# The effects of sitting on cerebrovascular and cognitive function

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# Abstract

Sedentary behaviour (SB) has emerged as a risk factor for cardiovascular morbidity and mortality, independent of physical activity (PA) levels. Despite associations between SB and cerebrovascular disease, little research has assessed the influence of SB on cerebrovascular function, comprising cerebral blood flow (CBF), cerebral autoregulation (CA) and cerebrovascular carbon dioxide reactivity (CVR). This is of upmost importance since the maintenance of cerebrovascular function appears critical for cognition, mood and the prevention of cerebrovascular diseases. Consequently, the overarching aims of this thesis were to explore the effects of sitting on cerebrovascular function, cognition and mood and to explore the effect of breaking up sitting on these parameters.

Study one assessed whether objectively measured workplace sitting and PA were associated with cognition and mood. Results showed workplace sitting was negatively associated with calm mood state, but not cognition. Standing and stepping whilst at work were positively associated with aspects of cognition (working memory and attention) and mood (positive affect and calm and content mood states), indicating PA throughout the workday should be encouraged as it may have beneficial effects on mental wellbeing and cognitive performance. In contrast to guidelines advising increasing light-intensity PA in the workplace, only moderate-intensity PA at work was positively associated with working memory, possibly indicating this higher intensity of PA should be encouraged during work hours to positively influence cognitive performance in desk workers.

Study two aimed to determine the acute effects of a prolonged sitting period on cerebrovascular function, cognition and mood in healthy desk workers. Uninterrupted sitting for six hours reduced CBF and impaired aspects of CA but had no effect on CVR. Decreases in positive affect, and the alert and content mood states were also observed, but these were not related to the concurrent changes in cerebrovascular function. There was no change in cognition following prolonged sitting. Results may have important implications for the long-term mental and physical health of individuals who are repeatedly exposed to periods of uninterrupted sitting.

Study three assessed the acute effects of breaking up sitting time on cerebrovascular function in healthy desk workers using two different walking break strategies. The decrease in CBF and CA observed following four hours of uninterrupted sitting was prevented using frequent, short duration walking breaks rather than less frequent, longer duration walking breaks. Results further demonstrate that prolonged uninterrupted sitting impairs cerebrovascular function and suggest that the frequency of the breaks used to interrupt sitting is an important component to preserve aspects of function. In contrast, both

walking break strategies caused a larger increase in CVR compared to prolonged sitting. This indicates that, for this aspect of cerebrovascular function, any duration or frequency of PA may have acute benefits.

Study four assessed whether using a computer-based prompting software designed to break up prolonged sitting at work altered cerebrovascular function, cognition and mood in healthy office workers. Following the intervention, workplace sitting was reduced and replaced predominantly by increased time spent standing. This reduction in sitting improved aspects of CA but had no influence on other measures of cerebrovascular function, cognition or mood. Results provide preliminary evidence that long-term reductions in SB may improve aspects of cerebrovascular function.

Overall, the major findings of this thesis are that prolonged, uninterrupted sitting acutely impairs aspects of cerebrovascular function, however this can be prevented by breaking up sitting with short duration, regular walking breaks. Prolonged sitting also acutely impairs aspects of mood but not cognition. Taken together this thesis provides the first evidence that SB negatively effects cerebrovascular function and further research should explore whether this leads to heightened cerebrovascular disease risk.

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*I Can't Get No Sleep*

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*The Only Way Is Essex*

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Finally, I would like to dedicate this thesis to my grandparents; they did not get to see either of their grandchildren begin their journeys at University and beyond, but I would like to think we have done them proud.

# Declaration

I declare that the work contained within this thesis is entirely my own.

***Publications directly based on the work described in this thesis***

**Carter, S. E.**, Draijer, R., Holder, S. M., Brown, L., Thijssen, D. H. J. and Hopkins, N. D. (2018). Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting. Journal of Applied Physiology, DOI: 10.1152/japplphysiol.00310.2018.

**Carter, S. E.**, Draijer, R., Holder, S. M., Brown, L., Thijssen, D. H. J. and Hopkins, N. D. (under review). Breaking up sitting with different frequencies and durations of physical activity: effects on femoral artery endothelial function. Journal of Science and Medicine in Sport.

