additionally, they extend their knee out in front of them forcing their heel into the ground in an attempt to slow themselves down. This causes excessive stress on the knee and lower back, and the runners stature appears very rigid. In contrast, during uphill running the tendency is to over-stride taking large steps, staring down at their feet as the body becomes slumped, leading to decreased running efficiency (Dunne, 2012; Padulo *et al.* 2013). Research investigating the biomechanics of locomotion tends only to consider level ground locomotion, neglecting the influences of gradients on kinematics. Locomotion consists of continuous raising and lowering, as well as accelerations and decelerations of the body (Minetti *et al.* 1994). Deviation from a constant velocity, occurs through the balance between positive (acceleration) and negative (braking) work from the muscles (Dunne. 2012; Gottschall and Kram, 2005). Equilibrium is present between these forces when running on level ground at a constant speed. However, grade-specific adjustments in locomotion have been reported, which impact the heel-strike force. The use of ground force reaction plates and motion analysis has illustrated how regulation of propulsion and braking forces occurs as the foot-strike adapts during graded running. Collectively, investigations have shown that biomechanics, neuromuscular and physiological alterations occur during long-term graded running (Vernillo *et al.* 2017).
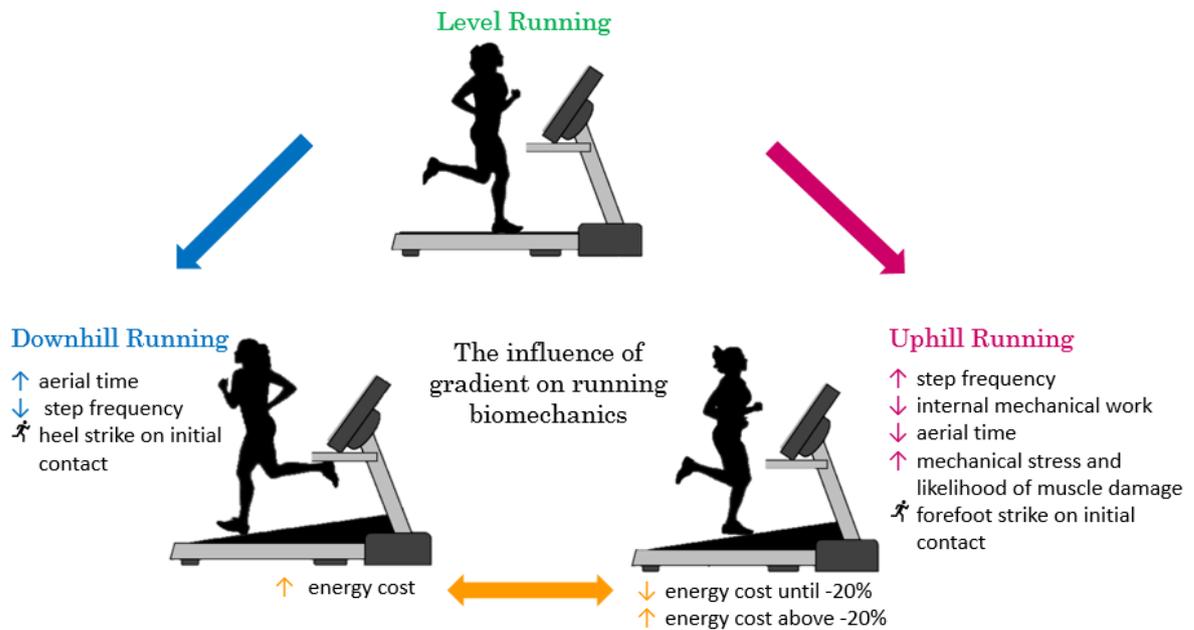
Figure 4: A schematic illustrating the influences of gradient on running kinematics.

The adoption of mid to forefoot-strike patterns have been evidenced to progressively occur with an increase in gradient. In contrast to a declining gradient where a rear-foot strike pattern becomes dominant (Gottschall and Kram, 2005; Lussiana *et al*. 2013). Gottschall and Kram (2005) demonstrated that all participants adopted a rear-foot striking pattern whilst running at negative gradients of -3, -6 and -9 degrees. However, as the gradient increased participants progressively started to initiate mid to forefoot-strike patterns. Clearly evidencing that downhill running enhances a rear-foot striking pattern as opposed to uphill running. This would potentially elevate the amplitude of the oscillations within the BP and CBF based on the beat phenomenon. Overall, displaying that gradient determines the foot-striking pattern, which refers to the part of the foot that makes the initial contact with the ground.

Uphill running is associated with an increase in step frequency and muscular work combined with a decrease in the swing phase compared to flat running (illustrated in schematic 3 above). Whereas during downhill running, the step frequency decreases

with an increase in the swing phase (Vernillo *et al*. 2017). A possible explanation for

the decrease in step frequency during downhill running in contrast to uphill running,

could be due to an increase in aerial time with a decline in the gradient. This occurs

when the belt speed is held constant, resulting in a reduced step frequency.

Specifically, Townshed *et al*. (2010) found that stride length during uphill running was

20.5% shorter, and 16.2% longer during downhill running, in comparison to flat over

ground running. This evidence indicates that stride length predominantly regulates

speed during graded over ground running. Therefore, both uphill and downhill

running may induce different oscillatory patterns within BP and CBF as a result of

different step frequencies. Although further research is required to investigate

whether step frequency has an influence over the oscillation frequency or wavelength

in CBF. This will develop an understanding of optimal exercise parameters to

improve cerebrovasculature health.

In over ground uphill running, a decrease of 0.1-0.3km/h for every 1% increase in

gradient has been reported (Mastroianni *et al*. 2000; Townshed *et al*. 2010). An

increase in power output at all joints, is required during uphill running to maintain

speed consistency (Vernillo *et al*. 2017). This increase in power is required for the

push-off phase, to raise the COM during locomotion, as well as compensating for the

reduced elastic energy storage within the muscles. Skeletal muscles work efficiently

during level-ground running, by storing elastic energy within the tendons during

landing and utilising this energy during take-off (Cavagna *et al*. 1977; Minetti *et al*.

1994; Gottschall and Kram, 2003). However, the under-utilisation of the stretch-

shortening cycle within the muscles occurs during uphill running, resulting in an

increased metabolic cost. The potential gravitational energy during uphill running

peaks at the end of the stance phase, where the COM is highest. Muscles are then required to generate a high amount of energy in order to push-off and raise the COM into the flight phase. Therefore, contributing to the elevated metabolic cost of uphill running, in comparison to downhill running. During downhill running, there is an increase in elastic energy, as the COM lowest point is at the end of the stance phase. This provides advanced elastic energy utilisation, therefore minimising metabolic costs (Snyder and Farley, 2011). This evidence indicates that if an individual was running at the same speed uphill and downhill the difference in metabolic cost needs to be taken into consideration when investigating CBF, due to the influence of PCO_2 on CBF.

Type and design of shoes worn, have been found to influence running performance. Traditional shoes weigh ~350g in comparison to a minimalist shoe, ~200g (Myers and Steudel, 1985; Frederick, 1984). Minimalist shoes are designed with increased flexibility and reduce weight, to enhance natural foot kinematics. Lussiana *et al.* (2013) discovered that traditional shoes impose an additional metabolic cost of 1.3% during graded running, compared to minimal shoes. The foremost explanation for this, was the elevated shoe mass of traditional shoes (Frederick, 1984; Divert *et al.* 2008). Of note, an increase of 100g on the foot has been reported to increase aerobic demand by 1%. Investigations have established that minimal shoes enhance mid-fore foot strike patterns, opposing the rear-foot strike patterns that are common in heavily cushioned traditional shoes (De Wit *et al.* 2000; Divert *et al.* 2005; Lussiana *et al.* 2013). This indicates that shoe design has an impact on metabolic demands and foot-strike patterns. Therefore, future investigations exploring the

impact of running on CBF and the oscillatory pattern might consider the type of shoes worn.

Overall, based on the aforementioned studies within the literature review, an increase in the heel-strike force will augment the amplitude of the pulse pressure oscillations within the mechanical wave. Together, this will potentially influence cerebral haemodynamic forces, modifying the stimulus for vasculature adaptations.

Entrainment

Running consists of rhythmic repetitive steps, which generates a locomotion rhythm that has been found to entrain to intrinsic oscillatory patterns (Niizeki and Saitoh, 2014). Exercise interventions observed synchronization occurred between running cadence and cardiac contraction (Palatini et al. 1989). Therefore, this section will explore the manifestation of entrainment during different running protocols and potential influencing factors. Collectively, increasing the understanding of how running impacts the systemic circulation, including interaction between the cardiac cycle and muscular contractions, and how this interaction impacts BP and potentially influences the pulse pressure oscillatory pattern within CBF. However, no studies have examined the impact of entrainment within CBF during running.

Cardio-locomotor synchronization (CLS) is the coupling between the cardiac cycle and locomotion rhythm that has been explored across a range of different rhythmic exercise modalities including cycling, hoping, running, skipping, walking and finger tapping (Kirby *et al.* 1989, 1990, 1991, 1992; Niizeki *et al.* 1993; Nomura *et al.* 2003). The exact underlying mechanisms behind the coordination between these rhythms remain unknown, although many are speculated. Nomura *et al.* (2006) examined the relationship between the contraction of the vastus lateralis muscle and the R-R

interval within the cardiac cycle. The protocol involved running to a buzzer to achieve a target HR of 160 beats per minute (bpm). Once at target HR, the buzzer rhythm started fluctuating to examine the impact of foot-strikes during different phases of the cardiac cycle. Results demonstrated a positive chronotropic effect when the foot-strike occurred within the first half of the cardiac cycle. However, minimal change occurred if the foot-strike occurred in the latter phase. Consequently, the timing of the footfall in accordance to the cardiac cycle may have an influence on the beat phenomenon, thus, impact the oscillatory pattern. Additionally, Nomura *et al.* (2006) reported CLS occurred within running but not cycling. Highlighting how running biomechanics induce a unique locomotor rhythm that has a large impact on intrinsic rhythms. Future investigations are required to explore how this phenomenon influences CBF and the implications on cerebrovasculature health.

Numerous studies have examined the implications of synchronization between HR and running cadence on performance. Phillips and Jin (2013) compared running performance of a 3-mile run between a self-paced and CLS condition. The CLS protocol involved matching the running cadence to auditory cues based on individuals HR, through the use of a portable HR monitor and audio device. The CLS condition induced a slower HR rise at the onset of exercise with a decrease in HR variability. Participants also self-reported finding the run easier in this trial. Collectively, an improvement in exercise performance occurred, indicating the CLS condition enabled the heart to operate more efficiently. Future studies should explore whether this protocol could be used as a training tool, improving performance through an enhancement of running economy. As well as investigating how inducing

CLS influences pulse pressure oscillations within CBF and the development of vascular adaptations.

Other methods have been utilized to endorse entrainment during locomotion. Takeuchi *et al.* (2014) investigated CLS during walking, using an ECG monitor that had a buzzer sound at each R wave. Therefore, participants walked at the frequency of their HR. An elevation in O_2 pulse and VO_2 occurred during the CLS protocol in comparison to self-paced walking. Thus, demonstrating an enhancement in walking efficiency enabling a greater amount of O_2 to be utilized. Although this occurred during walking, results are comparable to running. Moreover, increasing the body of evidence that inducing CLS during running, improves the efficiency of different physiological systems. Of note, research examining whether this enhancement of efficiency transcends into cerebral circulation is required. However, the first step is to identify whether stepping rate is related to pulse pressure oscillations in CBF. Running consists of rhythmic contractions of the exercising muscles that increase intramuscular pressure (IMP) inducing intermittent vascular occlusion and impeding blood flow to the active muscles (Niizeki, 2004; Kirby *et al.* 1989). Therefore, a reciprocal relationship (i.e. CLS) occurs between arterial pressures and peak IMP to optimize muscular perfusion and minimise cardiac afterload (Kirby *et al.* 1989). The contraction of the exercising muscles potentially occurs during diastole aiding venous return, thus increasing stroke volume through activation of the skeletal muscular pump (Takeuchi *et al.* 2014). In summary, CLS during running improves performance and running economy through the enhancement of cardiac efficiency as observed in the above studies. However, the underlying physiological mechanisms of CLS have not been determined, although a peripheral neural circuit origin in-conjunction with a

non-neural mechanism have been postulated (Nomura *et al.* 2006). Future research is essential, firstly to examine how running parameters impact CBF and the pulse pressure oscillations and secondly to then explore how CLS influences CBF alongside establishing whether a relationship exists between CLS and the beat phenomenon.

Summary

This section will synthesise all the current literature evidencing the potential influential factors on CBF during running, shown in figure 5. No studies have explored the beat phenomenon and whether the 'beats' transcend into cerebral circulation. Consequently, the driving factors influencing oscillation frequency and wavelength remain unknown. Although research has indicated that the resultant difference between step rate and HR equates to oscillation frequency (Palatini *et al.* 1989). Individual physiological characteristics may have substantial influence over the amplitude and frequency of oscillations within CBF. These factors include body morphology, corresponding to their stride length and stepping frequency, alongside an individual's fitness level. A taller individual may potentially have a greater stride length than a shorter individual whilst running at the same speed, theoretically inducing a reduction in oscillation frequency in comparison to a shorter individual. The weight and running style (e.g. natural toe runners vs. flat footed) of an individual may also influence the mechanical impact of the heel-strike, influencing the pulsatility of flow. A taller individual may weigh more, potentially inducing greater pressure changes, increasing the amplitude of the oscillations. Speed has been evidenced to modify the BP fluctuations which transmitted into CBF oscillations (at least up to 65%VO_{2peak}; Lyngeraa *et al.* 2013) as well as being associated with stride length and

stepping frequency. Therefore, running at a slow belt speed on a treadmill potentially induces short quick steps resulting in frequent and narrow oscillations within the CBF. However, elevating the speed would increase the stride length, reducing the stepping frequency producing an increase in oscillation wavelength.

Treadmill gradient has a large influence over manipulating the point of initial contact of a foot-strike. Running uphill on a treadmill could reduce the amplitude of oscillations within CBF, which may be related to the reduced heel-strike force as runners typically become more toe-weighted. This is in comparison to downhill running, where the initial contact is made with the rear of the foot potentially maximising heel-strike force and enhancing the mechanical wave. Thus, downhill running may induce a greater pulsatile flow, theoretically generating a superior stimulus for cerebrovasculature adaptations. However, the impact of these changes in oscillation properties on shear stress and cyclic strain are unknown. Both uphill and downhill footfall patterns could be simulated by running on level ground through initiating contact with the rear-foot and increasing the heel-strike force (simulating downhill running) or initiating ground contact with the forefoot (emulating uphill running). In summary, a simple way to explore this idea would be to compare flat, uphill and downhill running on a treadmill. The heel-strike force could therefore be manipulated, influencing the magnitude and frequency of the pulse pressure oscillations and resultant brain blood flow oscillations to be explored.

In addition, familiarisation of the treadmill could influence an individual's running gait and posture. A novice on a treadmill may possibly feel overwhelmed by running at a set pace. In current literature there appears to be two main driving factors of the oscillatory pattern which can be targeted through exercise interventions; 1. Speed, 2.

Gradient. Speed of the treadmill will have a direct effect on step frequency alongside HR and consequently oscillatory pattern. Whereas gradient will impact the heel-strike force, influencing the amplitude of pulse pressure oscillations. A multitude of factors may influence the oscillation profile, potentially influencing cerebral hemodynamic forces (i.e. shear stress and cyclic strain) and engender cerebrovasculature adaptations. However, further investigations are essential to confirm the relationship between the oscillatory pattern and cerebrovasculature adaptations. The first step in answering this question is to quantify the differences in CBF responses during different types of running.

Consequently, the overall purpose of this thesis is to characterise cerebral haemodynamic oscillations during different running parameters and styles. Therefore, the first aim of this study is to explore the use of a stepwise gradient change of the VO_{2peak} test, to investigate the impact of the increasing intensity (i.e. gradient) on MCAv oscillations (i.e. oscillation duration and frequency). The second aim is to compare CBF profiles during uphill and downhill running. The third aim is to explore how running styles impact the MCAv oscillatory pattern. Specifically, toe-weighted and heel-weighted running which mimics uphill and downhill running. The final aim of this study is to explore the influence step frequency has on MCAv oscillations.
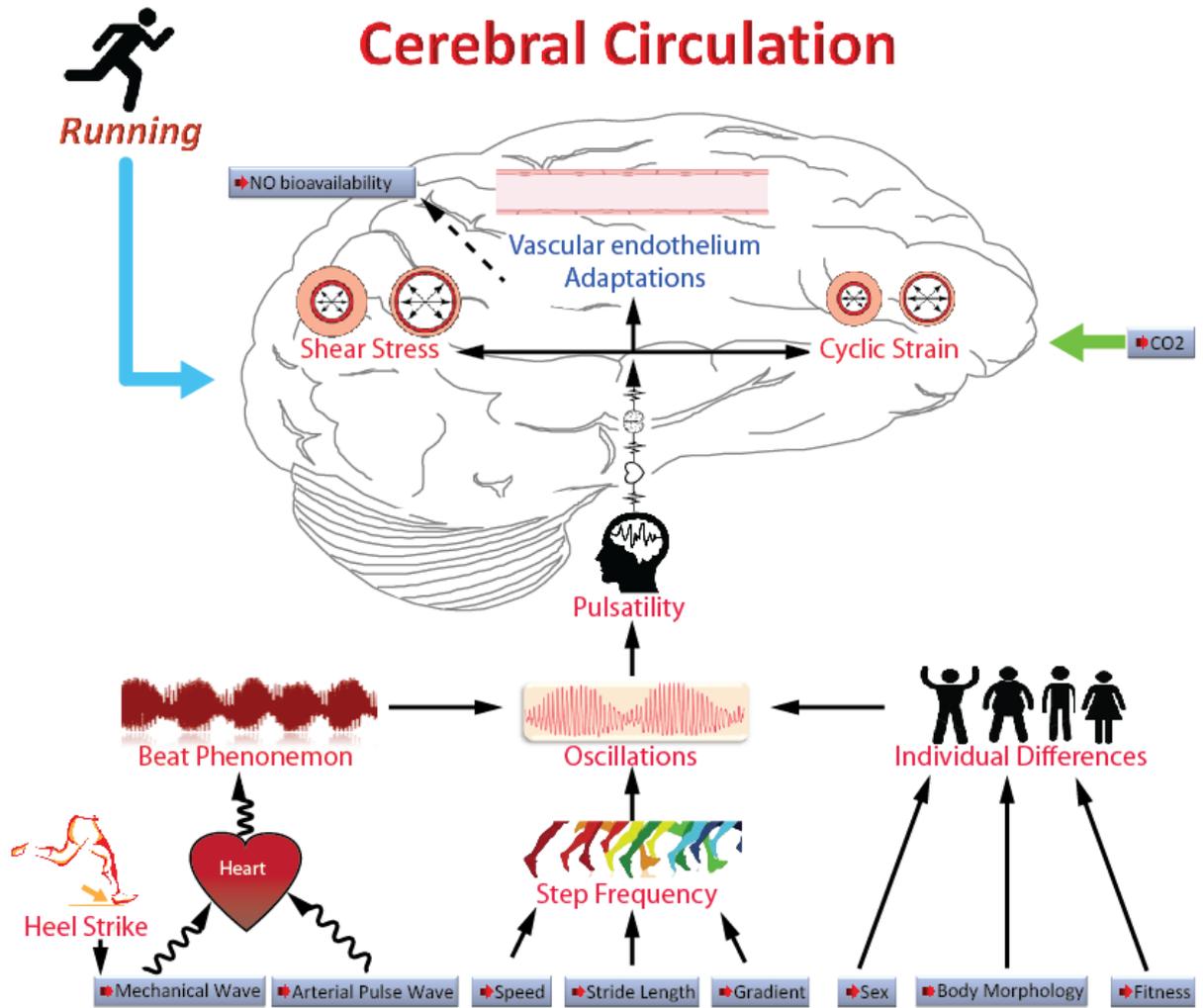
Figure 5: A schematic illustrating potential influential factors on cerebral blood flow during running.

METHODS

Sample Characteristics

Ten physically active participants (6 females and 4 males) were recruited from within the university community. The mean age was 27 ± 7 years (mean ± standard deviation), body mass was 69 ± 9 kg, height was 173 ± 7 cm, peak oxygen consumption (VO_{2peak}) was 44 ± 6 mL/kg /min. Prior to inclusion in the study, participants were invited for an initial screening session where the experimental protocol was carefully explained and there was an opportunity to ask questions and meet the researchers. Following informed consent, participants underwent a screening procedure that included completion of general health and physical activity questionnaires (General Practise Physical Activity Questionnaire, Department of Health, 2013). Participants were excluded if they self-reported having a sedentary lifestyle, being pregnant, or currently using prescribed medication apart from oral contraception, or had a history of metabolic, cardiovascular, pulmonary or neurological diseases. Ethical approval was sought and given from the University of Birmingham Ethical Review committee prior to commencement of study (code: ERN_17-1570).

Experimental design

The study consisted of a treadmill maximal oxygen consumption test (i.e. VO_{2peak}) followed by 3 submaximal exercise testing sessions that followed a randomised crossover design (as shown in figure 6 and 7). These four exercise sessions were conducted at least 48 hours apart, to allow sufficient recovery between sessions, and where possible all completed within two weeks.

Exercise sessions were held in an exercise laboratory within the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham and lasted an hour, of which ~30 minutes involved active exercise. Pre-experimental instructions included refraining from caffeine for 6 hours and alcohol consumption for 24 hours before testing. Pre-testing restrictions on food intake consisted of a large meal four hours prior and a light meal two hours prior to the exercise sessions. Participants were instructed to consume 0.5 litres of water within 4 hours and 0.25 litres within 15 minutes before testing, in accordance with the American College of Sports Medicine Hydration Guidelines (ACSM, 2007).

Study Procedures

Upon arrival of the VO_{2peak} test, participants' height and body mass were measured. Participants were then instrumented for measures of respiratory gases and volume, cerebral blood velocity, and prefrontal cortex and rectus femoris haemodynamics for the submaximal exercise protocols (detailed below; shown in figure 8). The menstrual cycle was not recorded. Participants TCD settings of depth and gain were recorded and then held constant across all visits whilst ensuring resting MCAv was of similar values. The position of the TCD probe was documented with the additional use of photographs if needed. Once instrumented, a seated resting baseline was collected for three minutes. Participants then stood or sat on either the treadmill or cycle ergometer before resting measures continued for a further three minutes once individuals were comfortable on the ergometer, maintaining the exercising posture. Prior to exercise commencement, all wires were safely secured to the modality by micropore tape, ensuring there was no tripping hazards or disruption to the transcranial doppler (TCD) signals. Participants then began exercising, starting with a

five-minute warm-up prior to commencing one of the four exercise protocols detailed below.

Exercise testing sessions

Treadmill maximal VO2peak protocol (see figure 6):

After instrumentation and baseline measures were collected, the test began with a 5-minute warm-up, with a treadmill incline of 1%. The participant controlled the speed during the warm-up, self-selecting a comfortable running speed to attain a rate of perceived exertion (RPE) (Borg, 1982) score of 11. Participants were then offered time to stretch, before a standardised ramp VO_{2peak} protocol began. Stage one of this ramp protocol consisted of the participant running for 3-minutes at the self-selected speed from the warm-up with the gradient at 1% incline. Thereafter, the gradient increased by 2% every 3-minutes until voluntary exhaustion, an RPE score of 20 or a plateau in VO_2. A 2-minute cool down followed at a walking speed of 5 km/h. Participants were then given an option to have a seated rest (~5-minutes), or to continue onto the familiarisation protocol, as well as the option to remove the face mask. Once recovered, participants practiced the toe- and heel-weighted running styles, as well as running in time to two audio-cue rhythms. This familiarisation consisted of 4 x 3-minute bouts in the following order: i) weighted toe-running; ii) weighted heel-running; iii) audio-cue rhythm of 145 bpm (beat per minute); iv) random audio-cue rhythms (145-185 bpm), and v) finishing with a 2-minute walk at 5 km/h. The exercise session was then terminated, equipment removed, and the participant was free to leave the laboratory.

Gradient running protocol (see figure 7):

Following resting measurements and a 5-minute warm-up to attain an RPE (Borg, 1982) score of 11, participants were offered time to stretch before the exercise session commenced. The gradient protocol involved running at a flat gradient followed by either uphill or downhill running that was determined in a randomised cross-over fashion. Participants started by running for 10-minutes at a gradient of 1% ('flat running'). For the uphill bout, the gradient was increased to 6%. After 5-minutes, the gradient was adjusted until participants achieved 65% of their VO_{2peak}. This speed and gradient were maintained for at least 10-minutes of continuous data collection. For the downhill bout, the initial gradient was -6% and after 5-minutes running at this gradient and speed, the gradient was adjusted until participants achieved 65% VO_{2peak}. Thereafter, belt speed and treadmill gradient were maintained for at least 10-minutes of continuous data collection. After completing each of the gradient bouts, participants concluded with a 2-minute cool down at a walking speed of 5 km/h and gradient of 1% before the test was terminated, equipment removed, and the participant was free to leave.

Step frequency and Foot-strike running protocol (see figure 7):

Following resting measurements, participants performed a 5-minute warm-up. During the warm-up, participants ran to an audio-cue of 145 bpm at a gradient of 1%, with the speed of the treadmill adjusted to achieve this step frequency, while maintaining an RPE (Borg, 1982) score of 11. The gradient and final speed of the warm-up was then maintained throughout the remainder of the session. Participants were then offered time to stretch before the commencement of the testing session. Participants then completed five, 5-minute bouts at a constant belt speed. The first bout was a flat

run (at 1% gradient), which was followed by toe-weighted and heel-weighted running, to achieve a change in the natural heel-to-toe biomechanics. The final two bouts consisted of running to two audio-cue rhythms. The first consisted of 145 bpm, while the latter consisted of a random audio-cue rhythm which included 30-second durations of rhythms that ranged between 145-185 bpm. At the end of the session participants finished with a 2-minute cool down at a walking speed of 5 km/h. The test was then terminated, the equipment removed, and the participant was free to leave.

30-minute cycling 65% VO2peak protocol (see figure 7):

Following baseline measurements, participants performed a 5-minute warm-up to attain an RPE (Borg, 1982) score of 11. Participants were then offered time to stretch before the commencement of the testing session. The session involved 30-minutes of continuous cycling at 65%VO2peak, as determined from the running VO2peak test. A stable cadence of 60-70 rpm (revolutions per minute) was maintained. The session finished with a 2-minute cool down at 50 watts. The test was then terminated, the equipment removed, and the participant was free to leave.
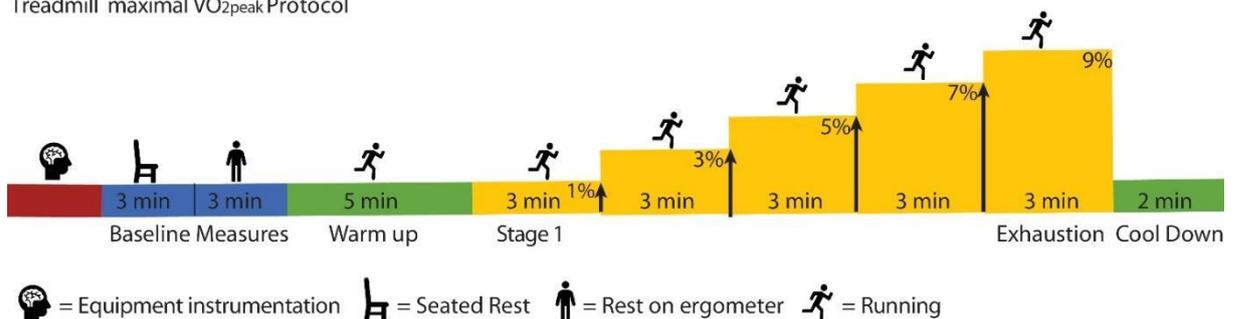
Figure 6: A schematic illustrating the treadmill maximal VO2peak protocol.
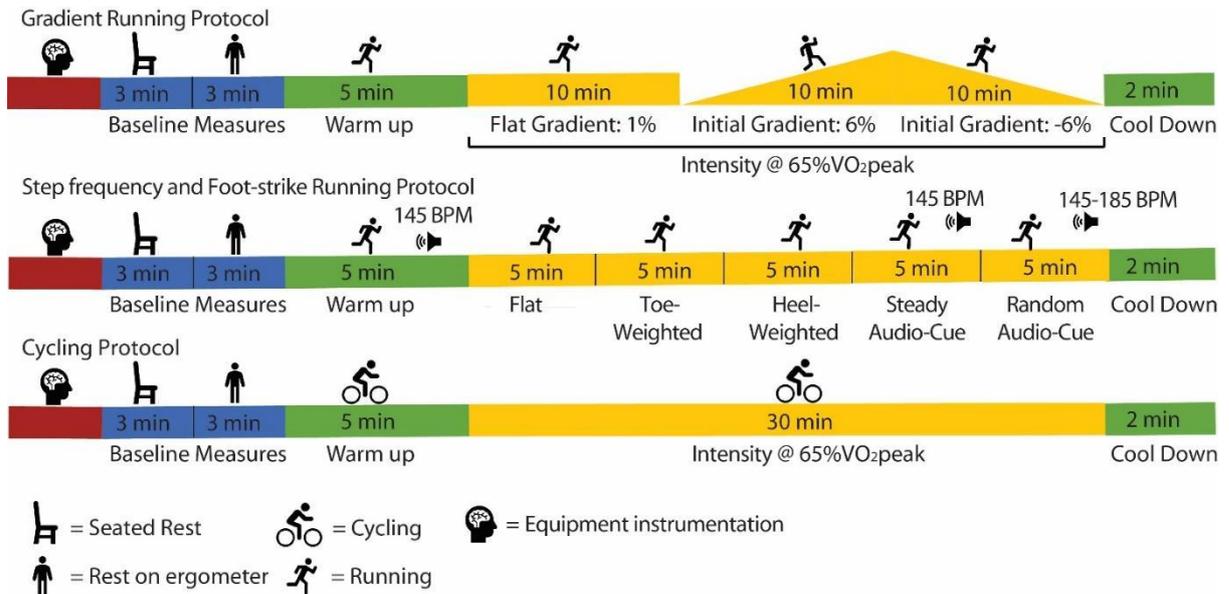
Figure 7: A schematic illustrating the 3 different exercise protocols.