**Carter, S. E.**, Draijer, R., Brown, L., Thijssen, D. H. J. and Hopkins, N. D. (under review). Acute effects of prolonged sitting on cerebrovascular function, cognition and mood. American Journal of Physiology-Heart and Circulatory Physiology.

***Other publications completed by the candidate during PhD tenure***

**Carter, S.,** Hartman, Y., Holder, S., Thijssen, D. H. J. and Hopkins, N. D. (2017). Sedentary behavior and cardiovascular disease risk: mediating mechanisms. Exercise and Sport Sciences Reviews, 45;2:80-86.

Thijssen, D. H. J., **Carter, S. E.** and Green, D. J. (2016). Arterial structure and function in vascular ageing: are you as old as your arteries? Journal of Physiology, 15;594(8):2275-84.

Holder, S. M., Low, D. A., Graves, L. E. F., **Carter, S. E.**, Brislane, Á. M. and Hopkins N. D. (in preparation). The effect of sedentary behaviour on cardiovascular biomarkers in active, healthy adults.

Brislane, Á., **Carter, S. E.** Holder, S. M., Jones, H., Low, D. and Hopkins, N. D. (in preparation). How does age and menopausal status affect peripheral and cerebrovascular adaptations across the female lifespan?

Roberts, K. A., Draijer, R., Thijssen, D. H, J., Hopkins, N. D., de Graaf, Y., Holder, S. M., **Carter, S. E.** and Low, D. A. (in preparation). Green tea attenuates the deleterious impact on peripheral vascular function associated with an unhealthy lifestyle.

***Oral communications***

**Carter, S. E.**, Draijer R., Brown, L., Thijssen, D. H. J. and Hopkins, N. D. Acute effects of a prolonged sitting period on cerebrovascular function, cognitive performance and mood. Okanagan Cardiovascular and Respiratory Symposium, Okanagan, British Columbia, Canada, 2018. **Runner Up: Best Postgraduate Oral Presentation**

**Carter, S. E.**, Draijer R., Brown, L., Thijssen, D. H. J. and Hopkins, N. D. Can prolonged sitting impair cerebral blood flow and autoregulation? Dick Thijssen Professorial Research Symposium, Liverpool, UK, 2017.

**Carter, S. E.**, Draijer R., Brown, L., Thijssen, D. H. J. and Hopkins, N. D. The effect of sedentary behaviour on cerebrovascular function, cognition and performance in adults. Research Institute for Sport and Exercise Sciences Sedentary Behaviour Seminar, Liverpool, UK, 2017.

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**Carter, S. E.**, Draijer, R., Brown, L., Thijssen, D. H. J. and Hopkins, N. D. Acute effects of a prolonged sitting period on cerebrovascular function, cognitive performance and mood. Northern Vascular Biology Forum, Liverpool, UK, 2017.

**Carter, S. E.**, Draijer, R., Holder, S. M., Thijssen, D. H. J. and Hopkins, N. D. The effect of breaking up prolonged sitting on cerebral blood flow. American College of Sports Medicine, Boston, USA, 2016.

***Invited communications***

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# Contents

# List of Abbreviations

Anterior Cerebral Arteries (ACA)

Area Under the Curve (AUC)

Attention Network Test (ANT)

Blood Pressure (BP)

Body Mass Index (BMI)

Carbon Dioxide ($CO_2$)

Cardiovascular Disease (CVD)

Cerebral Autoregulation (CA)

Cerebral Blood Flow (CBF)

Cerebral Blood Flow Velocity (CBFv)

Cerebrovascular Carbon Dioxide Reactivity (CVR)

Cerebrovascular Conductance (CVC)

Common Carotid Artery (CCA)

Fast Fourier Transformation (FFT)

Flow-mediated Dilation (FMD)

Heart Rate (HR)

Health and Work Questionnaire (HWQ)

High Frequency (HF)

International Physical Activity Questionnaire (IPAQ)

Intraclass Correlation Coefficient (ICC)