Measures

Bilateral middle cerebral artery blood velocity (MCAv) was measured using transcranial Doppler (TCD; Doppler box, DWL, Germany) as an index of CBF. Heart rate (HR) was derived from the systolic peak of the MCAv signal. Two ultrasound probes were placed above the zygomatic arch on both sides of the head and secured in place using an adjustable headband. Insonation depth, gain and filter settings were recorded on the first testing session and then replicated across all sessions. Ultrasound gel was used between probes and skin in order to attain a high-quality signal. Tissue oxygenation index (TOI) and tissue haemoglobin index (THI) were measured within the prefrontal lobe and the rectus femoris using near-infrared spectroscopy (NIRS; NIRO-200NX, Hamamatsu, Japan). A set of NIRS probes were placed over the middle of the rectus femoris (15cm proximal from the middle of the patella) in line with muscle fibres. The second set of NIRS probes were placed over the prefrontal cortex (proximal to the start of the participant's hair line, distal to their right eye). Excess leg hair was removed, followed by exfoliating and sterilising the

area before probe placement. Both sets of probes were covered with either tape or a

headband to minimise contamination of the signal from any external light sources.

An indirect calorimetry system (VyntusTM CPX, Carefusion, Germany) measured

respiration variables, which included ventilation rate, volume of oxygen consumption

(VO_2) and partial pressure of end-tidal carbon dioxide ($PETCO_2$) *via* a leak-free face

mask (V2 Series, Hans Rudolf Incorporated, USA). The participants were asked how

hard they felt they were exerting themselves at the end of each exercise stage, and

then stated a number on the Borg scale (Borg, 1982) corresponding to their RPE.

The running protocols were carried out on a treadmill (H/p/cosmos, Quasar) and

cycling on a cycle ergometer (Sport Excalibur, Lobe, The Netherlands).

MCAv and NIRS data were recorded *via* an analogue-to-digital converter

(PowerLaVb 16/35, ADInstruments, Australia) and displayed in real time on a

computer *via* LabChart software (Version 7, ADInstruments). RPE was manually

recorded and combined with the respiratory, cerebral blood velocity, rectus femoris

and prefrontal cortex haemodynamic data in electronic form within Excel version

2016 (Excel, Microsoft, USA).
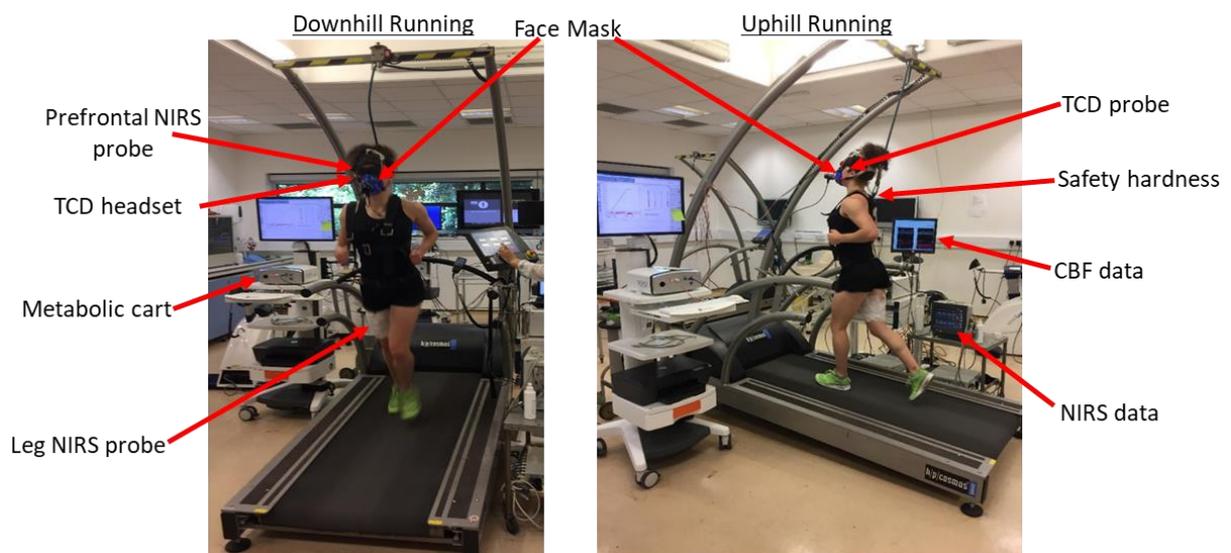
Figure 8: Annotated photographs illustrating the laboratory set-up during gradient, step frequency and foot-strike running protocols.

Statistical analysis

Data analysis

To determine participants' VO_{2peak}, a 30-second average of the respiratory data was taken around the highest consistent VO_2 value to determine VO_{2peak}. The VO_{2peak} was then extrapolated to determine 65% and used as an intensity target in the subsequent sessions. The impact of individuals' fitness on MCAv will be examined in the results section alongside the influence of running experience on the oscillatory pattern during the VO_{2peak} test. Participants running experience was determined by the physical activity questionnaire and verbal confirmation.

Respiratory and MCAv data for each stage of the VO_{2peak} test, step frequency and foot-strike running protocols included a 30-second (s) average at the end of each stage. For both cycling and gradient running protocols, respiratory and haemodynamic measures were averaged over 30 s at the target intensity (65% VO_{2peak}) towards the end of the 5- or 10-minute stages, with time points for all protocols matched up between these sets of data (time stamp markers simultaneously placed within these data sets during collection). MCAv, TOI and THI exercise data were reported as the change from resting baseline, as it is recommended with these approaches since absolute values can be confounded by small variations in probe placements between visits. In order to exclude artefacts, all doppler tracings were inspected and the hemisphere with minimum signal interference was chosen for analysing MCAv.

MCAv oscillation amplitude was determined by calculating the pulse pressure amplitude (PPA) using the equation PPA = Systole – Diastole. MCAv oscillation frequency was counted across the selected 30 s average periods, while the duration

of the oscillation cycle (i.e. wavelength) was averaged from the last 3 oscillations within each stage.

Statistical approach

After confirming normal distribution of data using Shapiro– Wilk normality tests. One-way ANOVA's were used to address how changing the heel-strike force and pattern during running affected the pulse pressure oscillations and MCAv profile. Specifically, separate one-way ANOVAs examined: i) comparison between resting data for each exercise session; ii) comparison between rest, 1% and maximal gradient achieved during the VO_{2peak} test; iii) comparison between rest, flat, uphill, downhill running and cycling at 65%VO_{2peak}; iv) comparison between rest, flat, toe-weighted and heel-weighted running, and v) comparison between rest and the different audio-cue rhythms. Pairwise comparisons of the different exercise bouts were performed and adjusted for multiple comparisons (Bonferroni). A Friedman repeated measures analysis of variance was completed on data not normally distributed. Statistical significance was accepted at p≤ .05. All statistical analysis was completed using SPSS version 25 for Windows (SPSS Inc. Chicago, IL).

RESULTS AND DISCUSSION

A total of 10 individuals were recruited and completed all 4 visits. However, resting data presented in table 3 represents 9 individuals due to insufficient TCD signal quality during rest prior to the VO_{2peak} test protocol. This issue continued in a further 2 participants throughout the exercise session, thus data from the VO_{2peak} test represents 7 participants. Also, 1 participant data was excluded from the step frequency and foot-strike protocol, for inadequate TCD signal quality. Additionally, poor signal quality occurred within the NIRS equipment, with 1 individual's data excluded during the gradient and another from the step frequency and foot-strike visits. Therefore, tissue oxygen index and tissue haemoglobin index of the rectus femoris (TOI_{leg}, THI_{leg}) data from both of these visits is for 9 participants. The different sample sizes are shown below each table.

Table 3: A summary of resting baseline data from each of the 4 visits

Dependant Variables	Resting Baseline					
	Treadmill VO_{2peak} test	Gradient Running	Step freq. & Foot-strike running	Cycling	Average	P value
MCAv (cm/s)	57.3±7.9	64.4±14.6	62.2±9.8	64.1±11.2	62.0±10.9	0.32
sMCAv (cm/s)	87.8±10.4	94.7±18.9	91.7±13.4	96.8±16.5	92.8±14.8	0.41
dMCAv (cm/s)	40.6±6.3	44.8±10.3	44.7±8.1	45.6±7.3	43.9±8.0	0.40
PPA (cm/s)	47.3±5.6	49.4±10.6	47.0±8.0	51.2±10	48.7±8.6	0.52
HR (bpm)	81±20	69±12	67±9	70±14	72±14	0.09
$PETCO_2$ (mmHg)	30.9±1.2	31.1±2.6	31.8±3.0	32.4±2.4	31.6±2.3	0.40
TOI_{head} (%)	x	68.5±4.2	68.6±6.3	68.1±4.1	68.4±4.9	0.91
THI_{head} (a.u.)	x	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	0.54
TOI_{leg} (%)	x	73.2±7.3	70.5±4.8	71.0±4.5	71.6±5.5	0.27
THI_{leg} (a.u.)	x	1±0.1	1±0.2	1.1±0.3	1.0±0.2	0.76

(Key: freq, frequency; MCAv, middle cerebral artery blood velocity; PPA, pulse pressure amplitude; sMCAv, systole middle cerebral artery blood velocity; dMCAv, diastole middle cerebral artery blood velocity; $PETCO_2$, partial pressure of end-tidal carbon dioxide; TOI_{head}, tissue oxygen index of prefrontal cortex; THI_{head}, tissue haemoglobin index of prefrontal cortex; TOI_{leg}, tissue oxygen index of rectus femoris; THI_{leg}, tissue haemoglobin index of rectus femoris; HR, heart rate; a.u., arbitrary unit) Values are means ± SD. n = 9 for MCAv, sMCAv, dMCAv, PPA, HR, $PETCO_2$, n = 10, TOIhead, THIhead, TOIleg THIleg.

Across the 4 visits, no significant difference occurred between resting baselines for any of the dependent variables (p>.05). Nevertheless, for each exercise session, data presented will be reported as change from the preceding baseline rest period.

Comparison between stage one (1% gradient) and the final stage (7-11% gradient) of the VO$_{2peak}$ test

MCAv increased from resting values at stage one and the final stage of the VO$_{2peak}$ test (p<.01; see table 4). MCAv showed a similar increase of ~14cm/s at both the start and end stages, despite increasing exercise intensity (p=.46). Of note, the 3 participants with a VO$_{2peak}$ of > 50 mL/min/kg increased MCAv by ~4cm/s from stage one to the final stage of their *VO$_{2peak}$ test* (p=.05), while the remaining participants (with an average VO$_{2peak}$ of 42±3 mL/min/kg) decreased MCAv by ~6cm/s (p=.02; shown in Figure 9). In contrast, all participants PETCO$_2$ significantly decreased by ~4 mmHg from stage one to the final stage of the VO$_{2peak}$ test (p<.05; table 4). Finally, HR significantly increased with intensity by ~27 bpm from stage one to the final stage of the VO$_{2peak}$ test (p=.02).
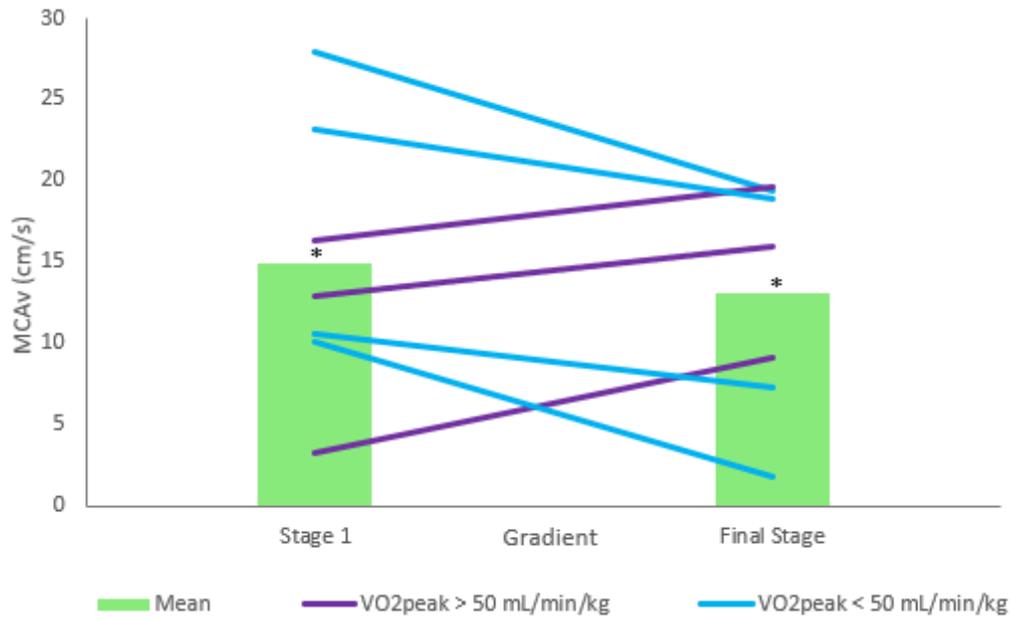
Figure 9: The change in participants middle cerebral artery blood velocity (MCAv, cm/s) during stage one (1% gradient) and the final stage (7-11% gradient) of a VO_{2peak} test (n=7).
*significant from rest (p<.05)
(Key: VO_{2peak} > 50mL/min/kg, peak oxygen consumption above 50 mL/min/kg; VO_{2peak} < 50 mL/min/kg, peak oxygen consumption below 50 mL/min/kg)
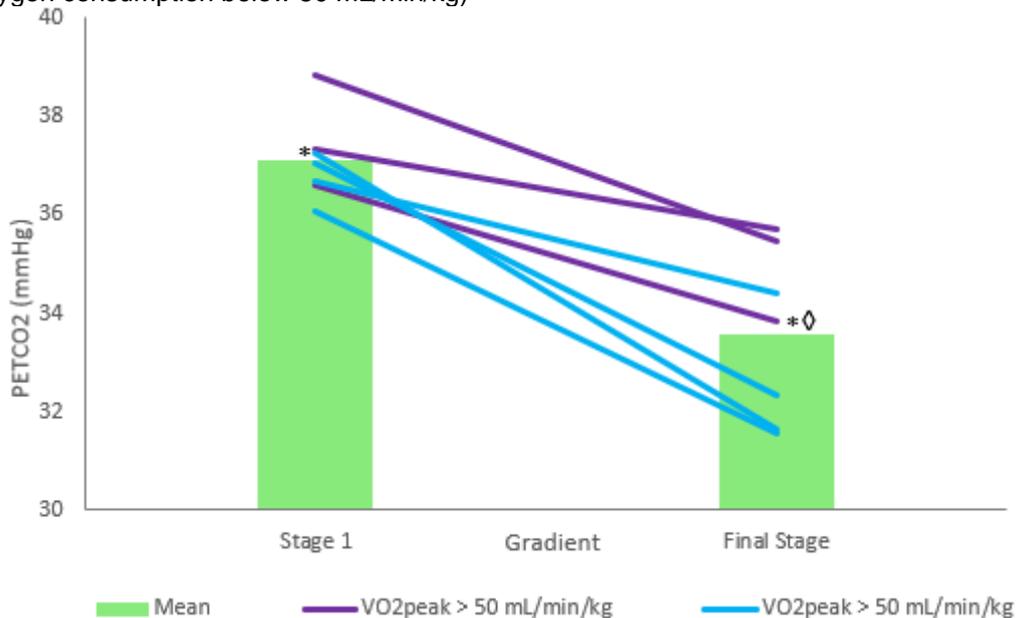
Figure 10: The change in participants partial pressure of end-tidal carbon dioxide ($PETCO_2$, mmHg) during stage one (1% gradient) and the final stage (7-11% gradient) of a VO_{2peak} test (n=7).
*significant from rest (p<.05); ◊ significant from 1% gradient (p<.05).
(Key: VO_2peak > 50mL/min/kg, peak oxygen consumption above 50 mL/min/kg; VO_2peak < 50 mL/min/kg, peak oxygen consumption below 50 mL/min/kg).

Table 4: The mean change from resting baseline in middle cerebral artery blood velocity (MCAv), partial pressure of end-tidal carbon dioxide (PETCO$_2$) and heart rate (HR) during stage one (1% gradient) and the final stage (7-11% gradient) of a VO$_{2peak}$ test.

Intensity	ΔMCAv	ΔPETCO$_2$	ΔHR
1%	14.9±8.3*	7.1±3.5*	75±12*
Maximal Intensity	13.1±7.1*	3.6±3.7*◊	102±20*◊

(Key: ΔMCAv, change in middle cerebral artery blood velocity; ΔPETCO$_2$, change in partial pressure of end-tidal carbon dioxide, ΔHR, change in heart rate).
*significant from rest (p<.05); ◊ significant from 1% gradient (p<.05).
Values are means ± SD. n = 7 for MCAv, PETCO$_2$; n = 6 for HR.
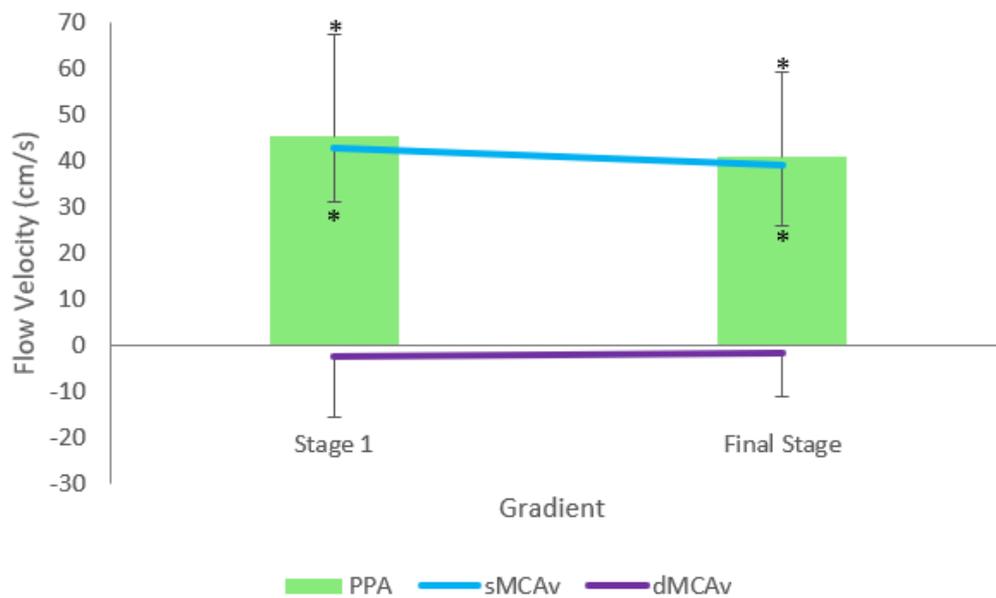
Figure 11: The mean change in pulse pressure amplitude (PPA, cm/s) during stage one (1% gradient) and the final stage (7-11% gradient) of a VO$_{2peak}$ test (n=7).
*significant from rest (p<.05).

Compared to resting baseline, sMCAv increased at stage one and the final stage of the VO$_{2peak}$ test (up by ~41 cm/s; p<.01) with no change occurring in dMCAv (p=.71). Consequently, this resulted in a similar rise in PPA of ~43cm/s above resting baseline at stage one and the final stage of the VO$_{2peak}$ test (p<.05, Figure 11). No significant difference occurred between stage one and the final stage for sMCA, dMCA and PPA (p>.05).
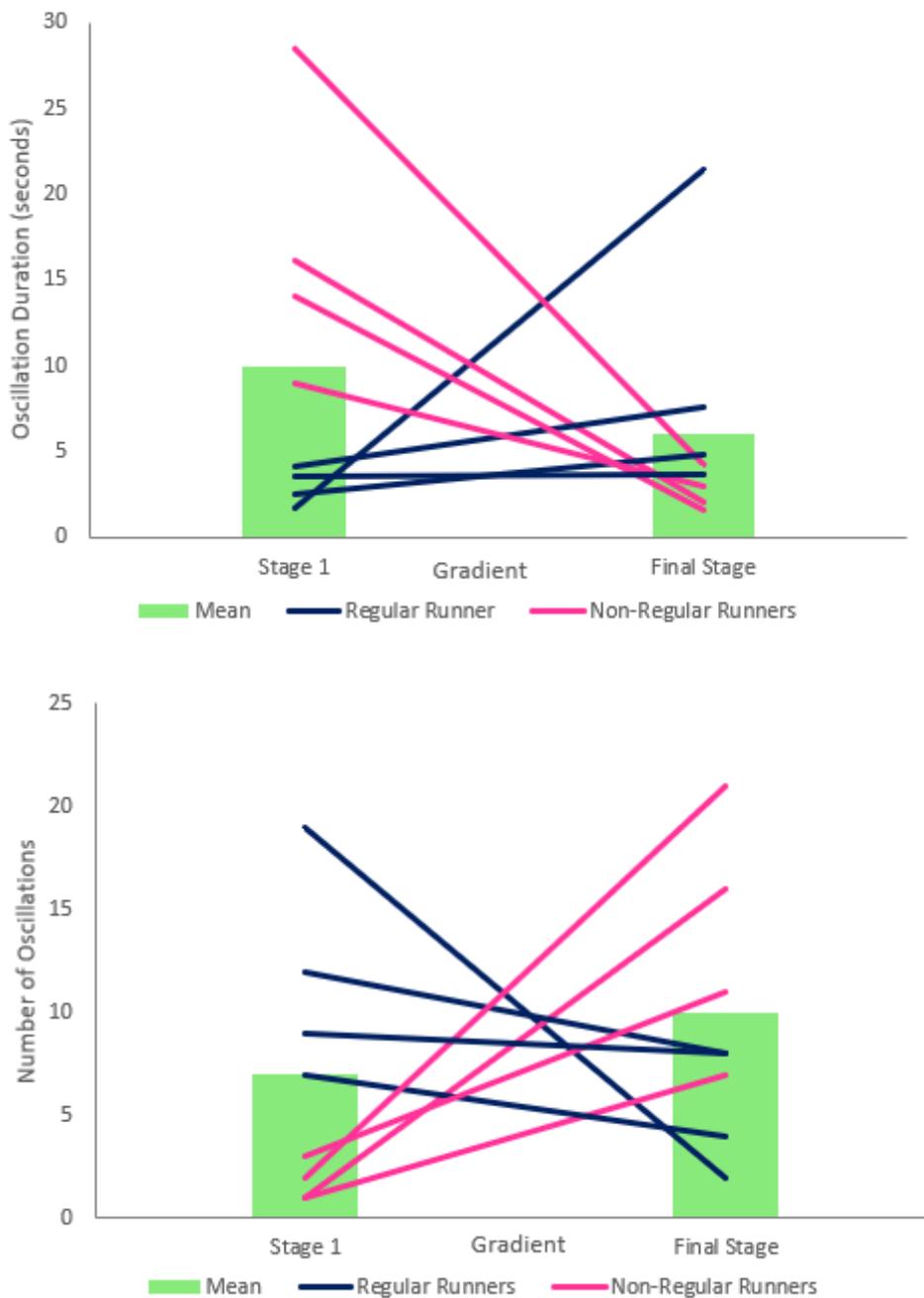
Figure 12: The oscillation wavelength duration (seconds, top panel) and number of oscillations (bottom panel) during stage one (1% gradient) and the final stage (7-11% gradient) of a VO_{2peak} test (n=8).

As a group, the oscillation wavelength duration decreased by ~4s, with an average

increase of 3 oscillations from stage one to the final stage of the VO_{2peak} test,

although no significant difference was reached for both measures (both p>.05; figure

12). However, as evident from figure 12, two different patterns emerged within the

dataset between participants, which was linked to how often they ran (self-reported). For 4 participants, wavelength duration increased (by 6 ± 9 s) while the number of oscillations decreased (by 6 ± 7) from stage one to the final stage of the VO_{2peak} test (p>.05; shown in dark blue). Whereas the remaining participants had the opposite pattern, with a decrease of ~14 s in wavelength duration, and an increase in oscillation number (12 ± 6) between stage one and the final stage of the VO_{2peak} test (p<.05; shown in pink).

Figures 13 and 14 show examples of these beat-to-beat flow velocity patterns. Figure 13 illustrates that, as the gradient elevated, the oscillation wavelength duration progressively decreased on average by ~4 s with an average increase of 4 oscillations per 2% incline. An increase in oscillation frequency of 15 occurred between stage one and the final stage of the VO_{2peak} test, with a decline of ~14 s in wavelength duration. Additionally, the largest range in wavelength duration occurred during flat running (1% gradient) of ~19 s and decreased to only ~1 s within the final two gradients (i.e. 7% and 9%). Figure 14 shows an example of when the gradient is elevated, the oscillation wavelength duration progressively increased (by ~3 s), with an average decrease of 4 oscillations per 2% incline. It was noted that during running at 9% gradient, the wavelength duration progressively increased, with a difference of ~9s between the shortest and longest wavelength at this gradient. The greatest increase in wavelength of ~8 s occurred between gradients 9% to 11%. The range in the wavelength duration, during the first four gradients was similar, with only an average difference of ~1 s, in comparison to an average difference of ~11 s within the last two gradients.
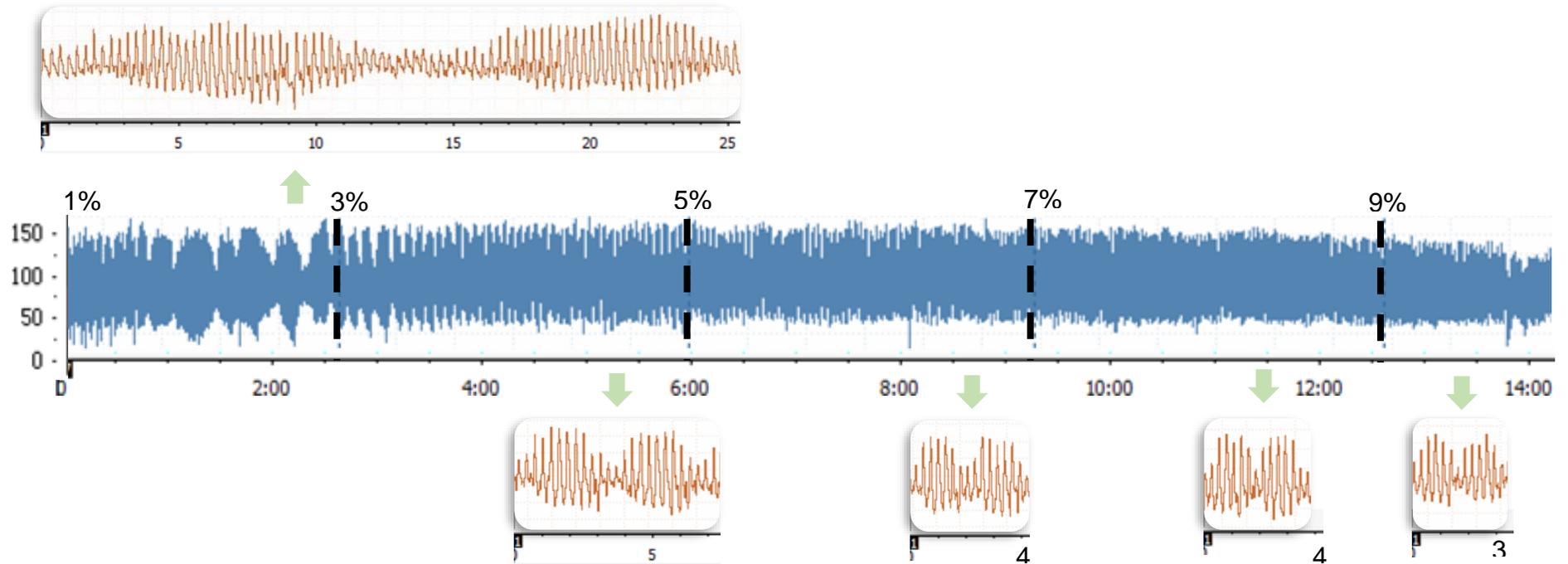
Figure 13: A continuous trace of an individual's middle cerebral artery blood velocity (MCAv, cm/s) profile during a treadmill VO_{2peak} test at 9km/h with gradient increasing 2% every 3-minutes. At each gradient there is an example of the MCAv trace across two oscillations, demonstrating a decline in the oscillation wavelength duration with an increase in gradient (scale of beat-to-beat MCAv data is the same for each panel).
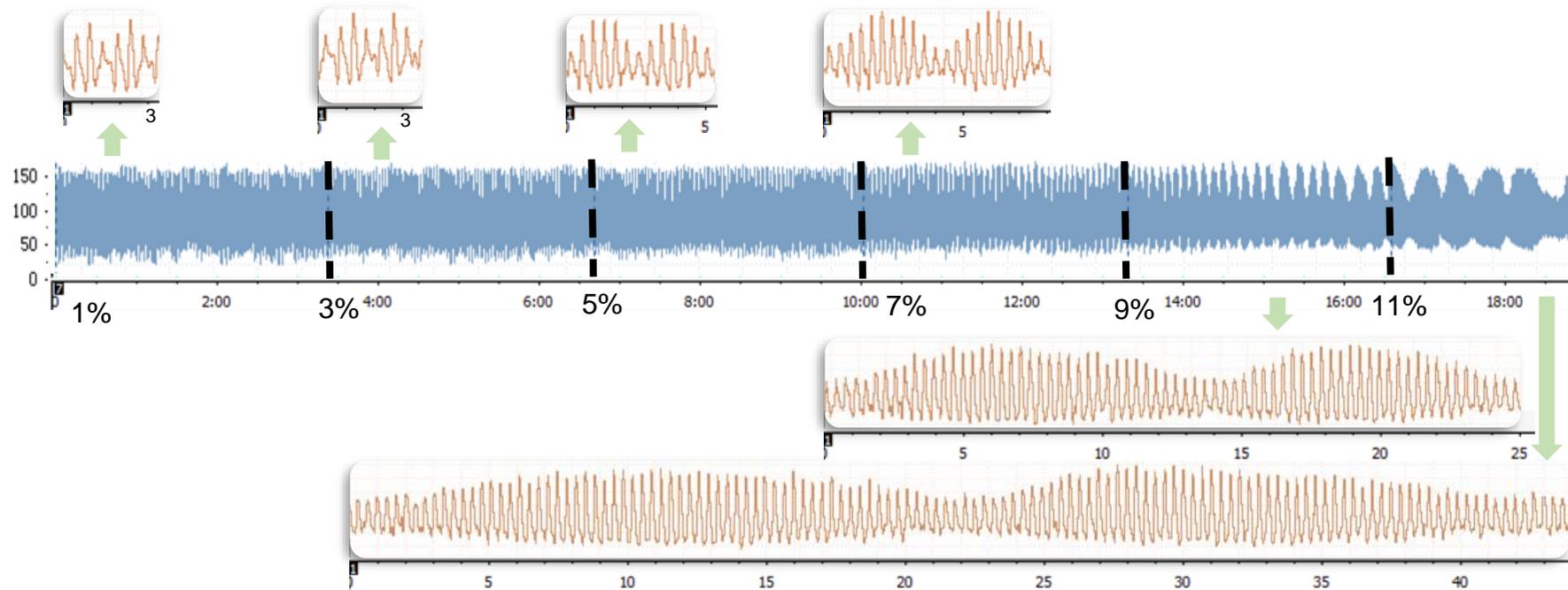
Figure 14: A continuous trace of an individual's middle cerebral artery blood velocity (MCAv, cm/s) profile during a treadmill VO$_{2peak}$ test at 11.5km/h with gradient increasing by 2% every 3-minutes. At each gradient there is an example of the MCAv trace across two oscillations, demonstrating the increased oscillation wavelength duration with an increase in gradient (scale of beat-to-beat MCAv data is the same for each panel).

The above findings from the running VO_{2peak} test protocol demonstrated a number of noteworthy observations. First, both $PETCO_2$ and MCAv, on average, increased from rest to stage one (1%) and the final stage of the VO_{2peak} test. $PETCO_2$ decreased from stage one to the final stage of the VO_{2peak} test, consistent with the inverted-U relationship for exercise intensity that has been shown in cycling-based exercise studies (e.g. Hellström *et al*. 1996; Brugniaux *et al*. 2014). However, there was no significant difference in MCAv values between both stages (see table 4). This appears to be the result of a unique fitness effect (as shown in Figure 9 and 10). Specifically, as individuals with a VO_{2peak} of greater than 50 mL/min/kg mean MCAv progressively increased from stage one until the end of their VO_{2peak} test, whereas $PETCO_2$ decreased during the final stages. In contrast, the MCAv response remained coupled with $PETCO_2$ in less fit participants (VO_{2peak} < 50 mL/min/kg, shown in figure 9 and 10). To date, the CBF profile during exercise has been predominantly researched within cycling, for which no fitness effect has been observed (or at least reported). Therefore, running may elicit a unique stress on the regulatory mechanisms governing CBF. That means the normally tightly coupled relationship between PCO_2 and CBF is uncoupled for fitter individuals running at high intensities. The novel finding that MCAv increases with intensity within fit individuals promotes the question what is driving this increase in blood flow.

As mentioned above, in fitter individuals the normally tightly coupled relationship between CBF and PCO_2 appears to be different during running at high intensities. During progressive intensity exercise, BP, HR, cardiac output alongside O_2 consumption are all known to increase, although at different magnitudes (Smith and Ainslie, 2017). In the current study, fit individuals reached higher exercise intensities,

where a greater pressor effect could have occurred from an elevation in BP and cardiac output driving an increase in CBF. Poiseuille's law states that CBF is influenced by arterial BP, coupled with vasculature resistance. Therefore, an increase in BP will elevate cerebral perfusion pressure, resulting in a direct impact on CBF (Hill and Gwinnutt, 2008). However arterial BP was not measured in the current study due to difficulties with movement artefact for the BP device. Nevertheless, rapid changes in perfusion pressures may challenge cerebral autoregulation, which takes normally takes ~3s to maintain a relatively stable CBF during changes in perfusion pressure (Ogoh and Ainslie, 2009). Additionally, a high level of cardio-respiratory fitness has been found to augment the latency of cerebral autoregulation with a reduced ability to dampen rapid fluctuations in MAP (Lind-Holst et al. 2011; Labrecque et al. 2017; Drapeau et al. 2019) as well as reduced cerebrovascular reactivity to CO_2 (Thomas et al. 2013). Together, this may explain the elevated MCAv response observed at the higher intensities for those working at high absolute workloads. Endurance athletes have a large maximal cardiac output resulting in a greater volume of blood available for competitive demands of active tissue (Basset and Howley, 2000; Yamaji and Miyashita, 1978). While cardiac output or blood volume was not assessed in these individuals, presumably high cardiac outputs in the fitter individuals may in part explain the progressive increase of MCAv with increasing intensity for this subgroup. Further research is needed to confirm such speculation.

Lyngeraa *et al*. (2013) reported that both BP and MCAv decreased during running at 75%HR reserve, which is equivalent to 65%VO_{2peak} (Swain *et al*. 1994). However, participants were recreational runners and the protocol did not involve high-intensity

exercise, potentially preventing the occurrence of the pressor effect and limiting the exploration of the CBF profile. Additionally, PETCO$_2$ was not measured, limiting the explanation behind the decrease of MCAv. Palatini *et al.* (1989) reported systolic BP gradually decreased during submaximal running. However, a sharp increase occurred during the final sprint within amateur runners, potentially indicating that BP increases during maximal intensity exercise that could result in an increase in CBF. These findings provide some support for the MCAv observations in the fitter participants tested here. Collectively, these findings indicate that there is a modality, intensity and fitness dependant relationship in regard to the CBF profile, however more research is needed to determine what mediates this effect.

A decrease in PPA was expected to occur with the progressive VO$_{2peak}$ stages, as the heel-strike force was hypothesised to reduce with the elevation of gradient. However, no change in PPA was reported between running at stage one and the final stage participants reached (shown in figure 11). Palatini *et al.* (1989) and Lyngeraa *et al.* (2013) both reported an increase in the amplitude of pulse pressure with increasing exercise intensity. To note, both these protocols involved increasing the speed rather than the gradient to elevate the intensity of exercise. Therefore, an alteration in belt speed (i.e. running cadence) could possibly elicit a greater impact on the pulsatility of blood flow than uphill running.

The rapid fluctuations in perfusion pressure are unique to running and induce a pulsatile flow. This potentially results in increased haemodynamic forces (i.e. sheer stress and cyclic strain) that may induce a stimulus for cerebrovasculature adaptations unlike cycling. Additionally, the vasoconstricted artery which is evidenced by the decline in PETCO$_2$ in fit individuals, would be experiencing an increase in

pressure and flow, potentially resulting in increased shear stress and cyclic strain, which may induce further vasculature benefits. The maximal increase in CBF is postulated to induce the greatest elevation of haemodynamic forces, which has previously been evidenced to occur at 65%VO_{2peak} intensity (e.g. Brugniaux *et al.* 2014). However, findings from the current study challenges the traditional paradigm, indicating that maximal intensity may induce a greatest MCAv response during running and therefore the optimal stimulus for cerebrovasculature adaptations in fit individuals. To note, only the first and last stage of the VO_{2peak} test was analysed. Therefore, the CBF profile across a range of exercise intensities was not explored within this investigation.

Cerebral oxygenation was not measured although would have enabled the observation as to whether oxygenation levels followed the same pattern as MCAv within the fit individuals. Current research during high-intensity cycling showed that cerebral oxygenation follows the decline in CBF (Curtelin *et al.* 2017). Future research is required to establish the relationship between cerebral oxygenation and CBF with intensity in fit compared to unfit individuals during running.

Similar to the PPA response, the oscillation profile was hypothesised to have a decrease in the number of oscillations alongside an increase in the wavelength duration with an increased treadmill gradient, due to inducing a toe-weighted running style. However, an interesting trend became apparent in the oscillatory pattern, which was that the oscillatory pattern seemed dependent on participants running experience (shown in figure 13 compared to figure 14). Specifically, while the regular runner's oscillatory profile was consistent with the hypothesis, participants less familiar with running had the opposite response (increased number of oscillations

and decreased wavelength duration, as shown in figures 13 and 14). This indicates that running experience impacts an individual's running style and cadence. Collectively, present findings and those by Lyngeraa and colleagues support the idea that an increase in intensity induces an increase in oscillation wavelength in regular runners. In contrast, a variety of factors could have been responsible for the unique oscillatory pattern observed within the non-regular runners. This includes inexperience with treadmill running that may have impacted their natural running cadence. In addition, less fit participants selected a slower running speed, which was potentially a consequence of being unaccustomed to running at a set cadence and were anxious about the VO_{2peak} test. These factors could have induced a shortened stride length with an increased step rate, potentially inducing a high frequency of narrow oscillations (as illustrated in figure 13). To note, environmental and psychological factors also differ between laboratory and outside running. In the current study, discrepancies in the rhythmic oscillatory pattern occurred during each gradient, which likely resulted from temporary variations in pace and corresponds with Palatini *et al*. (1989) findings. In contrast, however, Lyngeraa and colleagues reported that the oscillatory pattern remained relatively constant within each stage of intensity. Nevertheless, Lyngeraa *et al*. (2013) did observed that an alteration in belt speed disrupted the oscillation pattern within MCAv, demonstrating a potential association between step frequency and oscillations. Of note, previous exercise interventions have only explored the impact of an increase of belt speed on the CBF profile and not gradient.

Entrainment of HR and step rate is another potential explanation for an increase in wavelength duration with increasing intensity (Lyngeraa *et al*. 2013 and Palatini *et al*.

1989). The increase in wavelength duration found in regular runners could relate to entrainment between stepping rate and HR. Both Lyngeraa *et al.* (2013) and Palatini *et al.* (1989) found a decrease in the difference between these two rates with an elevation in exercise intensity, which resulted in an elongation of oscillation wavelength. Collectively, the increase in wavelength duration in both studies was related to HR and step rate approaching one another indicating entrainment between these two rhythms. A plethora of potential factors may have prevented entrainment from occurring in non-regular runners as previously mentioned. These include self-selecting a slow running pace, reduced motivation or early withdrawal before HR was able to increase towards the step rate. Interestingly, in non-regular runners a greater average difference in oscillation duration (i.e. difference between the shortest and longest wavelength) occurred during stage one of 29.8 ± 16.4 s, in comparison to only 0.6 ± 0.3 s during the final stage. By contrast, the mean in oscillation duration in regular runners was 2.1 ± 1.0 s in the first and 5.2 ± 5.6 s in the final stage. Therefore, indicating more discrepancies in step rate occurred during flat running, which decreased with an increase in intensity in non-regular runners. Or in terms of entrainment the difference in HR and step rate decreased with intensity, and the oscillatory pattern became more uniform, which is in agreement with the findings of Lyngeraa *et al.* (2013), as mentioned above.

The oscillation profile within CBF has not previously been explored, therefore further investigations are required to confirm the influence of intensity (i.e. speed and gradient) and the underlying mechanisms. However, both Palatini *et al.* (1989) and Rowell *et al.* (1986) confirmed that respiration is unrelated to the development of the rhythmic oscillations. For example, Palatini *et al.* (1989) demonstrated that breath-

holding during running did not have an influence on the arterial pressure oscillations, indicating that ventilation is not associated with the beat-to-beat changes of pulsatility. Therefore, the increase in pulsatility and wavelength duration observed with an increase in exercise intensity is likely not due to hyperventilation, or the biomechanics associated with rhythmical breathing and intrathoracic pressure changes.

Comparison of running at 65%VO$_{2peak}$ on different gradients (flat, uphill, downhill) and cycling at 65%VO$_{2peak}$

Table 5: The mean change in cerebral and leg haemodynamics, end-tidal carbon dioxide and heart rate responses from resting baseline during flat (1% gradient), uphill (6 to 11% gradient) and downhill running (-6 to -11% gradient), and cycling at 65%VO$_{2peak}$.

Modality	MCAv (Δcm/s)	PETCO$_2$ (ΔmmHg)	TOI$_{head}$ (Δ%)	THI$_{head}$ (Δau)	TOI$_{leg}$ (Δ%)	THI$_{leg}$ (Δau)	HR (Δbpm)
Cycling	9.3±8.1*	3.0±3.0	-2.5±4.9	0.1±0.1	-4.9±9.3	-0.4±0.1	72±23*
Flat	9.6±8.3*	6.0±3.4*	-4.5±5.0	0.0±0.1	-1.7±6.1	-0.8±0.2	75±8*
Uphill	11.3±8.6*	5.3±3.7*	-7.3±4.6*	-0.5±0.1	-2.8±6.8	-0.9±0.2	90±17*
Downhill	12.4±9.6*	2.1±3.7$^{×◊}$	-5.9±7.4	-0.3±0.3	-0.7±5.3	-0.7±0.2	83±26*

(Key: MCAv, middle cerebral artery blood velocity; PETCO$_2$, partial pressure of end-tidal carbon dioxide; TOI$_{head}$, tissue oxygen index of prefrontal cortex; THI$_{head}$, tissue haemoglobin index of prefrontal cortex; TOI$_{leg}$ tissue oxygen index of rectus femoris; THI$_{leg}$, tissue haemoglobin index of rectus femoris; HR, heart rate).
Values are means ± SD. n = 10 for MCAv, PETCO$_2$, TOIhead, THIhead; n = 9 for TOI$_{leg}$ and THI$_{leg}$; n = 8 for HR.
*significant from rest (p<.05); × significant from flat (p<.05); ◊ significant from uphill (p<.05).

Exercise induced a significant increase in MCAv from rest in all exercise bouts (table 3; p<.03), while increases in PETCO$_2$ were greater during flat and uphill running in compassion to downhill running (by ~4 mmHg; p<.01). TOI$_{head}$ significantly decreased from rest during uphill running (by ~7%; p<.01). No difference in the change of THI$_{head}$ occurred from rest in each of the exercise modalities (p=0.1). No difference in the change of both TOI$_{leg}$ and THI$_{leg}$ was present from rest in all modalities (p=.24

and p=.48, respectively). The exercise-induced increase in HR was similar between

all exercise bouts (p>.80).
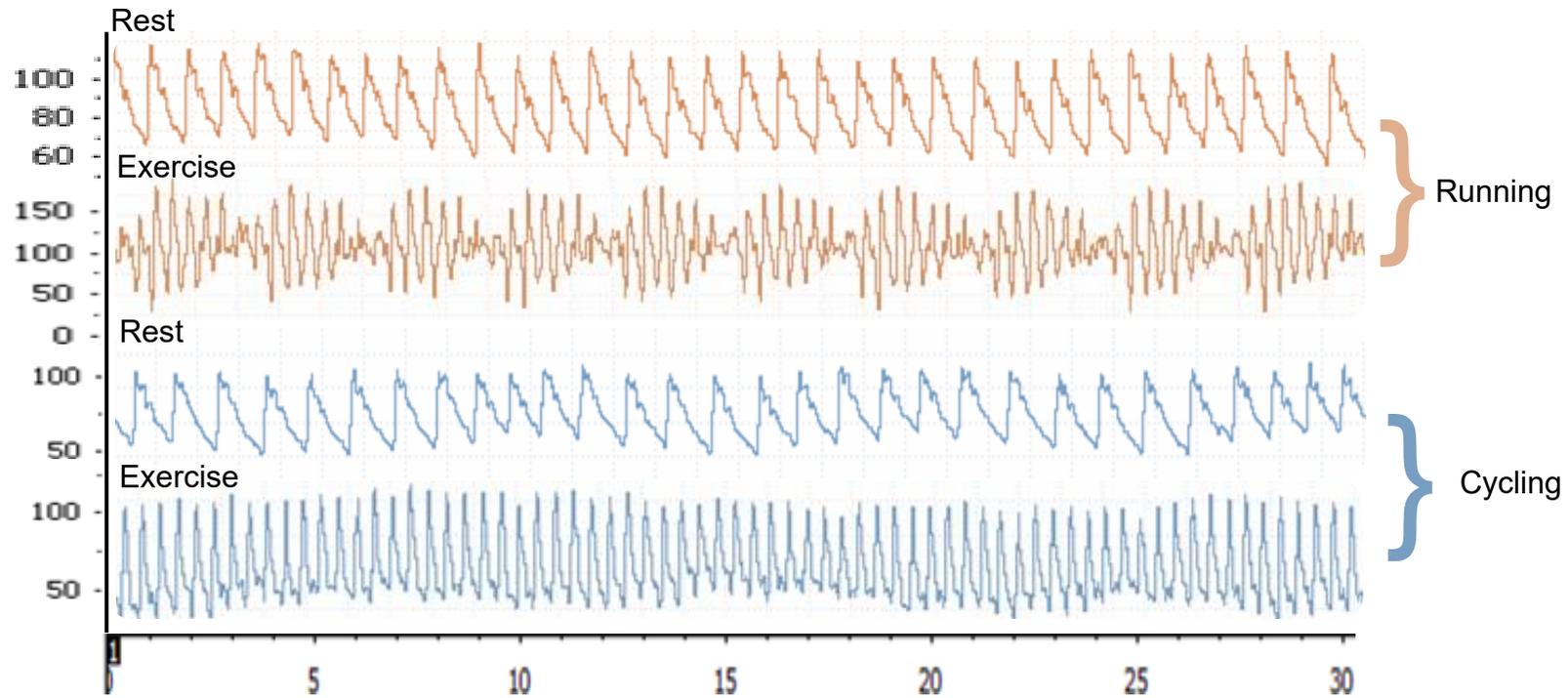
Figure 15: A 30-second trace of middle cerebral artery blood velocity (MCAv, cm/s) of an individual during resting baseline and exercise for both modalities.

Figure 15 is a representative trace of MCAv during resting baseline of both modalities. During exercise 10 clear oscillations are present within MCAv during running in contrast to cycling where they are non-existent, evidencing the difference in the beat-to-beat MCAv profile between these two modalities.
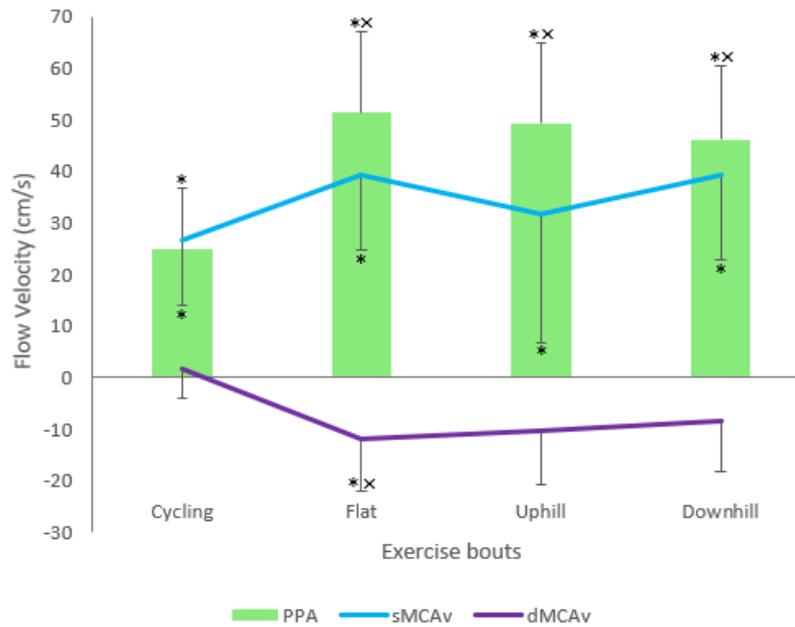
Figure 16: The mean change in pulse pressure amplitude (PPA, cm/s), systole middle cerebral blood artery velocity (sMCAv, cm/s) and diastole middle cerebral artery blood velocity (dMCAv, cm/s) from resting baseline, during flat (1% gradient), uphill (6-11% gradient) and downhill (-6-11% gradient) running in comparison to cycling at 65%VO_{2peak} (n=10).
*significant from rest (p<.05); × significant from cycling (p<.05).

Exercise induced a significant increase in PPA from rest in all exercise modalities (up by ~43 cm/s, p<.01). PPA was ~24cm/s higher during the running bouts compared to cycling (p<.01). A significant increase in sMCAv occurred in all exercise bouts from rest (p<.01). While the running modalities appeared to induce on average a greater increase in sMCAv (by ~10cm/s), although this difference between bouts did not reach significance (p>.14). There was a significant decrease of ~11cm/s for dMCAv between flat running and cycling (p<.05), while the average reduction in dMCAv for uphill and downhill running did not reach significance (p>.05). In contrast, cycling dMCAv was similar to resting values (p=1.0).
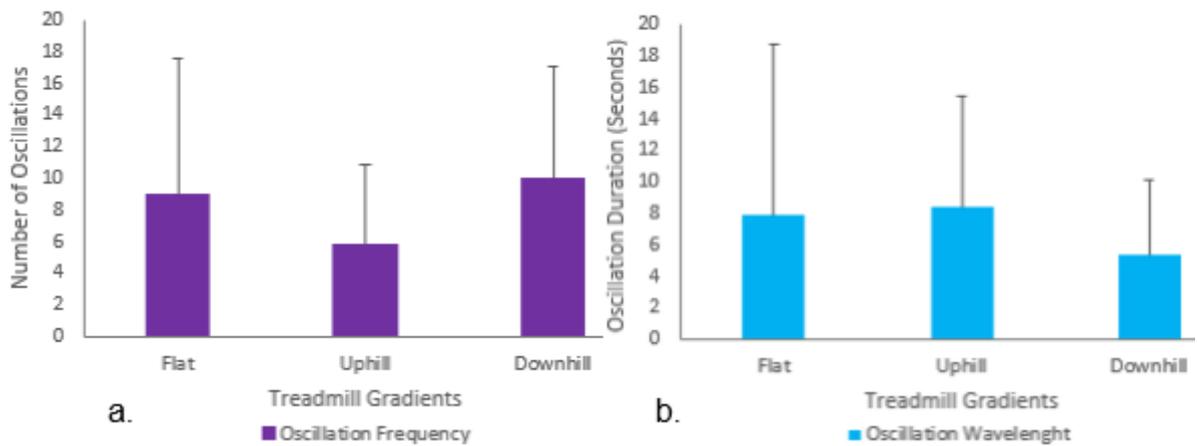
Figure 17: The change in oscillation frequency (a) and oscillation wavelength duration (seconds, b) during flat (1% gradient), uphill (6 to 11% gradient) and downhill (-6 to -11% gradient) running at 65%VO_{2peak} (n=9).

Oscillations were present during each running bout with a variety of oscillatory patterns between participants and gradients. Despite the on average differences between running bouts, oscillation wavelength duration (~7s; p=.61) and number of oscillations (~8; p=.21) was similar between each of the running gradients (see figure 17).
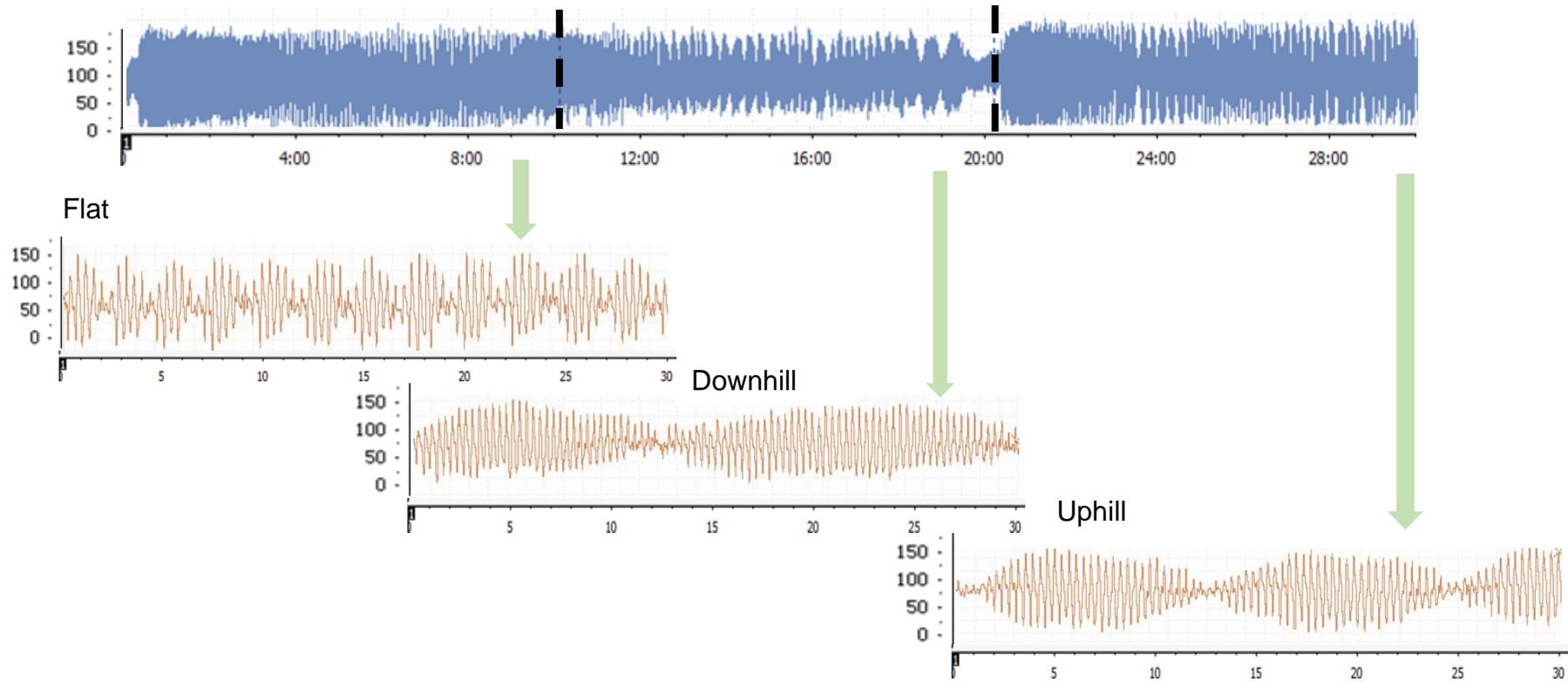
Figure 18: A 30-minute trace followed by a set of 30-second traces of an individual's middle cerebral artery blood velocity (MCAv, cm/s) during flat (1% gradient), uphill (6% gradient) and downhill (-6% gradient) running at 65%VO_{2peak}.