Low Frequency (LF)

Mean Arterial Pressure (MAP)

Metabolic Equivalents (METs)

Middle Cerebral Arteries (MCA)

Middle Cerebral Artery Blood Flow Velocity (MCAv)

Neurovascular Coupling (NVC)

Nitric Oxide (NO)

Partial Pressure of Arterial Carbon Dioxide ($PaCO_2$)

Partial Pressure of End-Tidal Carbon Dioxide ($PETCO_2$)

Physical Activity (PA)

Positive and Negative Affect Schedule (PANAS)

Posterior Cerebral Arteries (PCA)

Posterior Cerebral Artery Blood Flow Velocity (PCAv)

Rating of Perceived Exertion (RPE)

Reaction Time (RT)

Regions of Interest (ROI)

Sedentary Behaviour (SB)

Shear Rate (SR)

Standard Deviation (SD)

Transcranial Doppler Ultrasound (TCD)

Transfer Function Analysis (TFA)

Very Low Frequency (VLF)

Workforce Sitting Questionnaire (WSQ)

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# 1. Introduction

## 1.1. Background

Sedentary behaviour (SB) has emerged as an independent risk factor for cardiovascular and metabolic health (Dunstan et al., 2012; Healy et al., 2008). SB refers to any waking behaviour in a sitting, reclining or lying posture with an energy expenditure of less than 1.5 metabolic equivalents (Sedentary Behaviour Research Network, 2012; Tremblay et al., 2017) and includes activities such as workplace sitting, television viewing and computer use (Dunstan et al., 2012). The prevalence of SB is increasing, with the workplace identified as a key setting where most adults accrue SB since many light activity jobs now require extended sitting periods and are computer-based (Owen et al., 2010; Parry and Straker, 2013; Ryan et al., 2011). Indeed, UK office workers spend 60-65% of their work hours sitting which is not compensated with increased physical activity (PA) during leisure time (Clemes et al., 2014, 2016).

Associations between prolonged periods of SB and all-cause morbidity and mortality have been observed, which are not due to the lack of engagement in low-, moderate- or vigorous-intensity PA (Biswas et al., 2015). Indeed, there is a substantial body of prospective data on the associations of SB and the risk of developing diabetes mellitus and cardiovascular disease (CVD), as well as with overall mortality (Young et al., 2016). There is also some evidence that SB is associated with cerebrovascular diseases such as stroke (Chomistek et al., 2013; McDonnell et al., 2016). The potential mechanisms underlying the relationship between SB and CVD mortality and morbidity possibly relate to the impact of SB on traditional CVD risk factors. SB has negative connotations on body mass index, waist circumference (Campbell et al., 2018; Healy et al.,

2008; 2011), serum triglycerides, 2-hour plasma glucose, inflammatory markers (Healy et al., 2008; 2011) and blood pressure (Beunza et al., 2007; Gerage et al., 2015; Jakes et al., 2003; King et al., 2016). Additionally, recent experimental evidence suggests SB exerts direct and indirect effects on the vascular system itself. SB is associated with increased arterial stiffness (García-Hermoso et al., 2015) and carotid intima-media thickness (García-Hermoso et al., 2015). Furthermore, a single bout of prolonged sitting leads to acute lower limb peripheral conduit artery endothelial dysfunction (Restaino et al., 2015, 2016; Thosar et al., 2014; 2015), an early marker of atherosclerosis. However, to date research has focused on peripheral artery function and, despite some evidence that SB may be associated with cerebrovascular diseases, little experimental work has examined the direct effects of SB on cerebrovascular function.