Figure 18 demonstrates how the oscillation frequency and wavelength duration do not remain uniform within and between each of the gradients. The flat running 30-second trace portrays 12 oscillations, which decreases to two full oscillations during 30-seconds of uphill and downhill running. The largest range of wavelength duration within a gradient occurred during uphill running of ~38 s and decreased during downhill running to ~14 s and ~11 s in flat running. The number of oscillations within the last 30 s in both uphill (down by 9) and downhill (down by 10) running decreased, when compared to flat running. However, an increase in wavelength duration occurred from flat (up by ~23 s and ~9 s, respectively). Overall, the oscillatory pattern within the MCAv varied between the participants in relation to the treadmill gradient. An exercise-induced increase in MCAv was expected to occur during all running and cycling exercise bouts based on previous research (Brugniaux *et al.* 2014). MCAv increased in all exercise bouts from rest, however $PETCO_2$ only significantly increased during flat and uphill running. Additionally, $PETCO_2$ during flat and uphill running was significantly elevated in comparison to downhill running. The lower $PETCO_2$ during downhill running may have been a consequence of the unfamiliar nature of running downhill on a treadmill, since participants anecdotally reported being anxious during this protocol. This anxiety may have resulted in some level of hyperventilation and therefore explain the lowered $PETCO_2$ values for this exercise bout. Nevertheless, muscles have been reported to be 3-5 times more efficient producing negative work during downhill running, in comparison to producing positive work during uphill running (Abbot *et al.* 1952), therefore perhaps the lower $PETCO_2$ reflects lower metabolic demands. In addition, while $PETCO_2$ did not significantly increase during cycling, there was an on average 3 mmHg increase from rest, and

the known sensitivity of CBF to changes in arterial content of CO_2 (1 mmHg change in PCO_2 results in 3-4% increase in CBF, Battisti-Charbonney *et al.* 2011) suggests that any change in PCO_2 will have some impact of MCAv.

The NIRS data revealed that no oscillations were present during the exercise bouts. Therefore, cerebral regulatory mechanisms (e.g. cerebral autoregulation) appeared to regulate the observed pulsatile flow between the middle cerebral artery and the prefrontal cortex to reduce the impact of the fluctuations in pressure on the flow profile. Interestingly, an exercise-induced decrease in prefrontal tissue oxygenation (as index by TOI) occurred during uphill running, which is potentially due to participants increasing their concentration on movement. In support of this, the prefrontal cortex is involved in a variety of tasks involving activities related to cognition and planning of voluntary movement (Miller and Cohen, 2001; Sahyoun *et al.* 2004). This cortex has extensive connection with sensory and motor cortices and therefore is indirectly involved in motor command (Oussaidene *et al.* 2014). As mentioned above, the unfamiliar nature of the downhill treadmill running appeared to impact on participants' behaviour. This may also explain the lower TOI measures due to greater prefrontal engagement in the task as a result of the added concentration required compared to more familiar treadmill running.

In comparison to cycling, a higher PPA was expected to occur during running bouts due to the repetitive foot-strikes, with the largest increase occurring during downhill running due to inducing the greatest heel-strike force. In the current study, the transfer of weight between the feet during running elicited a greater increase in PPA over cycling, a non-weight bearing modality, as hypothesised. However, there was no difference in PPA between the three treadmill gradients. This is potentially due to the

un-familiarisation of gradient running, with individuals constantly changing their

running kinematics and gait patterns during the exercise bouts. An increase in

sMCAv occurred in all exercise bouts, with running causing a greater increase in

sMCAv than with cycling. A decrease in dMCAv from rest was present during flat

running as well as being significantly lower than cycling (shown in figure 16). On

average dMCAv during all the running bouts was 12cm/s lower than during cycling.

Similarly, Palatini *et al.* (1989) reported diastolic BP approached down to -17mmHg

during submaximal running. Whereas during cycling where HRs were comparable,

the lowest diastolic BP value was 40mmHg (Palatini *et al.* 1989). Together, both

findings of decreases in diastole measurements are unique to running and therefore

likely related to the large pressure oscillations induced from the foot-strikes.

However, this negative pressure could represent retrograde flow, where blood

transcends back towards the heart. Lower limb exercise is known to induce

retrograde flow (Green *et al.* 2002). Specifically, when continuous muscle

contractions occur at a fast velocity e.g. during running (Gonzales *et al.* 2008).

Investigations have observed within peripheral vasculature that an increase in

retrograde flow, and thus retrograde shear, results in a decrease of FMD (Thijssen *et al.* 2009). FMD is often used as an indicator of endothelial function as the measure is

largely dependent on NO release (Thijssen *et al.* 2009). Therefore, indicating that an

elevation in retrograde flow has a detrimental impact on endothelial function, which

may potentially extend to the cerebrovasculature. Overall, future investigations

should explore whether the MCAv profile induced from running elicits superior

haemodynamic forces compared to cycling, which may potentially produce enhanced

cerebrovasculature adaptations *via* greater release of neurovascular signalling (e.g. NO, vascular endothelial growth factor *etc*).

All running bouts induced an oscillating beat-to-beat profile, while no oscillations occurred during cycling. The amplitude of the oscillations was expected to decrease during uphill running due to the reduced heel-strike force, however, no statistical difference in the oscillation profile occurred between the three running gradients. To note, the greatest difference in the oscillation profile occurred between uphill and downhill running (shown in figure 17), so potentially the lack of statistical significance may be due to the small number of participants tested. Further, narrow and frequent oscillations were observed during downhill running. This is potentially associated with participants being unaccustomed to downhill treadmill running, which impacted their running style and cadence. Anecdotally, participants reported that they struggled to judge their foot placement on the treadmill, which seemed to shorten stride length and promote more toe-weighted running to act as a braking mechanism as individuals feared their foot was too close to the edge of the treadmill. A different oscillation profile may have developed if this experiment occurred outside and not on a treadmill as typically amateur runners drive their heel into the ground to reduce running speed (Dunne, 2012). Running outside in a natural environment would potentially induce the individual's natural running style and cadence, enabling a true examination of the relationship between gradient running and the oscillation profile. Although field studies are less tightly controlled and introduces portable and wireless technology requirements for measurement of the haemodynamics, the research would produce a greater real-life context (Aziz *et al.* 2017).

Wavelength duration differed between each gradient bout, with durations being ~23 s during flat running, ~26 s during uphill and ~10 s during downhill running. These data potentially indicate that individuals changed their running style or cadence during each gradient bout. Again, contrasting Lyngeraa *et al.* (2013) research that the oscillatory pattern remains relatively constant within an intensity. Of note, for the data presented within this thesis (e.g. figure 17b) an average of the last 3 oscillation wavelengths with each gradient bout was taken from each participant. The use of time and frequency domain analysis is potentially a more sophisticated approach to compare the oscillation profile between and within the different exercise bouts. This approach was used by Lyngeraa and colleagues, although as previously mentioned no variability of the oscillatory pattern was reported within their exercise bouts, and given the variability observed here, this would create potential issues for this analysis approach (Kurths *et al.* 1995).

Entrainment could be another potential explanation behind the oscillation profile, as the greatest increase in oscillation wavelength duration occurred during uphill running. Uphill running is the most metabolically demanding gradient (Synder and Farley, 2011), inducing a greater elevation in HR than either flat or downhill running. In the current study, HR may have been approaching uphill running step rate, resulting in a longer wavelength duration compared to downhill running. Therefore, entrainment between these two rhythms may have determined the oscillation wavelength duration with the heel-strike force influencing the oscillation amplitude. The exercise intensity target of each gradient was 65%VO_{2peak}, however this was challenging to achieve and maintain through alterations of speed and gradient. A trade-off between speed and gradient occurred during uphill running, due to the

increased metabolic demands of uphill running, and speed was often reduced below the participant's natural running pace. During downhill running individuals were often not comfortable to run at their natural running rhythm because of concerns about falling. Therefore, it was very challenging to achieve the intensity target, as muscles are more efficient at producing negative work during downhill running (Abbot *et al*. 1952). These complications impacted the individuals running cadence and ultimately the oscillatory pattern, which therefore limits the comparison of how gradient running influences the MCAv profile.

Rowell *et al.* (1968) observed pressure oscillations occurred within the radial artery and the aorta, albeit at a smaller magnitude during running, which indicates that the heart has to constantly pump against varying pressures during exercise. The diastolic phase of the heart is a passive process, which could potentially be impeded from pressure fluctuations of the mechanical wave resulting from each foot-strike. However, if entrainment occurs between the foot-strikes and the HR this could support the left ventricular ejection of blood, having a positive inotropic effect. Entrainment between these two rhythms has been found to improve cardiac efficiency inducing a 'hydraulic effect' (Phillips and Jin, 2013). Overall, this could result in a greater cardiac output which may transcend into the cerebral circulation supporting CBF and potentially increasing haemodynamic forces (i.e. sheer stress and cyclic strain), inducing a greater stimulus for cerebrovasculature adaptations. $PETCO_2$ was significantly greater during flat and uphill running compared to downhill running, although no difference occurred between the MCAv values. Future investigations could involve the use of $PETCO_2$ clamping, as a precaution to ensure PCO_2 is constant during the three running gradients and not influencing the CBF

oscillatory profile. Further research is essential to explore the influence of gradient running on the CBF and oscillation profile, with consideration of the potential impact this could elicit on the cerebrovasculature and in particular chronic adaptation. This information would increase current understanding of how to manipulate exercise parameters to optimise brain health benefits.

Comparison of flat, toe-weighted and heel-weighted running

Table 6: The mean change within a range of dependant variables from resting baseline during flat, toe and heel-weighted running at a self-selected speed.

Running Style	MCAv (Δcm/s)	PETCO$_2$ (ΔmmHg)	TOI$_{head}$ (Δ%)	THI$_{head}$ (Δau)	TOI$_{leg}$ (Δ%)	THI$_{leg}$ (Δau)	HR (Δbpm)
Flat	17.2±14.7*	5.5±3.2*	-0.7±3.3	0.0±0.1	0.9±4.2	-0.1±0.2	87±22*
Toe	13.5±12.9•	4.7±2.4*	-3.8±6.5	0.5±0.2	-1.7±10.8	-0.1±0.3	86±13*
Heel	17.2±15.5∞	3.4±1.8*	-4.4±7.3	0.8±0.3	-3.2±11.1	-0.1±0.4	89±10*

(Key: MCAv, middle cerebral artery blood velocity; PETCO$_2$, partial pressure of end-tidal carbon dioxide; TOI$_{head}$, tissue oxygen index of prefrontal cortex; THI$_{head}$, tissue haemoglobin index of prefrontal cortex; TOI$_{leg}$, tissue oxygen index of rectus femoris; THI$_{leg}$, tissue haemoglobin index of rectus femoris; HR, heart rate).
Values are means ± SD. n = 9 for MCAv, PETCO$_2$, TOI$_{head}$, THI$_{head}$, TOI$_{leg}$, THI$_{leg}$ and n = 7 for HR.
*significant from rest (p<.05) •change from rest (p=.08) ∞change from rest (p=.06).

Exercise induced a significant increase in PETCO$_2$ during all running styles from rest (p<.01) and in MCAv during flat running (p<.05). No difference occurred in both MCAv and PETCO$_2$ between running styles (p=1.0 and p>.40, respectively). No difference in the change of TOI$_{head}$, THI$_{head}$, TOI$_{leg}$ and THI$_{leg}$ occurred between each of the running styles (p>.05). Finally, HR change from rest was similar between all running styles (p=1.0).
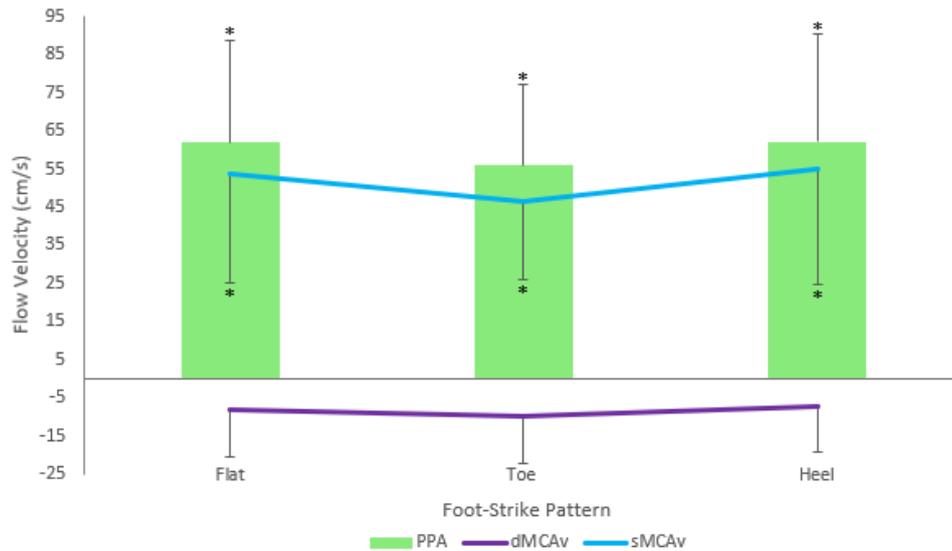
Figure 19: The mean change in pulse pressure amplitude (PPA, cm/s), systole middle cerebral blood artery velocity (sMCAv, cm/s) and diastole middle cerebral artery blood velocity (dMCAv, cm/s) from resting baseline, during flat running, then toe and heel-weighted running at a constant speed (n=9).
*significant from rest (p<.05).

Exercise induced increases in PPA (up by ~60 cm/s; p<.01) and sMCAv (up by

~52cm/s; p<.01) but remained similar between the three running styles (p=1.0).

dMCAv also remained relatively constant between all three running styles (decreased

from rest by ~8 cm/s; p=.07).
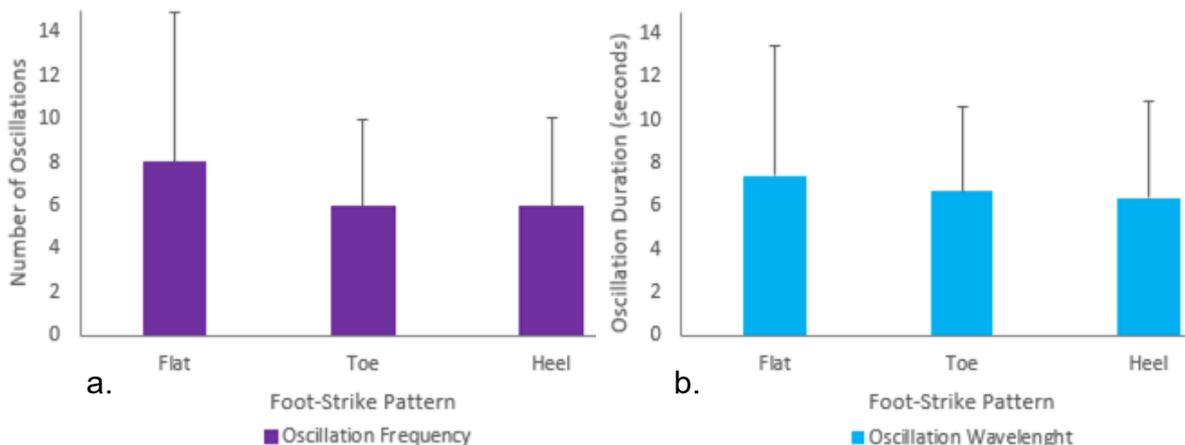
Figure 20: The change in oscillation frequency (a) and oscillation wavelength duration (seconds, b) during flat running, then toe and heel-weighted running at a constant speed (n=9).
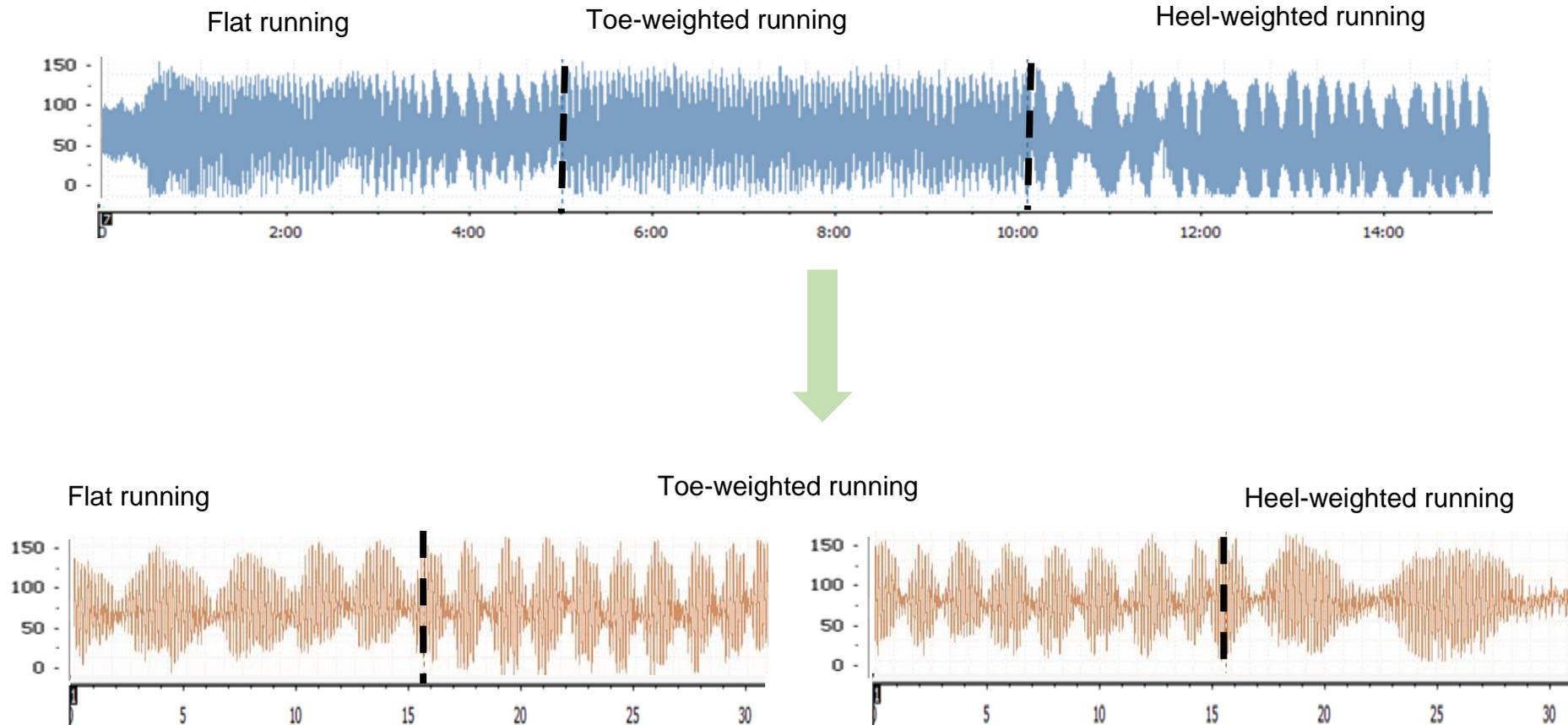
Figure 21: A 15-minute continuous trace followed by a set of 30-second traces individual's middle cerebral artery blood velocity (MCAv, cm/s) during flat, toe and heel-weighted running at a of 8.7km/h.

Figure 21 demonstrates the oscillatory pattern changes with the foot-strike pattern in one individual. This 15-minute trace shows that the oscillation wavelength instantly decreased while oscillation frequency increased when changing from flat to toe-weighted running. Thereafter, during heel-weighted running an immediate increase in oscillation wavelength occurred, resulting in a decrease of oscillation frequency. The first 30 s trace depicts 4 full oscillations within flat running, which instantaneously increased to 8 full oscillations within the first 15 s of toe-weighted running. Subsequently, the oscillation wavelength increased at the onset of heel-weighted running, oscillations decreased from 7 full to 2 full oscillations when moving from toe-weighted to heel-weighted running (represented in the second 30-second trace as well as in the 15-minute MCAv trace). Overall, the oscillatory pattern within the MCAv varied between the participants in relation to the different exercise bouts. Therefore, no change is apparent in the mean oscillatory pattern data.

A similar exercise-induced increase in MCAv and $PETCO_2$ occurred during all running styles. This was expected based on current literature, evidencing that individuals were exercising below their anaerobic threshold (Brugniaux *et al.* 2014). An exercise-induced increase also occurred in sMCAv for each running style resulting in an elevation of PPA, whilst dMCAv remained relatively constant. These data agree with the arterial BP data presented by Palatini *et al.* (1989). The running technique of an individual has been shown to influence the amplitude of pressure fluctuations (Palatini *et al.* 1989). PPA during heel-weighted running was expected to increase above toe-weighted running as a result of an increase in the heel-strike force. However, this was not observed here, potentially due to individuals being unaccustomed to heel-weighted running as well as treadmill running. Furthermore, it

was noted that individuals reverted back to their normal running style when they appeared to lose concentration, and this may have impacted the cerebral haemodynamic data recorded here. Nevertheless, PPA and sMCAv during toe-weighted running was slightly lower than during heel-weighted and flat running, which may relate to a reduced heel-strike force. Further research is needed to confirm or refute this hypothesis.

At the tissue level, both head and legs NIRS measures showed no oscillations within or between the different exercise bouts. There was an on average reduction in TOI_{head} for toe and heel-weighted running, which may reflect the greater concentration (and therefore prefrontal engagement) required for these running styles in comparison to flat running. The largest decrease in TOI_{leg} occurred during heel-weighted running, which potentially reflects a greater push-off force needed (and therefore greater muscle activation and metabolic need) to ensure a heavy landing on the heel. Nevertheless, no significant differences were evident, so more research and a larger participant sample is needed to further explore these potential causes. Interestingly, TOI_{head} during flat running of this protocol was a lot closer to resting values then during the gradient protocol. However, it is unclear why this occurred although potentially due to probe movement and placement. More research is required to confirm this data.

Heel-weighted running was hypothesised to induce an increase in the amplitude of the oscillations, in comparison to flat running as a result of an increase in heel-strike force, where the opposite was expected for the toe-weighted running due to the reduced heel-strike force. Oscillations were present within each of the three running styles, with a change in the oscillatory pattern occurring between the running styles.

However, no overall statistical difference in the oscillation pattern between the running styles was evident. As mentioned, individuals often required reminding to maintain toe or heel-weighted running, which seems likely to have had an influence on the oscillatory pattern (particularly its stability within a running style). Additional measures such as the use of ground force reaction plates would have helped examined this relationship between the oscillation profile and the heel-strike force. Finally, while figure 20 presents the mean oscillation wavelength duration and frequency responses for the final 30 s of each running style, it does not accurately represent the exercise bout where most participants oscillation wavelength duration and frequency peaked. Specifically, a total of 3 individual's wavelength duration peaked during toe-weighted running, and a further 3 during heel-weighted running. Therefore only 2 participants wavelength duration, peaked during flat running. However, these 2 individuals both had an exceptionally large average wavelength duration, of ~10 s above the average wavelength duration in each of the running bouts. Additionally, 3 individual's oscillation frequency peaked during heel-weighted running, as well as 3 participants during flat running with only 1 participant's maximal oscillation frequency occurring during toe-weight running. Of note, 3 participants were excluded from the above analysis as their oscillation wavelength duration and/or frequency were the same during two or all gradients. Overall, this is not reflected in the means of both measures. Therefore, this analytical approach may have been too simplistic to analyse the oscillatory pattern between the different running styles. The use of frequency domain analysis could be used in future investigations to explore the influence of the beat phenomenon on MCAv oscillations by measuring HR and step frequency. This would enable a more sophisticated

approach to examining the oscillation profile. However, this type of analysis is often

not sufficient to characterise complex fluctuations patterns in data (Kurths *et al.*

1995).

Comparison of a change in oscillatory pattern during running at a constant

speed at different step frequency
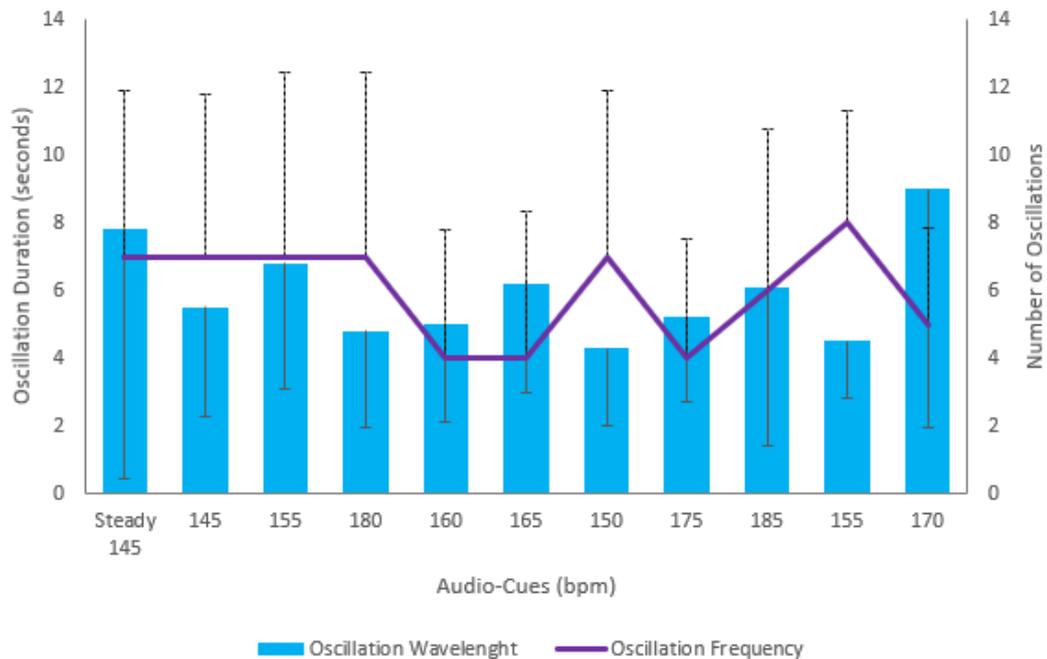
Figure 22: The change in oscillation frequency and oscillation wavelength duration
(seconds) when running at a constant speed with different step frequencies (n=9).

There was no significant difference in wavelength duration (p=.39) and oscillation

frequency (p=.26) with different step frequencies. An average of 6 oscillations

occurred across all step frequencies and ~6 s in wavelength durations (see figure

22). However, similar to the other conditions tested here, despite the lack of group

effects, there was some variation in the oscillation profiles within participants and

especially when changing step frequency (see figure 23 below).
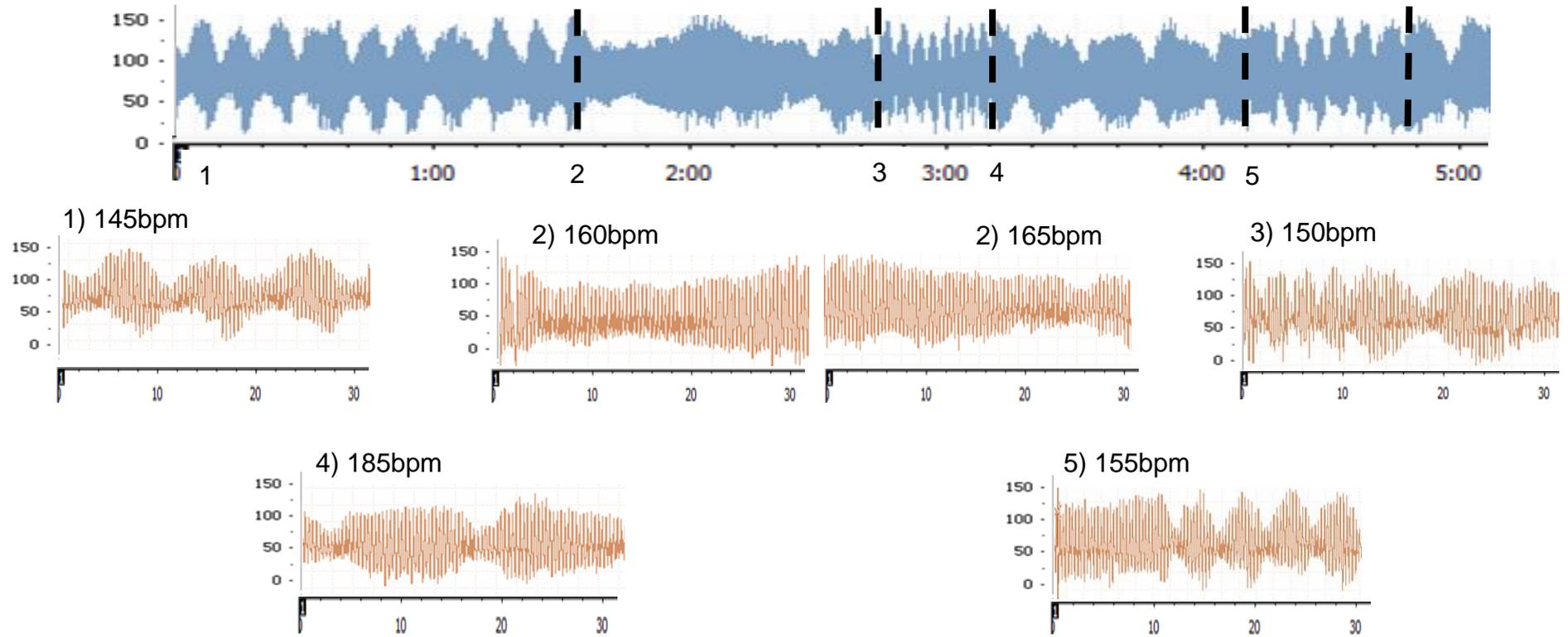
Figure 23: A 5-minute continuous trace followed by six 30-second traces of an individual's middle cerebral artery blood velocity (MCA, cm/s) profile during a variety of different step frequencies.

Figure 23 demonstrates that a change in step frequency has an impact on the oscillatory pattern within MCAv. However, a carryover effect is present, for example within 160bpm and 165bpm frequencies both traces consist of the same oscillation, with a large wavelength duration of 52 s. The 5-minute trace above consists of 10 different audio-cue step frequencies, although only 5 different oscillation patterns appear to occur (represented by the dash lines). These traces demonstrate that a clear change in the oscillatory pattern occurs throughout the 5-minute exercise bout although it does not coincide with the change in audio-cue step frequency. In addition, the set of 30 s traces above display a difference in oscillation appearance and frequency between the various audio-cues. Overall, the oscillatory pattern within the MCAv changed with differing step frequencies throughout the duration of the exercise bout for all participants.

The oscillatory pattern changed with differing audio-cue step frequencies in all individuals, indicating a direct impact of the step frequency on the CBF oscillatory pattern. However, due to the short duration of the audio-cue rhythms it was difficult to observe and report changes. The analysis of wavelength duration consisted of an average duration of the last 3 oscillations within an audio-cue bout. However, large wavelength durations were observed to occur over 1-to-2 audio-cue bouts and therefore any potential effect was missed as a consequence for the analysis approach applied here. Additionally, the audio-cue bouts may have been too short in duration for the participants to adjust to the audio-cue rhythm and then reach a steady state. The audio-cue rhythms were an exploratory part of the study, to observe whether a change in step frequency would impact the MCAv oscillations. Future investigations are required to continue to explore and quantify the impact of

step frequency on the oscillatory pattern. This can be achieved through increasing the duration of the exercise bouts, allowing a comparison of the oscillatory pattern between different audio-cue step frequencies, alongside enabling participants to adjust their running cadence and reach steady state.

The examination of HR and step rates could potentially reveal whether entrainment occurred during the audio-cue bouts. In future research a metronome could be used to explore this relationship, set at the participants' HR for individuals to run to and test whether inducing entrainment between the HR and step rate can be facilitated experimentally. A more advanced alternative followed by Takeuchi et al. (2014) involved participants wearing an ECG monitor that sounded with a buzzer with an occurrence of each R wave, participants then ran to the sound of the buzzer. Entrainment has not been explored in terms of cerebrovasculature benefits. More research is required to discover whether entrainment between these two rhythms induces a greater stimulus for cerebrovasculature adaptations in comparison to the rhythms opposing each other resulting in more frequent and narrow oscillations.
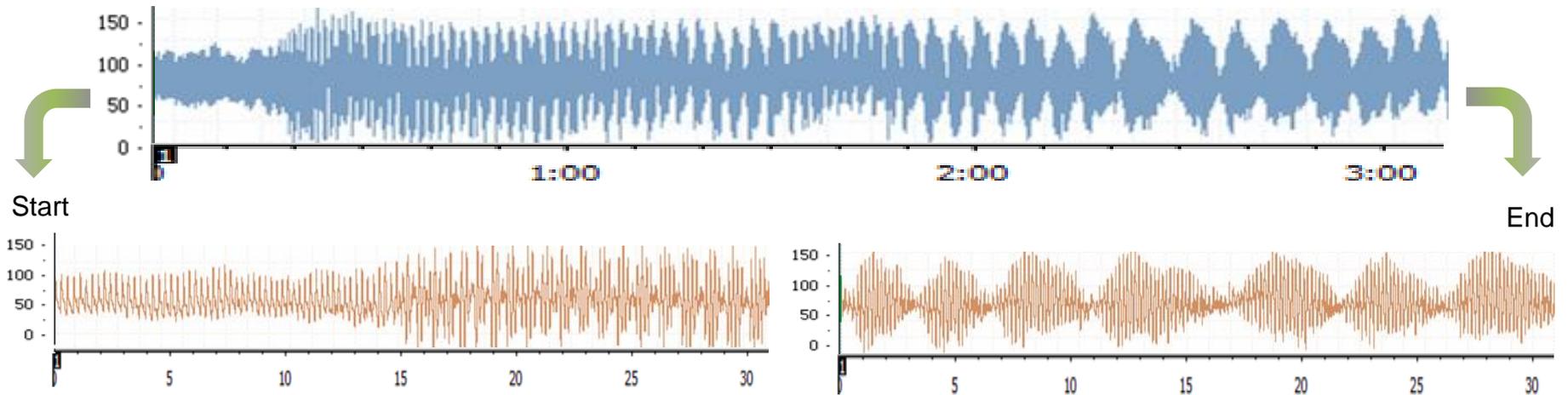
Figure 24: A 3-minute continuous trace of an individual's middle cerebral artery blood velocity (MCAv, cm/s) profile during a flat running bout at 65%VO_{2peak} and a constant step frequency.

Figure 24 is a representative trace of how oscillations develop within MCAv during flat treadmill running at a constant step frequency. The 3-minute trace depicts how the oscillation wavelength duration increases throughout the running bout. The set of 30-second traces clearly show the change in the oscillatory pattern from the start of the exercise bout in comparison to the end, with a difference of ~11 s in wavelength duration. Upon commencement no oscillations occurred (displayed as the first ~15 s). The oscillations then begin to develop but appear extremely narrow and hard to distinguish between ~15-20 s. Thereafter the oscillations become increasing more apparent throughout the remaining duration of the bout, as the oscillation wavelengths elongate. This is evidently displayed in the final 30 s trace depicting 7 clear oscillations. The set of continuous traces represent the general pattern observed within MCAv at the onset of treadmill running within all individuals.

METHODOLOGY CONSIDERATIONS

TCD was used as an index of flow, which makes the assumption that velocity represents flow. This is only a valid measurement if the calibre of the insonated vessel remains constant (Willie *et al*. 2011). A high signal quality during exercise was challenging to maintain, due to probes sensitivity towards movement. Additionally, the prefrontal cortex NIRS probes often impacted the signal quality of the TCD. This investigation did not directly measure HR or step frequency therefore the impact of these two rhythms on the oscillatory pattern or the relationship between the frequencies could not be examined.

A trade-off between speed and gradient occurred during uphill running within the gradient protocol. This often led to individuals running at a slower pace than their natural running rhythm and at a shallow gradient in order to achieve 65%VO_{2peak}. Additionally, to achieve this intensity during downhill running was challenging, as individuals were too nervous either to run at their natural running speed, or at a steep gradient as previously mentioned. A downhill familiarisation session would have increased the participant's confidence, and potentially enhanced an individual's natural running cadence during this protocol. As previously mentioned, the audio-cue durations were too short to enable a steady state adjustment to the rhythms and to investigate differences between the oscillatory pattern. Several participants found it difficult to adjust to the different audio-cue rhythms at a set speed of the treadmill. This would have not been an issue if this protocol occurred in a more natural environment that did not involve a treadmill. Individuals tend to run at a speed that is comfortable for them, however for participants unaccustomed to a treadmill it can be difficult to adjust to running at a set cadence. Additionally, a standardisation of shoes

could have occurred as different types of shoes are evidenced to impact foot-striking patterns and O_2 consumption.

Future investigations require a measurement of arterial BP, permitting an observation of whether pressure fluctuations in the peripheral vasculature transcend into the cerebral circulation, as Lyngeraa *et al.* (2013) documented. This would allow for a comparison of oscillation profiles between both vasculature beds. A transducer could be used as seen in Palatini *et al.* (1989) study alongside the TCD, enabling the observation of how running at different gradients impacts the mechanical and aortic waves and the overall CBF oscillatory pattern. Moreover, a treadmill with ground force plates would permit the analysis of the heel-strike force during different running conditions, enabling a comparison between whether peak heel-strike force coincides with peak PPA within CBF. Together potentially clarifying that an increase in heel-strike force is associated with an increase in haemodynamic forces inducing a greater stimulus for cerebrovasculature adaptations. The use of timing gates and a pedometer would have allowed step frequency to be quantified, to establish whether entrainment occurred with HR and had an influence over the oscillatory pattern. Phillips and Jin, 2013 used a novel iphone application which wirelessly connection to a heart monitor calculating a running pace, playing a 'tick tock' sound through earphones for the participants to run too, inducing entrainment between the two rhythms. A range of innovative entrainment-inducing methodology exists which can be used in and outside of the lab. The investigation of entrainment would improve the knowledge of the underlying mechanisms of how this unique oscillatory pattern is generated during running in CBF.

The use of two VO$_{2peak}$ tests with differing protocols (e.g., intensity is increased *via* speed vs. gradient) would allow observation of how the heel-strike force potentially influences oscillatory profile changes across different exercise intensities. The change of gradient may have a greater influence on the amplitude of the oscillations. In contrast, increasing the step rate may result in entrainment with HR, influencing the oscillation wavelength duration.

Unfortunately, the investigation did not involve the examination of sex differences between males and females and the mensural cycle was not taken into consideration. This was the result of time restrictions and would have required a larger cohort to enable formal examinations. However, sex hormones i.e. estradiol and progesterone both have vasoactive effects and levels fluctuate during follicular and luteal phases of the menstrual cycle. The changes in endogenous levels have been evidenced to have an impact on carotid arteries. In the follicular phase peak estrogen concentration has been reported with peak ICA blood flow velocity and inversely with ECA blood flow velocity returning towards baseline values (Krejza et al. 2001). Therefore, this may have a small impact on CBF response within females and resultant adaptations, which future research is required to explore.

CONCLUSION

In summary, running induces rhythmic oscillations in perfusion pressure which transcend into the cerebrovasculature, but do not transcend down the arterial tree towards the skin. The oscillatory pattern during running is associated with an individual's heel-strike force, step rate and HR. Further research is required to establish the relationship between these variables and how to manipulate them to induce the most beneficial effect on the cerebrovasculature. A multi-model approach

of intensities and modalities is required including considering an individual's fitness level in order to promote optimal brain health benefits. This should involve exploring the impact of flat, uphill and downhill running on PPA and the oscillatory pattern. Also, measurement of a neurotrophic factors (e.g. NO bioavailability) would enable quantification of which exercise parameters induces the greatest stimulus for adaptations.

APPENDICES

Appendix 1: Application for Ethical Review

UNIVERSITY OF BIRMINGHAM
APPLICATION FOR ETHICAL REVIEW

Who should use this form:

This form is to be completed by PIs or supervisors (for PGR student research) who have completed the University of Birmingham's Ethical Review of Research Self Assessment Form (SAF) and have decided that further ethical review and approval is required before the commencement of a given Research Project.

Please be aware that all new research projects undertaken by postgraduate research (PGR) students first registered as from 1st September 2008 will be subject to the University's Ethical Review Process. PGR students first registered before 1st September 2008 should refer to their Department/School/College for further advice.

Researchers in the following categories are to use this form:

1. The project is to be conducted by:
   - staff of the University of Birmingham; or
   - postgraduate research (PGR) students enrolled at the University of Birmingham (to be completed by the student's supervisor);
2. The project is to be conducted at the University of Birmingham by visiting researchers.

Students undertaking undergraduate projects and taught postgraduate (PGT) students should refer to their Department/School for advice.

NOTES:

➤ An electronic version of the completed form should be submitted to the Research Ethics Officer, at the following email address: aer-ethics@contacts.bham.ac.uk. Please **do not** submit paper copies.
➤ If, in any section, you find that you have insufficient space, or you wish to supply additional material not specifically requested by the form, please it in a separate file, clearly marked and attached to the submission email.
➤ If you have any queries about the form, please address them to the Research Ethics Team.

☐ **Before submitting, please tick this box to confirm that you have consulted and understood the following information and guidance and that you have taken it into account when completing your application:**

- **The information and guidance provided on the University's ethics webpages (https://intranet.birmingham.ac.uk/finance/accounting/Research-Support-Group/Research-Ethics/Ethical-Review-of-Research.aspx)**

- **The University's Code of Practice for Research (http://www.as.bham.ac.uk/legislation/docs/COP_Research.pdf)**

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UNIVERSITY OF BIRMINGHAM APPLICATION FOR ETHICAL REVIEW	OFFICE USE ONLY: Application No: Date Received:

1. TITLE OF PROJECT

Investigating the interaction between exercise mode and exercise intensity on brain vascular health.

2. THIS PROJECT IS:

University of Birmingham Staff Research project ☐
University of Birmingham Postgraduate Research (PGR) Student project ☒
Other ☐ (Please specify):

3. INVESTIGATORS

a) **PLEASE GIVE DETAILS OF THE PRINCIPAL INVESTIGATORS OR SUPERVISORS (FOR PGR STUDENT PROJECTS)**

Name: Title / first name / family name	Dr Samuel J.E. Lucas
Highest qualification & position held:	PhD, Senior Lecturer
School/Department	School of Sport, Exercise and Rehabilitation Sciences
Telephone:	+44 (0)121 414 7272
Email address:	▮▮▮▮▮▮▮▮▮▮

Name: Title / first name / family name	Dr Rebekah Lucas
Highest qualification & position held:	PhD, Lecturer
School/Department	School of Sport, Exercise and Rehabilitation Sciences
Telephone:	+44 (0)121 414 8748
Email address:	▮▮▮▮▮▮▮▮▮▮

b) **PLEASE GIVE DETAILS OF ANY CO-INVESTIGATORS OR CO-SUPERVISORS (FOR PGR STUDENT PROJECTS)**

Name: Title / first name / family name	Ms Claire Burley
Highest qualification & position held:	MSc; RA
School/Department	School of Sport, Exercise and Rehabilitation Sciences
Telephone:	
Email address:	▮▮▮▮▮▮▮▮▮▮

c) **In the case of PGR student projects, please give details of the student**

Name of student:	▮▮▮▮▮▮▮	Student No:	▮▮▮▮▮
Course of study:	MSc (by research)	Email address:	▮▮▮▮▮▮▮
Principal supervisor:	Dr Samuel Lucas		

Name of student:	Gabriella Imi	Student No:	▮▮▮▮▮
Course of study:	MSc (by research)	Email address:	▮▮▮▮▮▮▮
Principal supervisor:	Dr Samuel Lucas		

Name of student:	TBC	Student No:	
Course of study:	PhD	Email address:	
Principal supervisor:	Dr Samuel Lucas		

4. ESTIMATED START OF PROJECT   Date: December 1st 2017

     ESTIMATED END OF PROJECT   Date: September 1st 2021

5. FUNDING

List the funding sources (including internal sources) and give the status of each source.

Funding Body	Approved/Pending /To be submitted
Funds allocated to Masters projects / internal School funds and PGR bench fees	

If you are requesting a quick turnaround on your application, please explain the reasons below (including funding-related deadlines). You should be aware that whilst effort will be made in cases of genuine urgency, it will not always be possible for the Ethics Committees to meet such requests.

The initial study within this project is for MSc (by Research) students. These students have 1 year to complete their project, so being able to begin piloting and data collection as soon as possible is essential to ensure that they can submit their dissertation within the 1-year period (due 21/09/18).

6. SUMMARY OF PROJECT

Describe the purpose, background rationale for the proposed project, as well as the hypotheses/research questions to be examined and expected outcomes. This description should be in everyday language that is free from jargon. Please explain any technical terms or discipline-specific phrases.

Regular exercise enhances brain blood vasculature volume and flow, which has been associated with reducing the risk of developing neurodegenerative diseases such as dementia. Thus, exercise provides significant benefits to brain vascular structure and function. However, despite the extensive literature displaying the positive benefits exercise has on the brain (including other parameters of 'brain health'), there is limited understanding about *how* exercise (i.e., the underlying mechanisms) mediates the positive changes in the human brain. Shear stress and cyclic strain have been proposed as key mechanisms responsible for the vascular adaptations that occur in the brain from regular exercise; yet there are no studies to date examining whether there are differences between exercise intensities or exercise modalities for these adaptation stimuli, and thereby determining which form of exercise may produce the greatest stimulus for brain vascular adaptation.

Shear stress refers to the act of blood flow exerting a frictional force on the inner wall of a blood vessel and occurs during exercise due to the increased blood flow that is associated with undertaking strenuous physical activity (e.g. exercise training). Stimulation of acute shear stress (i.e. from a single bout) results in flow-mediated vasodilation and positive vascular adaptation, whereas chronic shear stress (i.e. repeated stimulus from weeks of training) is associated with positive structural changes as well as functional benefits; these include improved vascular responsiveness and vessel density. The key mechanism linked to shear stress's positive effects on the vasculature is the release of nitric oxide (NO) following upregulation of the enzyme endothelial nitric oxide synthase (eNOS). NO is a potent vasodilator that plays an important role in vascular remodelling and increased blood flow. It has been identified by Bolduc et al. (2013) as a likely contributor to the beneficial vascular adaptations within the brain that occur through exercise, yet remains largely untested within the human cerebrovasculature. Nevertheless, Tinken et al. (2010) have demonstrated that handgrip exercise-induced shear stress leads to an increase in flow-mediated dilation as well as positive remodelling of the vasculature within the brachial artery. These findings have positive implications for the role of shear stress and NO release in eliciting beneficial vascular adaptations for the brain. However, due to the complexity of the brain and the unique regulation of blood flow within it relative to the systemic circulation (especially related to the influence of carbon dioxide), specific investigation is required to demonstrate their roles within the cerebrovasculature.

Exercise-induced cyclic strain also elicits an increase in NO bioavailability via the upregulation of eNOS. The mechanism eliciting an endothelial response and the subsequent increase in NO through cyclic strain differs to that of shear stress. Cyclic strain refers to the shape and structure of the endothelium being manipulated by blood pressure-induced loading of the vessel wall. However, to our knowledge no research to date has directly tested the influence of different exercise parameters on cyclic strain. Consequently, how different exercise modalities or intensities influence cyclic strain's role in increasing NO bioavailability within the cerebrovasculature is unknown.

Against this background, given that shear stress and cyclic strain are the suggested mechanisms mediating brain vascular adaptations and both are linked to exercise-induced changes in brain blood

flow, it would be logical to assume that whichever exercise intensity or mode elicits the greatest increase in brain blood flow during exercise will also evoke the greatest stimulation of shear stress and cyclic strain (and thus NO release). Interestingly, brain blood flow during exercise is typically described as peaking at ~65% of maximum effort, which therefore may represent the 'optimal' exercise intensity for the shear stress and cyclic strain stimulus. The blood flow profile appears coupled to the changes in carbon dioxide content in the blood, also peaking at this intensity in line with the anaerobic threshold. However, these typically reported profiles of brain blood flow and carbon dioxide concentration have been established from cycling-based studies only, and different blood flow profiles for other exercise modalities (e.g. running and rowing) have recently been reported, which may influence this peak response and thus have implications for the optimal intensity between exercise modes. No study to date has formally compared the profiles of brain blood flow and carbon dioxide between different exercise modalities across a range of exercise intensities.

High intensity interval training (HIIT) exercise strategies have increased in popularity within the public sector, supported by research demonstrating superior metabolic, cardiorespiratory and systemic vascular benefits compared to the traditionally advocated exercise training model (30-minutes of moderate-intensity exercise (~65% of maximum effort) performed five times per week). Despite its increasing popularity and health promotion, the influence of HIIT on the brain remains largely unknown (Lucas et al. 2015). A recent study by Curtelin et al. (2017) reported that brain blood flow during a 30-second all-out cycling bout significantly decreased brain blood flow in comparison to rest. Based on these recent observations, all-out exercise (one promoted form of HIIT) may not be an effective way of increasing brain blood flow and thus, improving cerebrovascular health via shear stress, cyclic strain and upregulation of NO. The Curtelin et al. study did not measure blood NO content, so how a single bout of all-out HIIT influences this mediator of vascular adaptation is not clear, and given the complex interaction between changes in carbon dioxide (lower) and blood pressure (higher) that will influence brain blood vessel calibre and also drive shear and cyclic strain forces during HIIT exercise, it may not be as simple as an assumption that NO release will be optimal at the highest absolute brain blood flow. Furthermore, other forms of HIIT have also been promoted, most notably a 'clinical model' that promotes a more moderate intensity (~85% of maximum effort) for application in clinical populations, and which has shown similar superior positive metabolic, cardiorespiratory and systemic vascular benefits for less time commitment than the traditional exercise training model (Weston et al. 2013). How this clinical model of HIIT influences brain blood flow and the adaptive stimuli for vascular adaptation has not been tested, nor has there been a comparison of brain blood flow profiles between different exercise modalities during any HIIT exercise strategy.

Finally, running is associated with a 'beat phenomenon' that occurs due to the mechanical impact of the foot strike during the running gait pattern that shocks the blood vessels, generating a pressure wave that combines with arterial pressure waves and induces periodic pulse pressure oscillations (Palaini et al., 1989). These BP fluctuations are transmitted into brain blood flow oscillations, which appear to differ with changing exercise intensity (at least up to 75% of maximum; Lyngeraa et al., 2013). Furthermore, we have previously observed that uphill running on a treadmill reduces the magnitude and frequency of these oscillations, which may be related to the reduced heel strike force as runners typically become more toe weighted during uphill running. How these changing oscillations impact on shear stress and cyclic strain are unknown. A simple way to explore this question is to compare uphill and downhill running, through which we can reduce or enhance the heel strike force and consequently the magnitude and frequency of the pulse pressure oscillations and resultant brain blood flow oscillations.

Therefore, the overall aim of this project is to examine the effects of differing exercise modalities and intensities on brain blood flow and neurovascular adaptive signalling factors (e.g. NO, VEGF, IGF-1, BDNF). **Three separate studies** will be conducted to address this overall aim. **First**, we will profile brain blood flow and carbon dioxide responses across the full range of exercise intensity (including the clinical HIIT model) between cycling and running. **Second**, we will determine brain blood flow profiles and the resultant neurovascular adaptive signalling factors from three distinct exercise intensities that are linked to current physical activity health promotion; the traditional model (65% of maximum effort) and two promoted models of HIIT (clinical HIIT and all-out HIIT). **Third**, we will explore how changing the heel strike force and pattern during running affects the pulse pressure oscillations and what effect this has on brain blood flow.

Expected Outcomes and Implications: Findings from this work will improve our understanding of how exercise mediates improved brain vascular health. Specifically, the quantification of the different blood flow profiles and resultant release of neurovascular adaptive signalling factors induced via shear stress and cyclic strain forces that occur between the different exercise modalities and intensities (including HIIT) will help us better understand the how to optimise exercise for improved brain health.

7. **CONDUCT OF PROJECT**

Please give a description of the research methodology that will be used

The objectives for this project will be delivered via three separate but inter-related studies. **Study one** will directly compare the brain blood flow response during incremental cycling and running exercise, as well as during HIIT exercise for both exercise modalities. **Study two** will quantify brain blood flow profiles and the resultant neurovascular adaptive signalling factors from three distinct exercise intensities; 65% of maximum effort and two promoted models of HIIT (clinical HIIT and all-out HIIT). **Study three** will examine how changing the heel strike force and pattern during running affects the pulse pressure oscillations and brain blood flow.

Experimental approach

For each study 20 participants (aged 18-45) will take part in a randomised, crossover designed study. Interested participants will receive an information pack outlining each study prior to an initial screening visit (**visit 1**) where they will be asked to complete a General Health Questionnaire and a Physical Activity Questionnaire (see attached documents), and select the study they would like to volunteer for (i.e. study 1,2 or 3). The selected study will be explained in full detail and the participants will have the opportunity to ask any questions that they may have. If participants satisfy the inclusion criteria (detailed below) and are willing to take part, they will complete a consent form (attached) and will be scheduled for the experimental exercise sessions that correspond to the study they have volunteered for (detailed below); the first of which will occur at least 48 hours following this initial visit to allow volunteers additional time to consider their participation in the study. This initial session will also be used as a screening for the quality of their Doppler signal. If their acoustic Doppler window is poor, they will take no further part in the study from this point. During the initial visit participants will be informed that they are free to withdraw from the study at any time, and will be reminded of this at the start of the each testing session.

Specific details of each study:
Study one: Investigating the interaction between exercise mode and exercise intensity on cerebral blood flow during exercise

Once screened and recruited into the study, participants will be required to visit the laboratory **six times**. The first two visits will be for a cycling and a running VO_{2peak} test, completed in separate sessions at least 48 hours apart. Following these two visits, participants will be required to attend four exercise experimental sessions, the order of which will be randomised and counter-balanced across participants between the cycling and running incremental and HIIT protocols (detailed below). All running exercise sessions will be completed on the same motorised treadmill and all cycling exercise sessions will be carried out on the same cycle ergometer. Exercise sessions will take place in an exercise laboratory within SportExR depending on availability and convenience for the participants. Each visit will last approximately 1 hour, of which approximately 30 minutes will be exercise. There will be at least 48 hours between these sessions, but ideally no more than 2 weeks. Female participants will be able to participant at any time across their menstrual cycle.

Study one experimental protocols and measures
Maximal VO_{2peak} protocol (visits 2 and 3)
Treadmill protocol: Baseline resting measures will be collected before the start of the incremental test. The test will begin with a 5-minute warm-up, during which the treadmill belt speed will start at 7 km/h and will be increased throughout the warm up to achieve a rating of perceived exertion (RPE) (Borg, 1982) score of 11. Stretching time will then be offered to participants before a standardised ramp aerobic capacity protocol is initiated and completed. This involves increasing the treadmill belt speed 1 km/h every 3 minutes until a comfortable but challenging speed is reached, then increasing the gradient by 1% every 3 minutes until exhaustion or a RPE score of 20. Following the completion of the incremental test, participants will complete a 2-minute cool down at walking speed (5 km/h) before the test is terminated, the equipment is removed and the participant is free to leave. During this protocol, participants will be fitted with equipment to measure heart rate (HR; Polar monitor) and respiratory gases and volume (Vyntus™ CPX metabolic cart), to determine peak HR and VO_2 values, respectively.

Cycle protocol: Following completion of resting measurements, a 5-minute warm-up will take place at a self-selected cadence deemed comfortable by the participant at a low resistance (60 W). Resistance will be gradually increased upon the participant's request or to achieve a RPE score of 11; the option to stretch will then be offered to the participant before the test begins. The incremental aerobic capacity test entails a ramp protocol whereby the load increases by 20-35W (depended on

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body mass and perceived fitness) every 3 minutes until volitional exhaustion, a RPE score of 20 or when the cadence drops below 50 rev·min^{-1}. Cadence will be self-selected, in the range of 60-90 rev·min^{-1}. Following the incremental test's completion, a 2-minute cool down shall be completed at a low resistance (50-65 W) before the test is terminated, the equipment is removed and the participant is free to leave. During this protocol, participants will be fitted with equipment to measure HR (Polar monitor) and respiratory gases and volume (Vyntus^{TM} CPX metabolic cart), to determine peak HR and VO$_2$ values, respectively.

Exercise experimental testing (visits 4-7)
For all these visits, participants' willingness to continue in the study will be confirmed upon arrival to the laboratory, then baseline measures of body mass and hydration status (urine osmolarity, urine sample to be discarded immediately and not stored) will be obtained. Participants will then be fitted with equipment to measure brain blood flow (Transcranial Doppler), HR (Polar monitor) and respiratory gases and volume (Vyntus^{TM} CPX metabolic cart), and resting measures will be obtained in a seated then exercising-specific (seated on bike or standing on treadmill) posture before beginning the exercise test protocol. Resting and exercising blood pressure measures will also be obtained via an automated stress-testing blood pressure monitor (Tango, SunTech Medical) during both cycling protocols, and where possible for running protocols (our previous work has shown this is possible for some participants that have relatively small arm movements when running).

Incremental cycling protocol: This exercise test will commence at an initial rate of 60 W and participants will be asked to maintain a constant cadence of between 60-70 rev·min^{-1}. This will be maintained for 3 minutes in order to allow participants to adequately warm up. Following the warm up, the resistance load will be increased by an appropriate amount to achieve 35, 50, 65, 80 and 95% VO$_{2peak}$ (determined from exercise mode-specific maximal test), with each incremental step maintained for 3 minutes once target VO$_{2peak}$ is reached. After the final 3-minute stage (95% VO$_{2peak}$), participants will complete a ~2-minute cool down at 60 W before the exercise test is terminated, equipment removed and they are free to leave the laboratory.

Incremental treadmill running protocol: The treadmill test will begin with a 5-minute warm up at a self-selected pace. Following the warm up, the treadmill belt speed will be increased by an appropriate amount to achieve 35, 50, 65, 80 and 95% VO$_{2peak}$ (determined from exercise mode-specific maximal test), with each incremental step maintained for 3 minutes once target VO$_{2peak}$ is reached. Treadmill gradient will be maintained constant (at 2%) throughout. After the final 3-minute stage (95% VO$_{2peak}$), participants will complete a ~2-minute cool down (light jogging/walking) before the exercise test is terminated, equipment removed and they are free to leave the laboratory.

HIIT cycling protocol: The HIIT protocols are based on that described by Weston *et al.*, 2014; the 'clinical HIIT' model. Participants will begin with a 10-min warm up at 60% HR peak (as determined from maximal test). Participants will then perform 4x4-minute intervals at 90% HR peak, separated by 3 minutes of active recovery (~50 W of light intensity cycling). After the final effort interval, participants will complete a 5-minute cool down at 50% of HR peak before the test is terminated, equipment removed and they are free to leave the laboratory.

HIIT running protocol: As described by Weston *et al.*, (2014), participants will begin with a 10-minute warm up at 60% HR peak (as determined from maximal test). Participants will then perform 4x4-minute intervals at 90% HR peak, separated by 3 minutes of active recovery (light intensity walking). After the final effort interval, participants will complete a 5-minute cool down at 50% of HR peak before the test is terminated, equipment removed and they are free to leave the laboratory.

Study Two: Effect of exercise intensity on cerebral blood flow and release of neurovascular adaptive signalling factors.

Once screened and recruited into the study (as described above (i.e. visit one)), participants will be required to visit the laboratory four times. The first exercise session will be a cycling VO$_{2peak}$ test to determine participant's maximum oxygen capacity (i.e. VO$_{2peak}$) and HR, which will be used to set the target exercise intensities in the subsequent testing sessions. The remaining three exercise sessions will then be completed in a randomised fashion and involve: 1) a 30-minute cycle at 65% VO$_{2peak}$; 2) 4 x 30-second all-out cycling bouts (separated by 4.5 minutes rest; '**all-out HIIT**'), and 3) 4 x 4-minute cycling exercise bouts at 90% of HR maximum separated by 3-minute periods of active recovery ('**clinical HIIT**'). All exercise sessions will be carried out on the same cycle ergometer and take place in an exercise laboratory within SportExR depending on availability and convenience for the participants. Each visit will last approximately 1 hour, of which approximately 30 minutes will be exercise (including warm up and cool down). There will be at least 48 hours between these sessions, but ideally no more than two weeks. Female participants will be able to participant at any time across their menstrual cycle.

Study two experimental protocol and measures
Maximal cycling VO$_{2peak}$ protocol (visit 2): This will be identical to that described above in study one.

Exercise experimental testing (visits 3-5)
For all these visits, participants' willingness to continue in the study will be confirmed upon arrival to the laboratory, then baseline measures of body mass and hydration status (urine osmolarity, urine sample to be discarded immediately and not stored) will be obtained. Participants will then lie supine whilst having a cannula inserted into the antecubital vein to enable the extraction of multiple blood samples. Participants will then be fitted with equipment to measure brain blood flow (Transcranial Doppler), HR (Polar monitor), respiratory gases and volume (Vyntus™ CPX metabolic cart), and an automated stress-testing blood pressure monitor (Tango, SunTech Medical). Once the equipment is fitted, 3-minutes of seated resting measurements will be taken, including a blood sample drawn from the cannula.

65% VO$_{2peak}$ protocol: Following resting measurements and a 5-minute warm-up aimed at achieving a RPE score of 11, the participant will be given time to stretch before carrying out a continuous 30-minute cycling bout at 65% VO$_{2peak}$. Blood samples will be drawn at rest, immediately after the 30-minute exercise bout and then 15 minutes post exercise. Following this post-exercise blood draw, equipment will be removed and the participant will be free to leave the laboratory.

All-out HIIT protocol: Following resting measures and a 10-minute warm-up aimed at achieving a RPE score of 11, the participant will be given time to stretch before performing the 4 x 30-second all-out cycling efforts. These will compromise sprinting as fast as possible from start to finish with the cycle ergometer set in an isokinetic mode ensuring the same resistance is maintained throughout the test. Between repetitions of the all-out exercise, a 4.5-minute recovery period will be completed by the participant that will involve very light cycling (50 W). Blood samples will be obtained during rest, immediately following the first and fourth 30-second efforts and then 15 minutes post exercise. Following this post-exercise blood draw, equipment will be removed and the participant will be free to leave the laboratory.

Clinical HIIT protocol: This will be identical to that used in study one. Specifically, participants will begin with a 10-minute warm up at 60% HR peak (as determined from maximal test). Participants will then perform 4x4-minute intervals at 90% HR maximum, separated by 3 minute of active recovery (~50 W of light intensity cycling). After the final effort interval, participants will complete a 5-min cool down at 50% of heart rate peak. Blood samples will be taken at rest, after the first and fourth HIIT bouts, and then 15 minutes post exercise. Following this post-exercise blood draw, equipment will be removed and the participant will be free to leave the laboratory.

Study Three: Effect of uphill and downhill running on cerebral blood flow and release of neurovascular adaptive signalling factors.

Once screened and recruited into the study (as described above (i.e. visit one)), participants will be required to visit the laboratory **four times**. The first exercise session will be a running VO$_{2peak}$ test to determine participant's maximum oxygen capacity (i.e. VO$_{2peak}$) and HR, which will be used to set the target exercise intensities during up/downhill and flat running in subsequent testing sessions. This first exercise session will also serve as a familiarisation session for the participants to learn and practice toe and heel weighted running, and running in time with audio-cued rhythms. The remaining three sessions will then be completed in a randomised fashion and involve: 1) uphill, downhill and flat running for a total of 30 minutes (10 minutes per gradient) at 65%VO$_{2peak}$; 2) flat, toe and heel weighted, and audio-cued running for a total of 30 minutes; 3) a 30-minute cycling bout at 65% VO$_{2peak}$. All running exercise sessions will be carried out on the same motorised treadmill ergometer that has the reverse belt and downhill capability (H/P/Cosmos Quasar®). Exercise sessions will take place in an exercise laboratory within SportExR depending on availability and convenience for the participants. Each visit will last approximately 1 hour, of which 40 minutes will be exercise (including warm up and cool down). There will be at least 48 hours between these sessions, but ideally no more than 2 weeks. Female participants will be able to participant at any time across their menstrual cycle.

Study three experimental protocol and measures
Maximal running VO$_{2peak}$ protocol (visit 2): This will be identical to that described above in study one. In addition, once recovered (~5 mins), participants will complete a familiarisation session to practice the heel and toe weighted running style, as well as running in time with the audio-cue.

Exercise experimental testing (visits 3-5)
For all these visits, participants' willingness to continue in the study will be confirmed upon arrival to the laboratory, then baseline measures of body mass and hydration status (urine osmolarity, urine sample to be discarded immediately and not stored) will be obtained. Participants will be fitted with equipment to measure brain blood flow (Transcranial Doppler), pre-frontal cortex and rectus femoris haemodynamics (near-infrared spectroscopy), HR (Polar monitor), respiratory gases and volume (Vyntus™ CPX metabolic cart), and an automated stress-testing blood pressure monitor (Tango, SunTech Medical). Once the equipment is fitted, resting measures will be obtained in a seated then exercising-specific (standing on treadmill or seated on bike) posture before beginning the exercise test protocol.

Uphill/Downhill/Flat running protocol: Following resting measurements and a 5-minute warm-up aimed at achieving a RPE score of 11, the participant will be given time to stretch before starting the protocol. To control for the exercise intensity effect on brain blood flow, belt speed for the treadmill gradient will be altered to achieve 65% VO_{2peak} for each position, which will be maintained for 10 minutes at each gradient, overall consisting of ~35-minutes of continuous exercise. Pilot testing will finalise the specific parameters, but based on our past work we will initially use an uphill gradient of 10% and a downhill gradient of 15%. Participants will then complete a 2-minute cool down at walking speed (5 km/h) before the test is terminated, the equipment is removed and the participant is free to leave.

Toe/Heel/Audio-Cued running protocol: Following resting measurements and a 5-minute warm-up aimed at achieving a RPE score of 11, the participant will be given time to stretch before starting the protocol. Participants will complete five, 5-minute exercise bouts at a constant self-selected belt speed, selected on the basis of an RPE score of 14. The first 5-minute bout will be while running at a 1% gradient (flat), then participants will complete the next two bouts with emphasised toe and then heel (heavy footed) weighted running in order to change the natural heel-to-toe biomechanics. The final two, 5-minute bouts will be completed in time with an audio-cue rhythm played through speakers positioned next to the treadmill (one slower and one faster than the participant's natural stride frequency/length – determined during first the 5-minute bout), for which the participant will alter their stride length and stepping frequency to match this rhythm. Participants will then complete a 2-minute cool down at walking speed (5 km/h) before the test is terminated, the equipment is removed, and the participant is free to leave.

30-minute cycling 65% VO_{2peak} protocol: Following resting measurements and a 5-minute warm-up aimed at achieving a RPE score of 11, the participant will be given time to stretch before carrying out a continuous 30-minute cycling bout at 65% VO_{2peak} (taken from running maximal test.

<u>**Overview of measures for all studies**</u>
Brain blood flow (velocity) will be assessed using transcranial Doppler (Doppler box, DWL, Germany), near-infrared spectroscopy will measure pre-frontal cortex and rectus femoris haemodynamics (NIRO 200NX, Hamamatsu Photonics, Japan), HR will be monitored using telemetry (Polar), oxygen consumption (VO_2) and respiratory (ventilation rate and volume, partial pressure of end-tidal carbon dioxide) variables will be measured using an indirect calorimetry system (Vyntus™ CPX, Carefusion, Germany), and resting and exercising blood pressure will be measured via an automated stress-testing blood pressure monitor (Tango M2, SunTech Medical, USA). Calorimetry data will be obtained via a face mask, HR via a belt fitted around the chest, blood pressure via a cuff placed around the upper arm, and brain blood flow from ultrasound probes placed above the zygomatic arch on the left and right side of the head and secured via an adjustable headband. A small amount of ultrasound gel will be placed between the probes and the skin to obtain the highest quality images. At the end of each exercise stage/bout, participants will be asked to identify on a chart their ratings of perceived exertion via the Borg scale.

Venous blood samples obtained during study two will be drawn into 10-mL tubes via a cannula placed in the antecubital vein. Blood samples will be centrifuged and plasma/serum collected, labelled (according to assigned code) and stored (-80 freezer) for later analysis of NO_2/NO_3, BDNF, IGF-1 and VEGF using commercially available enzyme-linked immunosorbent assay [ELISA] kits. In addition, resting and exercising blood lactate levels will be obtained via a finger prick taken at the same time as the drawn venous blood samples to measure lactate metabolism (via Lactate Pro; blood samples tested immediately and not stored).

Statistical analysis
Repeated-measures ANOVA will be used to compare dependent variables of interest between the different exercise modalities and intensities.

General standardisation for participants across all studies: Prior to all exercise testing sessions, participants will be asked to refrain from eating a large meal 4 hours before arrival (a light meal is permitted so long as it is not less than 2 hours before arrival). In order to ensure adequate hydration status, participants will be advised to drink 0.5 litres of water within 4 hours of beginning testing and 0.25 litres of water within 15 minutes of testing, in accordance with the American College of Sports Medicine Hydration Guidelines (ACSM, 2011). They will also be asked to refrain from caffeine for 6 hours prior to testing, and refrain from vigorous exercise and the consumption of alcohol for 24 hours prior to testing.

All experimental procedures will be performed in accordance with the School of Sport, Exercise and Rehabilitation Sciences ethical, health and safety guidelines, and the researchers present have experience obtaining all these measures.

8. DOES THE PROJECT INVOLVE PARTICIPATION OF PEOPLE OTHER THAN THE RESEARCHERS AND SUPERVISORS?

Yes ☒ No ☐

Note: 'Participation' includes both active participation (such as when participants take part in an interview) and cases where participants take part in the study without their knowledge and consent at the time (for example, in crowd behaviour research).

If you have answered NO please go to Section 18. If you have answered YES to this question please complete all the following sections.

9. PARTICIPANTS AS THE SUBJECTS OF THE RESEARCH
Describe the number of participants and important characteristics (such as age, gender, location, affiliation, level of fitness, intellectual ability etc.). Specify any inclusion/exclusion criteria to be used.

It is anticipated that 20-25 participants will be recruited from the University of Birmingham community. They can be male, female, or non-binary genders aged between 18 and 45 years and physically active. The screening procedure will involve completion of a General Health Questionnaire and a Physical Activity Questionnaire (attached). Individuals with a history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease will be excluded from participation, as will those individuals using prescribed or over-the-counter medications, with the exception of oral conception. Females that are pregnant will be excluded. Individuals that self-report as being sedentary (i.e. have a sedentary job and do <1 hour of physical exercise per week) will also be excluded. Affiliation to the University of Birmingham is not necessary, however, the majority of participants are likely to be associated with the University (e.g. staff or students).

10. RECRUITMENT
Please state clearly how the participants will be identified, approached and recruited. Include any relationship between the investigator(s) and participant(s) (e.g. instructor-student).

Note: Attach a copy of any poster(s), advertisement(s) or letter(s) to be used for recruitment.

Participant recruitment will take place in the UK. The study will predominately be advertised via word-of-mouth, lecture shout-outs or advertisements (recruitment poster attached) posted in SportExR participant recruitment sites. Potential participants expressing an interest in the project or just in a single study will be contacted by the study investigators. Participants have the option of which study they wish to complete, including volunteering for all. Some participants may have an existing relationship with one or more of the investigators; however this will not be used to force participation in the study.

11. CONSENT

a) Describe the process that the investigator(s) will be using to obtain valid consent. If consent is not to be obtained explain why. If the participants are minors or for other reasons are not competent to consent, describe the proposed alternate source of consent, including any permission / information letter to be provided to the person(s) providing the consent.

Prospective participants will be provided with an information sheet (attached) outlining the study details and will be given sufficient time (information provided at least 48 hours prior to the first visit) to consider this information before making their decision whether or not participate in any of the described studies. The project investigators will in no way attempt to pressure or coerce any individual into taking part. Upon arrival for the first visit, participants will have the study explained to them in full and given the opportunity to ask any questions before being asked to sign the participant consent form and complete the General Health and Physical Activity questionnaire (attached). Participants must be able and willing to comply with all research requirements, but will be informed of their right to withdraw from the study at any point without giving a reason.

Note: Attach a copy of the Participant Information Sheet (if applicable), the Consent Form (if applicable), the content of any telephone script (if applicable) and any other material that will be used in the consent process.

b) Will the participants be deceived in any way about the purpose of the study?   Yes ☐ No ☒

If yes, please describe the nature and extent of the deception involved. Include how and when the deception will be revealed, and who will administer this feedback.

12. PARTICIPANT FEEDBACK

Explain what feedback/ information will be provided to the participants after participation in the research. (For example, a more complete description of the purpose of the research, or access to the results of the research).

A written summary of the research findings will be provided to all participants following completion of the study. Individual results will be made available to participants upon request. Study investigators also plan to disseminate the data to the wider scientific community through publication in high impact, peer reviewed, open access journals. If successful, the manuscript(s) will be made available to any participant interested in receiving this information.

13. PARTICIPANT WITHDRAWAL

a)   Describe how the participants will be informed of their right to withdraw from the project.

Participants will be made aware of their right to withdraw from the study at any point without having to give a reason, through verbal communication during the initial visit and again during each subsequent visit, as well as having read it in the participant information letter and consent form. Participants can withdraw at any time during the study and up to two weeks after their last laboratory visit. If a participant withdraws from the study, the data we have collected from them will be removed unless they give us permission to retain them.

Research hours completed up to the point of withdrawal will be credited to students that wish to use their participation for this degree course requirement.

b) Explain any consequences for the participant of withdrawing from the study and indicate what will be done with the participant's data if they withdraw.

There will be no consequence to any participant that withdraws from the study. In the event of a withdrawal, data collected will be retained for analysis with consent from the participant at the time of withdrawal. If consent is not given, the participant will be removed from the study and their details and data permanently deleted/destroyed.

14. COMPENSATION
Will participants receive compensation for participation?
      i) Financial   Yes ☐ No ☒
      ii) Non-financial   Yes ☒ No ☐
If **Yes** to **either** i) or ii) above, please provide details.

> Students taking part in the study may wish to use the hours committed to taking part for their degree course Research Hours allotment.

If participants choose to withdraw, how will you deal with compensation?

> Research hours completed up to the point of withdrawal will be credited to students that wish to use their participation for this degree course requirement.

15. CONFIDENTIALITY

a)   Will all participants be anonymous?   Yes ☐ No ☒

b)   Will all data be treated as confidential?   Yes ☒ No ☐

Note: Participants' identity/data will be confidential if an assigned ID code or number is used, but it will not be anonymous. Anonymous data cannot be traced back to an individual participant.

> Describe the procedures to be used to ensure anonymity of participants and/or confidentiality of data both during the conduct of the research and in the release of its findings.

> Participant details and data will be kept strictly confidential. Participants will be assigned a unique ID code under which their individual data will be recorded and stored. The research team will maintain a written record that links the identity of the participants to the assigned ID codes, which will be stored in a safe and secure location.

> If participant anonymity or confidentiality is not appropriate to this research project, explain, providing details of how all participants will be advised of the fact that data will not be anonymous or confidential.

> The storage, access and disposal of each participants data is outlined in the information sheet and will be verbally confirmed during their preliminary meeting with researchers.

16. STORAGE, ACCESS AND DISPOSAL OF DATA
Describe what research data will be stored, where, for what period of time, the measures that will be put in place to ensure security of the data, who will have access to the data, and the method and timing of disposal of the data.

> Data generated throughout the duration of the study will be managed in accordance with the terms and conditions of the Data Protection Act 1998. Data will be collated and stored in a password-protected electronic database and only those directly involved with the project will have access to the data. Following publication, data will be maintained in an easily understandable and accessible format so that it can be made available to academic researchers upon request. Data will be stored by the Principal Investigators of the project for a minimum of 10 years, in accordance with the University of Birmingham's data storage policy. If after this time the data is deemed to be of no further use to the academic world it will be permanently deleted.

17. OTHER APPROVALS REQUIRED? e.g. Criminal Records Bureau (CRB) checks or NHS R&D approvals.

      ☐   YES   ☐   NO   ☒   NOT APPLICABLE

If yes, please specify.

>

18. SIGNIFICANCE/BENEFITS
Outline the potential significance and/or benefits of the research

Potential benefits of taking part in this research include obtaining an accurate and reliable measure of maximal oxygen capacity (VO_{2peak}) as well as finding out brain blood flow responses to exercise. Furthermore, participants will have the opportunity take part in a study that uses world class equipment and facilities whilst improving our knowledge of the brain's responses to exercise.

Findings from this work will improve our understanding of how exercise mediates improved brain vascular health. Specifically, the quantification of the different blood flow profiles and resultant release of neurovascular adaptive signalling factors induced via shear stress and cyclic strain forces that occur between the different exercise modalities and intensities (including HIIT) will help us better understand the how to optimise exercise for improved brain health, and may therefore help to inform public health exercise guidelines.

19. RISKS

a) Outline any potential risks to **INDIVIDUALS**, including <u>research staff, research participants, other individuals not involved in the research</u> and the measures that will be taken to <u>minimise</u> any risks and the procedures to be adopted in the event of mishap

A complete risk assessment can be viewed on a separate risk assessment form, the contents of which are summarized below.

All the procedures outlined are well-established and widely used, and the staff and PGRs working on this project are experienced in performing the procedures detailed. Drs Sam and Rebekah Lucas have extensive experience of conducting research and participating in studies of this nature. Participants will be observed carefully by the investigators throughout each experimental test session and participants are encouraged to notify an investigator immediately if they have any bothersome sensations.

Transcranial Doppler is a non-invasive and painless procedure with which to assess blood flow velocity within the cerebrovascular (i.e., providing an index of CBF). No adverse effects have been reported to date. Nevertheless, we will follow best practice guidelines to minimise participant exposure to the ultrasound by stopping the ultrasound transmission and recording between each monitoring period.

Exercise testing.
Participants are required to perform exercise that involves an incremental exercise test to volitional exhaustion, as well as near-maximal efforts during repeated intervals (i.e., the HIIT protocol). Performing moderate or vigorous exercise carries the following risks that the participants will be made aware of through the Participant Information Sheet:
- Sensations of fatigue and physical exhaustion – this will be short-lived and will subside in a few minutes upon stopping exercise
- Fainting – often related to physical exhaustion and then suddenly stopping, this will be mitigated by the inclusion of a cool-down period immediately after the formal exercise test is complete to gradually bring participants back to normal
- Cardiovascular event (e.g., myocardial infarction or 'heart attack') – this is a small risk, particularly for healthy individuals who are accustomed to physical activity, which is an inclusion criteria for this study. This risk will also be minimised by the exclusion of participants with a history of cardiovascular disease.
- Excessive Exertion – participant's HR, VO_2 and RPE score will be monitored to ensure they achieved targeted intensities, particularly at the higher intensity bouts.

Blood samples: Venous blood samples will be taken by a trained and certified researcher proficient with this procedure, and in accordance with SportExR's Code of Practice for blood sampling. Risks associated with this procedure include some local discomfort, infection and sometimes bruising or discoloration. To minimise these risks, sterile equipment will be used, the site for the needle stick and cannula placement will be cleaned with an alcohol wipe, and covered with a sterile dressing during the experiment. Upon removal of the cannula, pressure will be applied following to minimise the chance of bruising. For researchers, there is the possible risk of hepatitis or HIV if there is a needle-stick injury. This risk is reduced by wearing gloves. All personnel that handle blood samples will wear gloves and be immunised against Hepatitis B. Standard laboratory safety standards for blood sampling and handling of blood samples will be maintained throughout this study.

b) Outline any potential risks to **THE ENVIRONMENT and/or SOCIETY** and the measures that will be taken to <u>minimise</u> any risks and the procedures to be adopted in the event of mishap.

> There are no known risks to the environment or society associated with the proposed study.

20. ARE THERE ANY OTHER ETHICAL ISSUES RAISED BY THE RESEARCH?

Yes ☐   **No** ☒

If yes, please specify

> N/A

21. EXPERT REVIEWER/OPINION

You may be asked to nominate an expert reviewer for certain types of project, including those of an interventional nature or those involving significant risks. If you anticipate that this may apply to your work and you would like to nominate an expert reviewer at this stage, please provide details below.

Name
Contact details (including email address)
Brief explanation of reasons for nominating and/or nominee's suitability

22. CHECKLIST

Please mark if the study involves any of the following:

- Vulnerable groups, such as children and young people aged under 18 years, those with learning disability, or cognitive impairments   ☐

- Research that induces or results in or causes anxiety, stress, pain or physical discomfort, or poses a risk of harm to participants (which is more than is expected from everyday life) ☐

- Risk to the personal safety of the researcher ☐

- Deception or research that is conducted without full and informed consent of the participants at time study is carried out   ☐

- Administration of a chemical agent or vaccines or other substances (including vitamins or food substances) to human participants.   ☐

- Production and/or use of genetically modified plants or microbes   ☐

- Results that may have an adverse impact on the environment or food safety   ☐

- Results that may be used to develop chemical or biological weapons   ☐

Please check that the following documents are attached to your application.

	ATTACHED	NOT APPLICABLE
Recruitment advertisement	☒	☐
Participant information sheet	☒	☐
Consent form	☒	☐
Questionnaire	☒	☐
Interview Schedule	☐	☒

23. DECLARATION BY APPLICANTS

I submit this application on the basis that the information it contains is confidential and will be used by the University of Birmingham for the purposes of ethical review and monitoring of the research project described herein, and to satisfy reporting requirements to regulatory bodies. The information will not be used for any other purpose without my prior consent.

I declare that:
- The information in this form together with any accompanying information is complete and correct to the best of my knowledge and belief and I take full responsibility for it.
- I undertake to abide by University Code of Practice for Research (http://www.as.bham.ac.uk/legislation/docs/COP_Research.pdf) alongside any other relevant professional bodies' codes of conduct and/or ethical guidelines.
- I will report any changes affecting the ethical aspects of the project to the University of Birmingham Research Ethics Officer.
- I will report any adverse or unforeseen events which occur to the relevant Ethics Committee via the University of Birmingham Research Ethics Officer.

Name of principal investigator/project supervisor:	Dr Samuel Lucas
Date:	12.04.2018

Please now save your completed form, print a copy for your records, and then email a copy to the Research Ethics Officer, at aer-ethics@contacts.bham.ac.uk. As noted above, please do not submit a paper copy.

Appendix 2: Participant Information Pack

UNIVERSITY OF
BIRMINGHAM

Investigating the interaction between exercise mode and exercise intensity on brain vascular health

Information Pack

This pack describes three studies that you can volunteer for. Each study examines a specific research question that relates to how differences in exercise mode and/or intensity may effect brain vascular health.

The Principal Investigator for this project is Dr Sam Lucas.
Email: s.j.e.lucas@bham.ac.uk
Phone: +44 121 414 7272

School of Sport, Exercise and Rehabilitation Sciences

Participant Information Sheet

Project title: Investigating the interaction between exercise mode and exercise intensity on brain vascular health (ERN_17-1570)

An invitation to take part:

Thank you for taking the time to read this leaflet. We would like to invite you to take part in this project, which contains three separate studies that you can choose to volunteer for. You can choose to volunteer for one, two or all three. Before you decide if you want to participate or not, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with friends or relatives, if you wish. Please ask us if there is anything that is not clear or if you would like more information.

1.   What is the purpose of each study?

Study 1: Investigating the interaction between exercise mode and exercise intensity on brain blood flow during exercise.

The purpose of this study is to compare brain blood flow responses during running and cycling exercise across a range of intensities, and to determine brain blood flow responses during a high-intensity interval training (HIIT) session for each of these modalities. HIIT has received a lot of attention recently and shows many positive effects for improved metabolic and cardiovascular health for much less time commitment than traditionally promoted aerobic-based training programmes. However, very little research has been done examining how HIIT affects the brain. This study will be the first to directly compare brain blood flow responses between exercise modes and during high-intensity interval training exercise.

Study 2: Effect of exercise intensity on brain blood flow and release of neurovascular adaptive signalling factors.

The purpose of this study is to establish which exercise intensity is the most effective at increasing brain blood flow and releasing blood markers of brain vascular adaptation. We will measure brain blood flow profiles and the resultant neurovascular adaptive signalling factors from three distinct exercise intensities that are linked to current physical activity health promotion; the traditional model (65% of maximum effort) and two promoted models of HIIT ['clinical HIIT' (4x4-min bouts of ~90% of maximum effort, separated by 3 minutes of rest) and 'all-out HIIT' (4 x 30-s sprints, separated by 4.5 minutes of rest). This study will identify the optimal exercise intensity to maximise the increase in brain blood flow and the accompanying release of blood-borne molecules linked to beneficial changes in brain function and structure.

Study 3: Effect of uphill and downhill running on brain blood flow and release of neurovascular adaptive signalling factors.

The purpose of this study is to examine the effect of altering pulse pressure oscillations in brain blood flow. These oscillations are induced from the impact of the foot strike during running, which produces a markedly different brain blood flow profile to that observed during other modes of exercise such as cycling. This study will compare uphill, downhill, flat, toe, heel (heavy-footed) running and include 2 audio-cue rhythms to alter your natural running pace, in order to assess the effect of changing the heel strike force and pattern on these oscillations and compare this against the brain blood flow profile generated from cycling exercise.

School of Sport, Exercise and Rehabilitation Sciences

2.   Why have I been chosen?

You have been chosen because you are:

- Aged between 18-45 years old (females can participate at any point across their menstrual cycle, but cannot be pregnant)
- Healthy and physically active (as defined by the Department of Health General Practise Physical Activity Questionnaire (2006))
- Have no history of cardiovascular, respiratory, metabolic or neurological disease

3.   Do I have to take part?

No. Taking part in this study is entirely voluntary. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form, but you are still free to withdraw at any time and without giving a reason. You should feel under no pressure to participate and if at any time you are asked questions that you are not comfortable with answering (e.g. those asked in the General Health Questionnaire) you are free to not disclose this information. Though please do bear in mind that all information collected will be kept strictly confidential. However if you do decide to withdrawal, any data collected relating to you will only be retained following your consent at the time of withdrawal.

4.   What will happen to me if I agree to take part?

You will be invited to complete a general health and physical activity questionnaire as part of the screening procedure for the study. You are encouraged to ask questions prior to and throughout the study protocol if there is anything you do not understand or feel uncomfortable with. You will then be asked to sign a consent form. Following provision of informed consent and providing the information provided in the questionnaires does not exclude you from the study, your participation in the study starts and you will be booked in for your data collection trials, to take place no less than 48 hours after the screening has been completed. If you do not meet our eligibility criteria, you will take no further part in the study and any information collected about you so far will be destroyed.

5.   What do I have to do for the measurements made during the experimental visits?

Before the experimental visits (**for studies 1, 2 and 3**)
You will be asked to refrain from eating a large meal 4 hours before arrival (a light meal is okay as long as it is not less than 2 hours before you arrive). You will also be asked to refrain from caffeine for at least 6 hours prior to testing, and refrain from vigorous exercise and the consumption of alcohol 24 hours prior to testing, as well as have a full night's sleep prior to your testing session. You are advised to drink approximately 0.5 litres of water within 4 hours of beginning testing and 0.25 litres of water within 15 minutes of testing in order to ensure adequate hydration.

Study 1

During the experimental visits
Following the initial screening visit (visit 1), we will ask you to attend **six** exercise sessions taking place within the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham at a time that is convenient to you, but similar across all testing sessions. There will be at least 48 hours between these sessions, but ideally no more than 2 weeks.

The first two sessions (visits 2 & 3) will involve completing a standardised incremental aerobic capacity protocol on a bike and a treadmill on separate days, which will allow us to determine your target exercise intensities for the subsequent exercise sessions. During the aerobic capacity tests,

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work rate will increase every 3 minutes, by either increasing the treadmill belt speed/gradient or by increasing the resistance load on the bike. You will be asked to continue the test until you cannot keep going (a point known as volitional exhaustion). Once this point has been reached, you will complete a 2-minute cool down. In total, you will complete approximately 30 minutes of exercise during each test. During these tests you will be fitted with equipment to measure your oxygen consumption and heart rate, as well as have your height and body mass measured before you start.

For the next four exercise sessions, upon arrival to the laboratory we will ask you to give us a urine sample to determine your hydration status and then we will measure your body mass and height. You will then sit resting while we put on the equipment to measure your brain blood flow, oxygen consumption, respiratory rate and volume, heart rate and blood pressure. We will collect resting measures before then asking you to perform exercise.

Visits 4 and 5 will involve completing a staged incremental protocol on a bike and a treadmill, the order of which will be randomised between all participants taking part in the study. For this test, work rate will increase every 3 minutes, by either increasing the treadmill belt speed or by increasing the resistance load on the bike. You will be asked to complete 5 x 3-min stages at 35, 50, 65, 80 and 95% of your aerobic capacity, as determined from the preceding exercise capacity tests. Following the last stage of exercise (at 95%), you will complete a 2-minute cool down. In total, you will complete approximately 30 minutes of exercise.

The final two sessions (visits 6 & 7) will involve high-intensity-interval exercise (i.e., HIIT), with the mode of exercise being either running on a treadmill or cycling. Based on published guidelines for this type of training, we will ask you to complete four, 4-minute intervals exercising at 90% of your heart rate peak, determined from the preceding exercise capacity tests. Between the effort intervals you will complete 3 minutes of light exercise. These intervals will be preceded and followed by a 10-minute warm-up and 5-minute cool-down period of light-to-moderate intensity exercise. For these HIIT sessions, you will complete a total of 43 minutes of exercise.

Study 2

<u>During the experimental visits</u>

Following the initial screening visit (visit 1), we will ask you to attend **four** exercise sessions taking place within the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham at a time that is convenient to you, but similar across all testing sessions. There will be at least 48 hours between these sessions, but ideally no more than 2 weeks.

The first testing session will involve completing a standardised incremental aerobic capacity protocol on a bike, which will allow us to determine your target exercise intensities for the subsequent exercise sessions. For this test work rate will increase every 3 minutes by increasing the resistance load on the bike, and you will be asked to continue the test until you cannot keep going (a point known as volitional exhaustion). Once this point has been reached, you will complete a 2-minute cool down before leaving the laboratory. In total, you will complete approximately 30 minutes of exercise. During this test you will be fitted with equipment to measure your oxygen consumption and heart rate, as well as have your height and body mass measured before you start.

The next three sessions will occur in a random order.
1) <u>Clinical HIIT protocol:</u> Upon arrival to the laboratory we will ask you to give us a urine sample to determine your hydration status and then we will measure your body mass and height. You will then sit resting while we put on the equipment to measure your brain blood flow, oxygen

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consumption, respiratory rate and volume, heart rate and blood pressure. We will also place a cannula in a vein on your arm to allow us to take blood samples before, during (after 1st and 4th HIIT bout) and following the exercise session, which will be paired with finger prick blood sample measures of blood lactate. We will collect resting measures before then asking you to perform exercise.

Based on published guidelines for this type of training, we will ask you to complete four, 4-minute intervals exercising at 90% of your heart rate peak, which will be determined from your aerobic capacity test (first exercise session). Between the effort intervals you will complete 3 minutes of light exercise. These HIIT intervals will be preceded and followed by a 10-minute warm-up and 5-minute cool-down period of light-to-moderate intensity exercise. In total, you will complete 43 minutes of exercise. Once the final blood sample is obtained (15 minutes after the last exercise effort bout), we will remove the equipment and you will be free to leave the laboratory.

2) <u>All-out HIIT protocol:</u> As for the Clinical HIIT protocol, we will ask you to provide a urine sample upon arrival and measure your body mass and height. You will be fitted with equipment to measure your brain blood flow, oxygen consumption, respiratory rate and volume, heart rate and blood pressure. We will also place a cannula in a vein on your arm to allow us to take blood samples before, during (after 1st and 4th HIIT bout) and following the exercise session, which will be paired with finger prick blood sample measures of blood lactate. We will collect resting measures before then asking you to perform exercise.

The all-out HIIT protocol involves four all-out 30-second sprints, with 4.5-minutes of active recovery separating the exercise bouts. This HIIT protocol is well established and commonly used to examine the health benefits of short duration, high intensity exercise on health (albeit none on examining the effect on the brain to date). As with the clinical HIIT protocol, these HIIT intervals will be preceded and followed by a 10-minute warm-up and 5-minute cool-down period of light-to-moderate intensity exercise. In total, you will complete 35 minutes of exercise. Once the final blood sample is obtained (15 minutes after the last exercise effort bout), we will remove the equipment and you will be free to leave the laboratory.

3) <u>Traditional exercise training:</u> As for the HIIT protocols, we will ask you to provide a urine sample upon arrival and measure your body mass and height. You will be fitted with equipment to measure your brain blood flow, oxygen consumption, respiratory rate and volume, heart rate and blood pressure. We will also place a cannula in a vein on your arm to allow us to take blood samples before, immediately following the 30-minute exercise bout and 15 minutes following the exercise, which will be paired with finger prick blood sample measures of blood lactate. We will collect resting measures before then asking you to perform exercise.

Based on the traditionally recommended guidelines for engaging in regular physical activity, we will ask you to cycle at a moderate intensity (65% VO_{2peak}) for 30 minutes, preceded and followed by a 5-minute warm-up and cool-down of light intensity exercise. In total, you will complete 40 minutes of exercise. Once the final blood sample is obtained (15 minutes after the exercise bout), we will remove the equipment and you will be free to leave the laboratory.

Study 3

<u>During the experimental visits</u>
Following the initial screening visit (visit 1), we will ask you to attend four exercise sessions taking place within the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham at a time that is convenient to you, but similar across all testing sessions. There will be at least 48 hours between these sessions, but ideally no more than 2 weeks.

The first exercise testing session (visit 2) will involve completing a standardised incremental aerobic capacity protocol on a treadmill, which will allow us to determine your target exercise intensity (i.e., 65% VO$_{2peak}$) for the subsequent exercise sessions. For this test, work rate will increase every 3 minutes by increasing the treadmill belt speed/gradient, and you will be asked to continue the test until you cannot keep going (a point known as volitional exhaustion). Once this point has been reached, you will complete a 2-minute cool down and recovery period. Once recovered, we will ask you to complete a short familiarisation session, which will enable us to set the belt speed and gradients for the subsequent testing sessions (during up, downhill, flat and toe/heel running), as well as give you the opportunity to learn and practice the various running protocols (toe and heel weighted, and running to an audio-cue). In total, you will complete approximately 40 minutes of exercise. During this test you will be fitted with equipment to measure your oxygen consumption and heart rate, as well as have your height and body mass measured before you start.

The next three sessions (visits 3 – 5) will be:
1) A protocol of three, 10-minute exercise bouts of: uphill, downhill and flat running (30 minutes in total);
2) A protocol of five, 5-minute bouts of: flat, toe and heel weighted running, and 2 sets of running in time with an audio-cued rhythm (25 minutes in total)
3) A 30-minute cycle protocol.

1) <u>Uphill/downhill/flat running protocol:</u> Upon arrival to the laboratory we will ask you to give us a urine sample to determine your hydration status and then we will measure your body mass and height. You will then sit resting while we put on the equipment to measure your brain blood flow and oxygenation, thigh muscle blood flow and oxygenation, oxygen consumption, respiratory rate and volume, heart rate and blood pressure.

   After a 5-minute warm up, we will increase the gradient of the treadmill to 10% and adjust the belt speed until you reach our target moderate exercise intensity of 65% VO$_{2peak}$. We will ask you to run at this intensity for 10 minutes (altering the treadmill belt speed throughout to maintain this moderate level of exercise). Next, we will set the treadmill to downhill mode, which will require you to stop running briefly while the treadmill belt is stopped and re-started in reverse. We will set the downhill gradient at 15% and ask you to start walking on the treadmill, adjusting the belt speed until you reach our target moderate exercise intensity of 65% VO$_{2peak}$ that you will maintain for 10 minutes. The final 10-minute bout will be done with the treadmill set at a 1% gradient to represent flat running. We will again adjust the belt speed to maintain our target moderate exercise intensity of 65% VO$_{2peak}$. You will finish this session with a light intensity 5-minute cool down period

2) Toe/Heel and Audio-Cue Running protocol: The set-up to this session will be identical to the pervious protocol except for the treadmill being at 1% gradient throughout. The first 5-minutes will consist of a self-selected speed at 1% gradient. This speed will be maintained for the subsequent four 5-minute bouts consisting of toe- and then heel-weighted running, followed by 2 audio-cued bouts for which well will ask you to adjust your stride length and frequency to match the rhythm of the audio-cues. These cues will be slightly slower and faster than your natural running stride rhythm, which we will ascertain from the first 5-minute exercise bout. The session will finish with a 2-minute cool down of walking (~5km/h).

3) Cycling protocol: For this session, all the measures we take will be the same as the running protocols, but instead of running we will ask you to cycle at the same moderate intensity for 30 minutes.

Details of measures obtained

- **Brain blood flow using transcranial Doppler:** Blood flow in major arteries supplying the brain will be assessed by transcranial Doppler. This consists of placing an ultrasound probe in the area above the cheekbone. A small amount of ultrasound gel will be placed between the probe and your skin to obtain the highest quality images. The probe is fixed in place using an adjustable headband.
- **Heart rate:** This will be monitored using telemetry via a belt fitted around your chest.
- **Oxygen consumption:** The content of your expired breath will be measured for the concentration of carbon dioxide and oxygen. This is measured from sampling the air inside a fitted face mask that you will wear during the tests. The volume of air you breath will also be measured via a turbine connected to the face mask.
- **Blood pressure:** This will be monitored via an automated stress-testing blood pressure monitor using a cuff placed around your upper arm.
- **Brain (pre-frontal cortex) and thigh muscle (Rectus Femoris) blood flow and oxygenation:** Oxygenated and deoxygenated haemoglobin tissue content in the pre-frontal cortex and rectus femoris will be assessed non-invasively using near infrared spectroscopy (NIRS). The NIRS equipment consists of two sets of light-emitting laser diodes and light-detecting photodiodes. One set will be placed on the left side of the forehead, just below the hair line (pre-frontal cortex) with a sweat band placed over the probes and around the head to minimise light exposure from environmental surroundings. The other set will be placed on the quadricep thigh muscle (rectus femoris), midway between the patella and anterior superior iliac spine and in line with the muscle fibres. Tape will be place over the probes to prevent any light exposure from environmental surroundings and to ensure probe placement is maintained throughout the exercise session.
- **Perceived exertion:** This will be measured using a chart called the Borg 15 point RPE (Rate of Perceived Exertion) Scale, which you will be shown prior to testing and asked to point to at set intervals during testing.
- **Urine sample:** A singular urine sample will be obtained at the beginning of each testing session to measure hydration. This will involve urinating into a container which will be tested for osmolality.
- **Blood samples:** For study two only, blood samples will be obtained via a cannula inserted into the forearm by an experienced and well-trained phlebotomist. Small samples (~10 mL each) will be drawn while you are at rest, performing exercise and 15 minutes after finishing the exercise. These blood samples are necessary to enable us to measure and compare blood markers of brain vascular structural and functional changes between the different exercise intensities.

6. **What are the possible disadvantages and risks of taking part?**

Performing moderate or vigorous exercise carries the following risks that we feel you should be made aware of, as well as some of the things we are doing to minimise these risks:

- Sensations of fatigue and physical exhaustion – this will be short-lived and will subside in a few minutes upon ceasing exercise
- Fainting – often related to physical exhaustion and then suddenly stopping, this will be mitigated by the inclusion of a cool-down period immediately after the formal exercise test is

complete to gradually bring you back to normal
- Cardiovascular event (e.g., myocardial infarction or 'heart attack') – this is a small risk, particularly for healthy individuals who are accustomed to physical activity. We will also ensure that you are warmed up and cooled down appropriately around the exercise tests and will be monitoring your heart rate and general disposition when exercising to minimise the risk of a cardiovascular event.

Trained investigators will supervise the exercise tests, and there will be at least one CPR-certified investigator with automated external defibrillator (AED) training present during testing (the AED is located in the corridor outside where the exercise testing will take place). If at any time during the test you want to stop, you can signal as instructed and the test will be stopped. Investigators will observe you carefully throughout the study and you are encouraged to notify an investigator immediately if you have any worrisome symptoms in addition to those symptoms described above.

Blood sampling: The insertion of a needle for cannula placement can cause some local pain and discomfort, and sometimes bruising and discoloration after it is removed. The needle insertion and cannula placement for blood sampling will be done by a trained researcher proficient with this procedure, and in accordance with School's Code of Practice for blood sampling. Furthermore, to minimise risks stated above as well as any risk of infection, sterile equipment will be used, the site for the needle stick and cannula placement will be cleaned with an alcohol wipe, and covered with a sterile dressing during the experiment. Upon removal of the cannula, pressure will be applied for ~2 minutes to minimise the chance of bruising.

7.   What are the possible benefits of taking part?

At your request, you will be able to obtain an accurate and reliable measure of your maximal oxygen capacity (VO_{2peak}), as well as find out about your brain blood flow responses to different exercise modalities and intensities. You will have the opportunity to take part in a study that uses world class equipment and facilities whilst improving our knowledge of the brain's responses to exercise.

8.   Will my taking part in this study be kept confidential?

Yes, your participation in this study will be kept confidential. Both hard copies and electronic data collected during the study will only be accessible to responsible employees from the University of Birmingham. Your data will be stored for a minimum of 10 years in accordance with the University of Birmingham's policies on password protected systems accessible only to research personnel associated with this project.

9.   What will happen to the results of the research study?

The results of this project may be published anonymously in a scientific journal and within a postgraduate research thesis; however names of participants will never be published.

10.   Who is organising and funding the research?

The School of Sport, Exercise and Rehabilitation Sciences is funding the research. Drs Sam and Rebekah Lucas and MSc by Research students, Rhodri Furlong and Gabriella Imi, are organising the research as part of ongoing research studies within the area of brain health and exercise.

11.   Can I obtain feedback from the study?

Yes, if you wish to know the results of the study you took part in a summary of the results can be provided once the study has concluded. On the Consent Form there is a space to indicate if you would like to receive a study summary.

School of Sport, Exercise and Rehabilitation Sciences

12.   What will happen if I wish to withdraw from the study?

You are free to withdraw from the study at any time, including following data collection, without giving a reason. If the data collected until the time of withdrawal could be used, you will specifically be asked to give your consent to having the data included in any analysis. Additionally, you can withdraw your data from the study for up to two weeks following completion of the data collection, by notifying us via email or telephone. If you withdraw and do not consent to having the data collected so far included in the analysis, the data will be permanently deleted/destroyed.

13.   Do you have any further questions?

If you have any further questions about the study please feel free to contact:

Dr Sam Lucas (+44 121 414 7272): ███████████████

████████████████████████

Ms Gabriella Imi: █████████████████

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Appendix 3: Participant Consent Form

School of Sport, Exercise and Rehabilitation Sciences

Participant Consent Form

Study Title: *Investigating the interaction between exercise mode and exercise intensity on brain vascular health.*

Investigators: Dr Sam Lucas, Dr Rebekah Lucas, Ms Claire Burley, Mr Rhodri Furlong, Ms Gabriella Imi

Participant Name & ID: _____

Participant Address: _____

Telephone Number: _____   Date of Birth: _____

Initial each box

1. I have read the study information sheet and have discussed the experiment with one of the above named investigators, who have explained the procedures to my satisfaction.

2. I understand that I am volunteering to participate in the experiment by my choice and that I may stop and withdraw from the experiment at any time.

    a. I am volunteering for study 1: [ ]   (Initial this box to choose study 1)

    b. I am volunteering for study 2: [ ]   (Initial this box to choose study 2)

    c. I am volunteering for study 3: [ ]   (Initial this box to choose study 3)

3. I confirm that I have not been treated for any cardiovascular, metabolic, neurological or respiratory conditions in the past.

4. I understand that the data collected during the study may be looked at by responsible individuals from the University of Birmingham where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data and understand that any information will be kept strictly confidential.

5. I understand that my digital data will be stored for a minimum of 10 years in accordance with University of Birmingham policies on password protected systems accessible only to research personnel associated with this study. I agree to this.

6. I understand that my questionnaire data will be stored for a minimum of 10 years in accordance with University of Birmingham policies in a locked cabinet only accessible to the research personnel associated with this study. I agree to this.

7. I would like to receive a summary of the study findings   Yes / No (circle your response)

8. I agree to participate in this study.

_____   _____   _____
Name of Participant (PRINT)   Date   Signature

_____   _____   _____
Name of Researcher (PRINT)   Date   Signature

Appendix 4: General Health Questionnaire

The University of Birmingham

School of Sport, Exercise and Rehabilitation Sciences

General Health Questionnaire

Name: ....

Address: ....

....

....

Phone: ....

Name of the responsible investigator for the study:

....

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1.	You are.......	Male	Female
2.	What is your exact date of birth? Day........... Month...........Year......... So your age is............................ Years		
3.	When did you last see your doctor? In the: Last week............ Last month.......... Last six months............ Year................ More than a year...........		
4.	Are you currently taking any medication?	YES	NO
5.	Has your doctor ever advised you not to take vigorous exercise?	YES	NO
6.	Has your doctor ever said you have "heart trouble"?	YES	NO
7.	Has your doctor ever said you have high blood pressure?	YES	NO
8.	Have you ever taken medication for blood pressure or your heart?	YES	NO
9.			

	Do you feel pain in your chest when you undertake physical activity?	YES	NO
10.	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
11.	Has your doctor (or anyone else) said that you have a raised blood cholesterol?	YES	NO
12.	Have you had a cold or feverish illness in the last month?	YES	NO
13.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14.	a) Do you suffer from back pain b) if so, does it ever prevent you from exercising?	YES YES	NO NO
15.	Do you suffer from asthma?	YES	NO
16.	Do you have any joint or bone problems which may be made worse by exercise?	YES	NO
17.	Has your doctor ever said you have diabetes?	YES	NO
18.	Have you ever had viral hepatitis?	YES	NO
19.	If you are female, to your knowledge, are you pregnant?	YES	NO
20.	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
21.	Are you accustomed to vigorous exercise (an hour or so a week)?	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signed: ....

Date: ....

Appendix 5: General Practice Physical Activity Questionnaire

NHS

General Practice Physical Activity Questionnaire (GPPAQ)

Date:

Name:

1. Please tell us the type and amount of physical activity involved in your work.

		Please mark one box only
a	I am not in employment (e.g. retired, retired for health reasons, unemployed, full-time carer etc.)	
b	I spend most of my time at work sitting (such as in an office)	
c	I spend most of my time at work standing or walking. However, my work does not require much intense physical effort (e.g. shop assistant, hairdresser, security guard, child-minder, etc.)	
d	My work involves definite physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter, cleaner, hospital nurse, gardener, postal delivery workers etc.)	
e	My work involves vigorous physical activity including handling of very heavy objects (e.g. scaffolder, construction worker, refuse collector, etc.)	

2. During the _last week,_ how many hours did you spend on each of the following activities?
   Please answer whether you are in employment or not

Please mark one box only on each row

		None	**Some but less than 1 hour**	**1 hour but less than 3 hours**	**3 hours or more**
a	Physical exercise such as swimming, jogging, aerobics, football, tennis, gym workout etc.				
b	Cycling, including cycling to work and during leisure time				
c	Walking, including walking to work, shopping, for pleasure etc.				
d	Housework/Childcare				
e	Gardening/DIY				

3. How would you describe your usual walking pace? Please mark one box only.

☐ Slow pace (i.e. less than 3 mph)   ☐ Steady average pace

☐ Brisk pace   ☐ Fast pace (i.e. over 4mph)

Appendix 6: Borg Scale

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

LIST OF REFERENCES

Aaslid, R., Lindegaard, K.F., Sorteberg, W. and Nornes, H. (1989) 'Cerebral autoregulation dynamics in humans', *Stroke*, *20* (1), pp. 45-52.

Abbott, B.C., Bigland, B., and Ritchie, J.M. (1952) 'The physiological cost of negative work', *Journal of Physiology*, 117 (3), pp. 380-390.

Alexander, R.M. (1992) *The human machine*. New York: Columbia University Press.

Anderson, T. (1996) 'Biomechanics and running economy', *Sports Medicine*, 22 (2), pp. 76–89.

Ando, J., Yamamoto, K. (2009) 'Vascular mechanobiology: endothelial cell responses to fluid shear stress', *Circulation Journal*, 73 (11), pp. 1983-1992.

Awolesi, M.A., Sessa, W.C., and Sumpio, B.E., (1995) 'Cyclic strain upregulates nitric oxide synthase in cultured bovine aortic endothelial cells', *Journal of Clinical Investigation,* 96 (3), pp. 1449–1454.

Aziz, H.A. (2017) 'Comparison between Field Research and Controlled Laboratory Research', *Archives of Clinical and Biomedical Research,* 1 (2), pp. 101-104.

Babcock, M.C., Lefferts, W.K., Hughes, W.E., Fitzgerald, K.L., Leyer, B.K., Redmond, J.G., and Heffernan, K.S. (2015) 'Acute effect of high-intensity cycling exercise on carotid artery hemodynamic pulsatility', *European Journal of Applied Physiology,* 115 (5), pp. 1037-1045.

Bailey, D.M., Marley, C.J., Brugniaux, J.V., Hodson, D., New, K.J., Ogoh, S., and Ainslie, P.N. (2013) 'Elevated Aerobic Fitness Sustained Throughout the Adult Lifespan Is Associated With Improved Cerebral Hemodynamics', *Stroke,* 44 (11), pp. 3235-3238.

Ballerman, B.J., Dardik, A., Eng, E., and Liu, A. (1998) 'Shear stress and the endothelium', *Kidney International*, 54, pp.100-108.

Barnes, K.R., and Kilding, A.E. (2015) 'Running economy: measurement, norms, and determining factors', *Sports Medicine–Open*. 1 (1), pp. 1-15.

Basset, D.R., and Howley, E.T., (2000) 'Limiting factors for maximum oxygen uptake and determinants of endurance performance', *Medicine and Science in Sports and Exercise*, 32 (1), pp. 70–84.

Battisti-Charbonney, A., Fisher, J., and Duffin, J. (2011) 'The cerebrovascular response to carbon dioxide in humans', *The Journal of Physiology*, 589 (12), pp. 3039-3048.

Bergholm, R., Makimattila, S., Valkonen, M., Liu, M.L., Lahdenpera, S., Taskinen, M.R., Sovijarvi, A., Malmberg, P., and Yki-Jarvinen, H. (1999) 'Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilation in vivo', *Atherosclerosis*, 145 (2), pp.341–349.

Bhambhani, Y., Malik, R., and Mookerjee, S. (2007) 'Cerebral oxygenation declines at exercise intensities above the respiratory compensation threshold', *Respiratory Physiology & Neurobiology*, 156 (2), pp. 196–202.

Birk, G.K., Dawson, E.A., Batterham, A.M., Atkinson, G., Cable, T., Thijssen, D.H., and Green, D.J. (2013) 'Effects of exercise intensity on flow mediated dilation in healthy humans', *International Journal of Sports Medicine*, 34 (5), pp. 409-414.

Birukov, K.G. (2009) 'Cyclic Stretch, Reactive Oxygen Species, and Vascular Remodeling', *Antioxidants and Redox Signaling*, 11 (7), pp. 1651-1667.

Borg, G.A., (1982) 'Psychophysical bases of perceived exertion', *Medical and Science in Sports and Exercise*, 14 (5), pp.377-381.

Boushel, R., Langberg, H., Olesen, J., Gonzales-Alonzo, J., Bülow, J. and Kjaer, M. (2001) 'Monitoring tissue oxygen availability with near infrared spectroscopy (NIRS) in health and disease', *Scandinavian journal of medicine & science in sports*, 11 (4), pp. 213-222.

Brassard, P., Ferland-Dutil, H., Smirl, J.D., Paquette, M., Le Blanc, O., Malenfant, S., and Ainslie, P.N. (2017) 'Evidence for hysteresis in the cerebral pressure-flow relationship in healthy men', *American Journal of Physiology. Heart and Circulation Physiology*, 312 (4), pp. H701-H704.

Brugniaux, J.V., Marley, C.J., Hodspn, D.A., New, K.J., and Bailey, D.M. (2014) 'Acute exercise stress reveals cerebrovascular benefits associated with moderate gains in cardiorespiratory fitness', *Journal of Cerebral Flow & Metabolism*, 34 (12), pp. 1873-1876.

Burley, C.V., Bailey, D.M., Marley, C.J., and Lucas, S.J.E. (2016) 'Brain train to combat brain drain; focus on exercise strategies that optimize neuroprotection', *Experimental Physiology,* 101 (9), pp. 1178-1184.

Cavagna, G.A., Saibene, F.P., and Margaria, R. (1977) 'Mechanical work in running'*, Journal of Applied Physiology*, 19 (2), pp. 249–256.

Cavanagh, P.R., and Williams, K.R. (1982) 'The effect of stride length variation on oxygen uptake during distance running', *Medicine and Science in Sports and Exercise*, 14 (1), pp. 30–35.

Cipolla, M.J., 2009. 'The cerebral circulation', *Integrated systems physiology: From molecule to function*, 1 (1), pp.1-59.

Clarkson, P., Montgomery, H.E., Mullen, M.J., Donald, A.E., Powe, A.J., Bull, T., Jubb, M., World, M., and Deanfield, J.E. (1999) 'Exercise Training Enhances

Endothelial Function in Young Men'*, Journal of the American College of Cardiology*, 33 (5), pp. 1379-1385.

Colombe, S.J., Erickson, K.I., Raz, N., Webb, A.G., Cohen, N.J., McAuley, E., and Kramer, A.F. (2003) 'Aerobic fitness reduces brain tissue loss in aging humans', *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 58 (2), pp. 176-180.

Colombe, S.J., Erickson, K.I., Scalf, P.E., Kim, J.S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D.X., Hu, L., and Kramer, A.F. (2006) 'Aerobic exercise training increases brain volume in aging humans', *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 61 (11), pp. 1166-1170.

Curtelin, D., Morales-Alamo, D., Torres-Peralta, R., Rasmussen, P., Martin-Rincon, M., Perez-Valera, M., Siebenmann, C., Pérez-Suárez, I., Cherouveim, E., Sheel, A.W., Lundby, C., and Calbet, J.A. (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 and Metabolism*, 38 (1), pp. 136-150.

Davenport, M.H., Hogan, D.B., Eskes, G.A., Longman, R.S., Poulin, M.J. (2012) 'Cerebrovascular Reserve: The Link Between Fitness and Cognitive Function?', *Exercise and Sports Science Reviews*, 40 (3), pp. 153-158.

Davies, K.J.A., Quintanilha, A.T., Brooks, G.A., and Packer, L. (1982) 'Free radicals and tissue damage produced by exercise', *Biochemical and Biophysical Research Communications*, 107 (4), pp. 1198–1205.

Deary, I.J., Corley, J., Gow, A.J., Harris, S.E., Houlihan, L.M., Marioni, R.E., Penke, L., Rafnsson, S.B., and Starr, J.M. (2009) 'Age-associated cognitive decline', *British Medicine Bulletin*, 92 (1), pp. 135–152.

De Wit, B., De Clercq, D., and Aerts, P. (2000) 'Biomechanical analysis of the stance phase during barefoot and shod running', *Journal of Biomechanics*, 33 (3), pp. 269-278.

Dinenno, F.A., Tanaka, H., Monahan, K.D., Clevenger, C.M., Eskurza, I., DeSouza, C.A., and Seals, D.R. (2001) 'Regular endurance exercise induces expansive arterial remodelling in the trained limbs of healthy men', *Journal of Physiology*, 534 (1), pp. 287–295.

Divert, C., Mornieux, G., Baur, H., Mayer, F., and Belli, A. (2005) 'Mechanical comparison of barefoot and shod running', *International Journal of Sports Medicine*, 26 (7), pp. 593–598.

Divert, C., Mornieux, G., Freychat, P., Baly, L., Mayer, F., and Belli, A. (2008) 'Barefoot-shod running differences: shoe or mass effect?', *International Journal of Sports Medicine*, 29 (6), pp. 512–518.

Drapeau, A., Labrecque, L., Imhoff, S., Paquette, M., Le Blanc, O., Malenfant, S. and Brassard, P. (2019) 'Six weeks of high-intensity interval training to exhaustion attenuates dynamic cerebral autoregulation without influencing resting cerebral blood velocity in young fit men', *Physiological reports*, 7 (15).

Dunne, J. (2012) *Running technique uphill & downhill running technique*. Available at: https://www.kinetic-revolution.com/uphill-downhill-running-technique/ (Accessed: 15 April 2018).

Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabof, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E., and Kramer, A.F. (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), pp. 3017-3022.

Eston, R.G., Mickleborough, J., and Baltzopoulos, V. (1995) 'Eccentric activation and muscle damage: biomechanical and physiological consideration during downhill running', *British Journal of Sports Medicine*, 29 (2), pp. 89-94.

Faraci, F.M., and Heistad, D.D. (1990) 'Regulation of large cerebral arteries and cerebral microvascular pressure', *Circulation Research*, 66 (1), pp. 8–17.

Farris, D. J., and Sawicki, G. S., (2012) 'The mechanics and energetics of human walking and running: a joint level perspective', *Journal of the Royal Society Interface*, 9 (66), pp. 110-118.

Faull, O.K., Cotter, J.D., and Lucas, S.J.E. (2015) 'Cerebrovascular responses during rowing: Do circadian rhythms explain morning and afternoon performance differences?', *Scandinavian Journal of Medical Science and Sports*, 25 (4), pp. 467-475.

Frederick, E.C. (1984) 'Physiological and ergonomics factors in running shoe design', *Applied Ergonomics*, 15 (4), pp. 281–287.

Gonzales, J.U., Thompson, B.C., Thistlethwaite, J.R., and Scheuermann, B.W. (2008) 'Role of retrograde flow in the shear stimulus associated with exercise blood flow', *Clinical Physiology and Functional Imaging*, 28 (5), pp. 318-325.

González-Alonso, J., Dalsgaard, M.K., Osada, T., Volianitis, S., Dawson, E.A., Yoshiga, C.C., and Secher, N.H. (2004) 'Brain and central haemodynamics and oxygenationduring maximal exercise in humans', *Journal of Physiology,* 557 (1), pp. 331–342.

Goto, C., Higashi, Y., Kimura, M., Noma, K., Nakagawa, K., Kawamura, M., Chayama, K., Yoshizumi, M., and Nara, I. (2003) 'Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress', *Circulation*, 108 (5), pp. 530-535.

Gottschall, J.S., and Kram, R. (2003) 'Energy cost and muscular activity required for propulsion during walking', *Journal of Applied Physiology (1985),* 94 (5), pp. 1766-1772.

Gottschall, J.S., and Kram, R. (2005) 'Ground reaction forces during downhill and uphill running', *Journal of Biomechanics,* 38 (3), pp. 445-452.

Green, D.J. (2009) 'Exercise Training as Vascular Medicine: Direct Impacts on the Vasculature in Humans', *Exercise and Sport Sciences Reviews*, 37 (4), pp. 196-202.

Green, D.J., Cable, N.T., Rankin, J.M., and Taylor, R.P. (1994) 'Modification of forearm resistance vessels by exercise training in young men', *Journal of Applied Physiology*, 77 (4), pp. 1829-1833.

Green, D.J., Cheetham, C., Reed, C., Dembo, L., and O'Driscoll, G. (2002) 'Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry', *Journal of Applied Physiology*, 93 (1), pp. 361-368.

Green, D.J., Fowler, D.T., O'Driscoll, J.G., Blanksby, B.A., and Taylor, R.R. (1996) 'Endothelium-derived nitric oxide activity in forearm vessels of tennis players', *Journal of Applied Physiology (1985),* 81 (2), pp. 943-948.

Green, D.J., Hopman, M.T.E., Padilla, J., Laughlin, M.H., and Thijssen, D.H.J. (2017) 'Vascular Adaptation to Exercise in Humans: Role of Hemodynamic Stimuli', *American Physiological Society*, 97 (2), pp. 495-528.

Green, D.J., Maiorana, A., O'Driscoll, G., and Taylor, R. (2004) 'Effect of exercise training on endothelium-derived nitric oxide function in humans', *Journal of Physiology*, 561 (1), pp. 1-25.

Hallal, P.C., Andersen, L.B., Bull, F.C., Guthold, R., Haskell, W., and Ekelund, U. (2012) 'Global physical activity levels: surveillance progress, pitfalls, and prospects', *The Lancet*, 380 (9838), pp. 247–257.

Hambrecht, R., Adams, V., Erbs, S., Linke, A., Krankel, N., Shu, Y., Baither, Y., Gielen, S., Thiele, H., Gummert, J.F., Mohr, F.W., and Schuler, G. (2003) 'Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase', *Circulation*, 107 (25), pp. 3152-3148.

Heckmann, J.G., Hilz, M.J., Mück-Weymann, M., and Neundörfer, B. (2000) 'Transcranial Doppler sonography–ergometer test for the non-invasive assessment of cerebrovascular autoregulation in humans', *Journal of the Neurological Sciences*, 177 (1), pp. 41-47.

Hellström, G., Fischer-Colbrie, W., Wahlgren, N.G., and Jogestrand, T. (1996) 'Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise', *Journal of Applied Physiology*, 81 (1), pp. 413-421.

Hill, L. and Gwinnutt, C. (2008) 'Cerebral Blood Flow and Intracranial Pressure', *Update in Anaesthesia*, 24 (2), pp. 30-35.

Huonker, M., Schmid, A., Schmidt-Trucksass, A., Grathwohl, D., and Keul, J. (2003) 'Size and blood flow of central and peripheral arteries in highly trained able-bodied and disabled athletes', *Journal of Applied Physiology (1985),* 95 (2), pp. 685-691.

Irace, C., Gnasso, A., Cirillo, F., Leonardo, G., Ciamei, M., Crivaro, A., Renzulli, A., and Cotrufo, M. (2002) 'Arterial remodelling of the common carotid artery after aortic valve replacement in patients with aortic stenosis', *Stroke*, 33 (10), pp. 2446-2450.

Kety, S.S., and Schmidt, C.F. (1948) 'The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men'*, Journal of Clinical Investigation*, 27 (4), pp. 484–492.

Killgore, W.D.S., 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 (1). p. 3457.

Kirby, R.L., Atkinson, S.M., Donville, J.E., Urdang, M.F., Stanley, D.A., Gupta, S.K., and MacLeod, D.A. (1992) 'Failure of accentuated vertical body movements to induce cardiac-locomotor coupling', *Journal of Applied Physiology*, 72 (4), pp. 1368–1374.

Kirby, R.L., Carr, S.E., and MacLeod, D.A. (1990) 'Cardiac-locomotor coupling while finger tapping', *Perceptual Motor Skills*, 71 (3), pp. 1099–1104.

Kirby, R.L., Gupta, S.K., Carr, S.E., and MacLeod, D.A. (1991) 'Cardiaclocomotor coupling while finger tapping: Part II. A cross-over control study', *Perceptual Motor Skills*, 73 (3), pp. 831–834.

Kirby, R.L., Nugent, S.T., Marlow, R.W., MacLeod, D.A., and Marble, A.E. (1989) 'Coupling of cardiac and locomotor rhythms', *Journal of Applied Physiology*, 66 (1), pp. 323–329.

Kramer, A.F., Hahn, S., and Gopher, D. (1999) 'Task coordination and aging: explorations of executive control processes in the task switching paradigm', *Acta Psychologica*, 101 (2-3), pp. 339-378.

Krejza, J., Mariak, Z., Huba, M., Wolczynski, S., and Lewko, J. (2001) 'Effect of endogenous estrogen on blood flow through carotid arteries', *Stroke*, 32 (1), pp. 30-36.

Kurths, J., Voss, A., Saparin, P., Witt, A., Kleiner, H.J., and Wessel, N. (1995) 'Quantitative analysis of heart rate variability', *Chaos: An Interdisciplinary Journal of Nonlinear Science*, 5 (1), pp. 88-94.

Labrecque, L., Rahimaly, K., Imhoff, S., Paquette, M., Le Blanc, O., Malenfant, S., Lucas, S.J., Bailey, D.M., Smirl, J.D. and Brassard, P. (2017) 'Diminished dynamic cerebral autoregulatory capacity with forced oscillations in mean arterial pressure with elevated cardiorespiratory fitness', *Physiological reports*, *5* (21), p. 13486.

Laughlin, M.H. (1985) 'Endothelium-mediated control of coronary vascular tone after chronic exercise training', *Medicine and Science in Sports and Exercise*, 27 (8), pp. 1135–1144.

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), pp. 588-600.

Lautenschlager, N.T., Cox, K., and Cyarto, E.V. (2012) 'The influence of exercise on brain aging and dementia', *Biochemica et Biophysica Acta*, 1822 (3), pp. 474-481.

Lefferts, W.K., Augustine, J.A., and Heffernan, K.S. (2014) 'Effect of acute resistance exercise on carotid artery stiffness and cerebral blood flow pulsatility', *Frontiers in Physiology*, 5, p. 101.

Levine, B.D., Giller, C.A., Lane, L.D., Buckey, J.C., and Blomqvist, C.G. (1994) 'Cerebral Versus Systemic Hemodynamics During Graded Orthostatic Stress in Humans', *Circulation*, 90 (1), pp. 298-306.

Lind-Holst, M., Cotter, J.D., Helge, J.W., Boushel, R., Augustesen, H., Van Lieshout, J.J. and Pott, F.C. (2011) 'Cerebral autoregulation dynamics in endurance-trained individuals', *Journal of Applied Physiology*, 110 (5), pp.1327-1333.

Linke, A., Schoene, N., Gielen, S., Hofer, J., Erbs, S., Schuler, G., and Hambrecht, R. (2001) 'Endothelial Dysfunction in Patients with Chronic Heart Failure: Systemic Effects of Lower-Limb Exercise Training', *Journal of the American College of Cardiology*, 37 (2), pp. 392-397.

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

Lucas, S.J.E., Cotter, J.D., Brassard, P., and Bailey, D.M. (2015) 'High-intensity interval exercise and cerebrovascular health: curiosity, cause and consequence', *Journal of Cerebral Blood Flow and Metabolism*, 35 (5), pp. 902-911.

Lussiana, T., Fabre, N., Hébert-Losier, K., and Mourot, L. (2013) 'Effect of slope and footwear on running economy and kinematics', *Scandinavian Journal of Medicine and Science in Sports*, 23 (4), pp. e246-253.

Lussiana, T., Hebert-Losier, K., and Mourot, L. (2015) 'Effect of minimal shoes and slope on vertical and leg stiffness during running', *Journal of Sport and Health Science*, 4 (2), pp. 195-202.

Lyngeraa, T.S., Pedersen, L.M., Mantoni, T., Belhage, B., Rasmussen, L.S., van Lieshout, J.J., and Pott, F.C. (2013) 'Middle cerebral artery blood velocity during running', *Scandinavian Journal of Medicine and Science in Sports*, 23 (1), pp. 32-37.

Mastroianni, G.R., Zupan, M.F., Chuba, D.M., Berger, R.C., and Wile, A.L. (2000) 'Voluntary pacing and energy cost of off-road cycling and running', *Applied Ergonomics*, 31 (5), pp. 479-485.

Miller, E.K., and Cohen, J.D. (2001) 'An integrative theory of prefrontal cortex function', *Annual Review of Neuroscience,* 24 (1), pp. 167–202.

Minetti, A.E., Ardigo, L.P., and Saibene, F. (1994) 'Mechanical determinants of the minimum energy cost of gradient running in humans', *Journal of Experimental Biology*, 195 (1), pp. 211-225.

Mitchell, G.F., van Buchem, M.A., Sigurdsson, S., Gotal, J.D., Jonsdottir, M.K., Kjartansson, O., Garcia, M., Aspelund, T., Harris, T.B., Gudnason, V., and Launer, L.J. (2011) 'Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/ Environment Susceptibility – Reykjavik Study', *Brain*, 134 (11), pp. 3398-3407.

Miyachi, M., Tanaka, H., Yamamota, K., Yoshioka, A., Takahashi, K., and Onodera, S. (2001) 'Effects of one-legged endurance training on femoral arterial and venous size in healthy humans', *Journal of Applied Physiology (1985)*, 90 (6), pp. 2439-2444.

Moraine, J.J., Lamotte, M., Berré, J., Niset, G., Leduc, A., and Naeije, R. (1993) 'Relationship of middle cerebral artery blood flow velocity to intensity during dynamic exercise in normal subjects', *European Journal of Applied Physiology*, 67 (1), pp. 35-38.

Myers, J., and Ashley, E. (1997) 'Dangerous Curves: A Perspective on Exercise, Lactate, and the Anaerobic Threshold', *Chest*, 111 (3), pp. 787-795.

Myers, M.J., and Steudel, K. (1985) 'Effect of limb mass and its distribution on the energetic cost of running', *The Journal of Experimental Biology*, 116 (1), pp. 363–373.

NHS: The general practice physical activity questionnaire (GPPAQ). 2013.

Niizeki, K. (2004) 'Intramuscular pressure-induced inhibition of cardiac contraction: implications for cardiac-locomotor synchronization', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 288 (3), pp. R645-650.

Niizeki, K., Kawahara, K., and Miyamoto, Y. (1993) 'Interaction among cardiac, respiratory, and locomotor rhythms during cardiolocomotor synchronization', *Journal of Applied Physiology*, 75 (4), pp. 1815-1821.

Niizeki, K., and Saitoh, T. (2014) 'Cardiolocomotor phase synchronization during rhythmic exercise', *Journal of Physical Fitness and Sports Medicine*, 3 (1), pp. 11-20.

Nishiyasu, T., Sone, R., Tan, N., Maekawa, T., and Kondo, N. (2001) 'Effects of rhythmic muscle compression on arterial blood pressure at rest and during dynamic exercise in humans', *Acta Physiologica Scandinavica*, 173 (3), pp. 287-295.

Nomura, K., Takei, Y., and Yanagida, Y. (2003) 'Comparison of cardio-locomotor synchronization during running and cycling', *European Journal of Applied Physiology*, 89(3-4), pp. 221-229.

Nomura, K., Takei, Y., Yoshida, M., and Yanagida, Y. (2006) 'Phase-dependent chronotropic response of the heart during running in humans', *European Journal of Applied Physiology*, 97 (2), pp. 240-247.

Norton, S., Matthews, F.E., Barnes, D., Yaffe, K., and Brayne, C. (2014) 'Potential for primary prevention of Alzheimer's disease: an analysis of population-based data', *Lancet Neurology*, 13 (8), pp. 788-794.

Novacheck, T.F. (1998) 'The biomechanics of running', *Gait Posture*, 7 (1), pp. 77-95.

Ogoh, S., and Ainslie, P.N. (2009) 'Regulatory Mechanisms of Cerebral Blood Flow During Exercise: New Concepts', *Exercise and Sport Sciences Reviews*, 37 (3), pp.123-129.

Olmedillas, H., González-Agüero, A. Moreno, L. A., Casajus, J. A., and Vicente-Rodríguez, G. (2012) 'Cycling and bone health: a systemic review', *BMC Medicine*, 10 (1), p. 168.

Oussaidene, K., Prieur, F., Bougault, V., Borel, B., Matran, R., and Mucci, P. (2013) 'Cerebral oxygenation during hyperoxia-induced increase in exercise tolerance for untrained men', *European Journal of Applied Physiology*, 113 (8), pp. 2047–2056.

Oussaidene, K., Prieur, F., Tagougui, S., Abaidia, A., Matran, R., and Mucci, P. (2014) 'Aerobic fitness influences cerebral oxygenation response to maximal exercise in healthy subjects', *Respiratory Physiology & Neurobiology*, 205, pp. 53-60.

Padilla, J., Simmons, G.H., Bender, S.B., Arce-Esquivel, A.A., Whyte, J.J., and Laughlin, M.H. (2011) 'Vascular Effects of Exercise: Endothelial Adaptations Beyond Active Muscle Beds', *American Physiological Society*, 26 (3), pp.132-145.

Padulo, J., Powell, D., Milia, R., and Ardigo, L.P. (2013) 'A Paradigm of Uphill Running', *PLoS One*, 8 (7), p. e69006.

Palatini, P., Mormino, P., Mos, L., Di Marco, A., Munari, L., Del Torre, M., Valle, F., Pessina, A.C. and Dal Palù, C. (1988) 'Blood pressure changes during physical exercise (the beat phenomenon)', *Journal of Hypertension Supplement*, 6 (4), pp. 88-90.

Palatini, P., Mos, L., Mormino, P., Di Marco, A., Munari, L., Fazio, G., Giuliano, G., Pessina, A.C., and Dal Palù, C. (1989) 'Blood pressure changes during running in humans: the "beat" phenomenon', *Journal of Applied Physiology*, 67 (1), pp. 52-59.

Papaioannou, T.G., and Stefanadis, C. (2005) 'Vascular wall shear stress: basic principles and methods', *Hellenic Journal of Cardiology*, 46 (1), pp. 9-15.

Perez-Suarez, I., Martin-Rincon, M., Gonzalez-Henriquez, J.J., Fezzardi, C., Perez-Regalado, S., Galvan-Alvarez, V., Juan-Habib, J.W., Morales-Alamo, D., and Calbet, J.A. (2018) 'Accuracy and precision of the COSMED K5 portable analyser', *Frontiers in physiology*, 9, p.1764.

Phillips, B., and Jin, Y. (2013) 'Effect of Adaptive Paced Cardiolocomotor Synchronization during Running: A Preliminary Study', *Journal of Sports Science and Medicine*, 12 (3), pp. 381-387.

Pott, F., Knudsen, L., Nowak, M., Nielsen, H.B., and Hanel, B. (1997) 'Middle cerebral artery blood velocity during rowing', *Acta Physiologica Scandinavica,* 160 (3), pp. 251-255.

Querido, J.S., and Sheel, W. (2007) 'Regulation of Cerebral Blood Flow During Exercise', *Sports Medicine*, 37 (9), pp. 765-782.

Rakobowchuk, M., Tanguay, S., Burgomaster, K.A., Howarth, K.R., Gibala, J.G., and MacDonald, M.J. (2008) 'Sprint interval and traditional endurance training induce similar improvements in peripheral arterial stiffness and flow mediated dilation in healthy humans', *American Journal of Physiology*, *Regulatory, Integrative and Comparative*, 295 (1), pp. R236-R242.

Rowell, L.B., Brengelmann, G.L., Blackmon, R.A., and Murray, J.A. (1968) 'Disparities between aortic and peripheral pulse pressures induced by upright exercise and vasomotor changes in man', *Circulation*, 37 (6), pp. 954-964.

Rowley, N.J., Dawson, E.A., Birk, G.K., Cable, N.T., George, K., Whyte, G., Thijssen, D.H., and Green D.J. (2011) 'Exercise and arterial adaptation in humans: uncoupling localized and systemic Effects', *Journal of Applied Physiology (1985),* 110 (5), pp. 1190–1195.

Rowley, N.J., Dawson, E.A., Hopman, M.T., George, K., Whyte, G.P., Thijssen, D.H., and Green D.J. (2012) 'Conduit diameter and wall remodelling in elite athletes and spinal cord injury', *Medicine and Science in Sports and Exercise,* 44 (5), pp. 844–849.

Rupp, T., and Perrey, S. (2008) 'Prefrontal cortex oxygenation and neuromuscular responses to exhaustive exercise', *European Journal of Applied Physiology*, 102 (2), pp. 153–163.

Sahyoun, C., Floyer-Lea, A., Johansen-Berg, H., and Matthews, P.M. (2004) 'Towards an understanding of gait control: brain activation during the anticipation' *NeuroImage*, 21 (2), pp. 568-575.

Sawka, M.N., Burke, L.M., Eichner, E.R., Maughan, R.J., Montain, S.J., and Stachenfeld, N.S. 'American College of Sports Medicine position stand. Exercise and fluid replacement', *Medical and Science in Sports and Exercise*, 39 (2), pp. 377-90.

Schad, J.F., Meltzer, K.R., Hicks, M.R., Beutler, D.S., Cao, T.V., and Standley, P.R. (2011) 'Cyclic strain upregulates VEGF and attenuates proliferation of vascular smooth muscle cells', *Vascular Cell*, 3 (21). p. 21.

Sinoway, L.I., Hendrickson, C., Davidson, W.R., Prophet, S., and Zelis, R. (1989) 'Characteristics of flow-mediated brachial artery vasodilation in human subjects', *Circulation Research*, 64 (1), pp.32–42.

Sinoway, L.I., Musch, T.I., Minotti, J.R., and Zelis, R. (1986) 'Enhanced maximal metabolic vasodilatation in the dominant forearms of tennis players', *Journal of Applied Physiology (1985),* 61 (2), pp. 673–678.

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

Snyder, K.L., and Farley, C.T. (2011) 'Energetically optimal stride frequency in running: the effects of incline and decline', *The Journal of Experimental Biology*, 214(12), pp. 2089-2095.

Stringer, W., Wasserman, K., Casaburi, R., Porszasz, J., Maehara, K., and French, W. (1994) 'Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise', *Journal of Applied Physiology (1985)*, 76 (4), pp.1462–1467.

Subudhi, A.W., Dimmen, A.C., and Roach, R.C. (2007) 'Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise', *Journal of Applied Physiology*, 103 (1), pp. 177–183.

Subudhi, A.W., Lorenz, M.C., Fulco, C.S., and Roach, R.C. (2008) 'Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance', *Journal of Applied Physiology*, 294 (1), pp. H164–H171.

Subudhi, A.W., Olin, J.T., Dimmen, A.C., Polaner, D.M., Kayser, B., and Roach, R.C. (2011) 'Does cerebral oxygen delivery limit incremental exercise performance?', *Journal of Applied Physiology*, 111 (6), pp. 1727–1734.

Swain, D.P., Abernathy, K.S., Smith, C.S., Lee, S.J., and Bunn, S.A. (1994) 'Target heart rates for the development of cardiorespiratory fitness', *Medicine & Science in Sports & Exercise*, 26 (1), pp. 112-116.

Takeuchi, S., Nishida, Y., and Mizushima, T. (2014) 'Effects of Synchronization between Cardiac and Locomotor Rhythms on Oxygen Pulse during Walking', *Journal of Sports Science and Medicine*, 13 (4), pp. 881-887.

Tang, S.T., Su, H., Zhange, Q., Tang, H.Q., Wang, C.J., Zhou, Q., Wei, W., Zhu, H.Q., and Wang, Y. (2016) 'Melatonin Attenuates Aortic Endothelial Permeability and Arteriosclerosis in Streptozotocin-Induced Diabetic Rats: Possible Role of MLCK- and MLCP-Dependent MLC Phosphorylation', *Journal of Cardiovascular Pharmacology and Therapeutics*, 21 (1), pp. 82-92.

Thijssen, D.H.J., Dawson, E.A., Black, M.A., Hopman, N.T, and Green, D.J. (2009) 'Brachial artery blood flow responses to different modalities of lower limb exercise', *Medicine and Science in Sports and Exercise*, 41 (5), pp. 1072-1079.

Thijssen, D.H.J., Dawson, E.A., Tinken, T.M., Cable, N.T., and Green, D.J. (2009) 'Retrograde flow and shear rate acutely impair endothelial function in humans', *Hypertension*, 53 (6), pp. 986-992.

Thomas, B.P., Yezhuvath, U.S., Tseng, B.Y., Liu, P., Levine, B.D., Zhang, R. and Lu, H. (2013), 'Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO2', *Journal of Magnetic Resonance Imaging*, *38* (5), pp. 1177-1183.

Tinken, T.M., Thijssen, D.H., Black, M.A., Cable, N.T., and Green, D.J. (2008) 'Time course of change in vasodilator function and capacity in response to exercise training in humans', *Journal of Physiology*, 586 (20), pp. 5003-5912.

Tinken, T.M., Thijssen, D.H., Hopkins, N., Black, M.A., Dawson, E.A., Minson, C.T., Newcomer, S.C., Laughlin, M.H., Cable, N.T., and Green, D.J. (2009) 'Impact of shear rate modulation on vascular function in humans', *Hypertension*, 54 (2), pp. 278-363.

Tomoto, T., Sugawara, J., Nogami, Y., Aonuma, K., and Maeda, S. (2015) 'The influence of central arterial compliance on cerebrovascular hemodynamics: insights from endurance training intervention', *Journal of Applied Physiology (1985)*, 119 (5), pp. 445-451.

Topper, J.N., and Gimbrone, M.A. (1999) 'Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype', *Molecular Medicine Today*, 5 (1), pp. 40-46.

Townshend, A.D., Worringham, C.J., and Stewart, I.B. (2010) 'Spontaneous pacing during overground hill running', *Medicine and Science in Sports and Exercise*, 42 (1), pp. 160-169.

Tzeng, Y.C., and Ainslie, P.N. (2014) 'Blood pressure regulation IX: cerebral autoregulation under blood pressure challenges', *European Journal of Applied Physiology*, 114 (3), pp. 545-559.

Ündar, A., Masai, T., Frazier, O.H., and Fraser, J.C. (1999) 'Pulsatile and nonpulsatile flows can be quantified in terms of energy equivalent pressure during cardiopulmonary bypass for direct comparisons', *American Society for Artificial Internal Organs Journal*, 45 (6), pp. 610-614.

Vernillo, G., Giandolini, M., Edwards, W.B., Morin, J.B., Samozino, P., Horvaism, N., and Millet, G.Y. 'Biomechanics and Physiology of Uphill and Downhill Running', *Sports Medicine*, 47 (4), pp. 615-629.

Wagshul, M.E., Eide, P.K., and Madsen, J.R. (2011) 'The pulsating brain: A review of experimental and clinical studies of intracranial pulsatility', *Fluids and Barriers of the CNS*, 8 (5). p. 5.

Vyaire Medical (2019) Cardiopulmonary Exercise Testing Special Edition. Available at: https://intl.vyaire.com/products/vyntus-cpx-metabolic-cart

Willie, C.K., Colino, F.L., Bailey, D.M., Tzeng, Y.C., Binsted, G., Jones, L.W., Haykowsky, M.J., Bellapart, J., Ogoh, S., Smith, K.J., Smirl, J.D., Day, T.A., Lucas, S.J., Eller, L.K., and Ainslie, P.N. (2011) 'Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function', *Journal of Neuroscience Methods*, 196 (2), pp. 221-237.

Willie, C.K., Tzeng, Y.C., Fisher, J.A., and Ainslie, P.N. (2014) 'Integrative regulation of human brain blood flow', *Journal of Physiology*, 592 (5), pp. 841-859.

Yamaji, K., and Miyashita, M. (1978) 'Differences in cardio-respiratory responses to exhaustive exercise between athletes and non-athletes', *European Journal of Applied Physiology*, 38 (4), pp. 233–238.

Zeppilli, P., Vanniculli, R., Dello Russso, A., Picani, C., Palmieri, V., Cameli, S., Corsetti, R., and Pietrangeli, L. (1955) 'Echocardiographic size of conductance vessels in athletes and sedentary people', *International Journal of Sports Medicine*, 16 (1), pp. 38-44.