Cerebrovascular function describes the mechanisms regulating cerebral blood flow (CBF) to maintain constant cerebral perfusion (Willie et al., 2014), preserving normal brain function (Willie et al., 2011) and preventing the risk of ischemic brain injury and damage (Tzeng and Ainslie, 2014; Wheeler et al., 2017; Willie et al., 2014). Impairments in cerebrovascular function can cause reduced cognitive functioning (Bertsch et al., 2009; Marshall et al., 2001), neurodegenerative diseases including dementia, Alzheimer's disease and stroke (Gommer et al., 2012; Keage et al., 2012; Wolters et al., 2017), and mood disorders such as depression (Honda et al., 2014; Nobler et al., 2002; Videbech, 2000) and bipolar disorder (Benabarre et al., 2005; Dev et al., 2015). Minimal research has considered the potential impact of SB on cerebrovascular function, cognition and mood. Physically active adults exhibit improved

cerebrovascular function compared to sedentary adults (Ainslie et al., 2008; Bailey et al., 2013; Brown et al., 2010) however, individuals were classified as sedentary if they did not complete regular PA and physical inactivity is distinct from SB. Mood appears to also be influenced by SB since experimentally increased SB enhanced negative mood state (Edwards and Loprinzi, 2016a; Endrighi et al., 2016), but the mechanisms underlying this response are unknown. Additionally, a systematic review recently concluded SB is negatively associated with cognitive function, however, as highlighted by the authors, all included studies were observational in design and used subjective assessments of SB, whilst some misclassified SB as a lack of PA (Falck et al., 2017). However, recently some evidence indicates SB may affect cognition since SB was associated with reduced thickness of the medial temporal lobe, which is thought to be the site of atrophy during cognitive decline (Siddarth et al., 2018). Collectively, to date in the few studies that have examined the influence of SB on cerebrovascular function, cognition and mood, most have not actually assessed SB or the research designs adopted mean causality cannot be determined, highlighting the need for further experimental research.

Following on from research demonstrating the potential detrimental health effects of SB, evidence is emerging that breaking up periods of prolonged SB with short PA bouts can improve cardiometabolic health and CVD risk factors. Laboratory studies have observed breaking up sitting with walking breaks prevents the detrimental impact of prolonged sitting on glucose metabolism (Bailey and Locke, 2015; Dunstan et al., 2012; Peddie et al., 2013), lower limb endothelial function (Thosar et al., 2015) and blood pressure (Larsen et al.,

2014). Similarly, breaking up sitting also improves mood and potentially cognition. Mood state increased when sitting was interrupted with hourly treadmill walking bouts (Bergouignan et al., 2016), whilst four days of following a free-living 'sit less' strategy also increased pleasantness (Duvivier et al., 2017). Acute improvements in cognitive performance were observed when breaking up eight hours of sitting with either standing, walking or cycling bouts (Mullane et al., 2017), however this has not been universally observed (Bergouignan et al., 2016; Duvivier et al., 2017; Wennberg et al., 2016). Furthermore, the mechanisms underlying these observations have not been investigated, but owing to the previously discussed influence of cerebrovascular function on mood and cognition, changes in cerebrovascular function may contribute.

In summary, SB has emerged as independent risk factor for cardiometabolic and cardiovascular morbidity and mortality. Research investigating the mechanisms underlying this risk to date have focused on traditional CVD risk factors and peripheral artery function. Despite associations between SB and cerebrovascular disease, little research has assessed the influence of SB on cerebrovascular function. This is of upmost importance since the regulation of cerebrovascular function may be critical for cognitive performance, mood and the prevention of cerebrovascular disease development. The inclusion of PA breaks during prolonged sedentary periods can improve mood and potentially cognition, however the mechanisms explaining this response are unknown, but may relate to changes in cerebrovascular function. Consequently, the overarching aims of this thesis are to investigate the effects of sitting on

cerebrovascular function, cognition and mood, and to explore the effect of breaking up sitting on cerebrovascular function, cognition and mood.

## 1.2. Aims and Objectives

The overall aims of this thesis are to:

1. Assess the relationship between workplace SB and PA, cognition and mood.
2. Determine the acute effects of a prolonged, uninterrupted sitting period on cerebrovascular function, cognition and mood.
3. Determine the acute effects of breaking up prolonged sitting with short bouts of light-intensity PA on cerebrovascular function.
4. Assess the changes in cerebrovascular function, cognition and mood following an 8-week intervention designed to break up prolonged sitting at work.

The aims outlined above will be achieved through the following objectives:

In line with Aim 1:

1. Objectively measure workplace SB and PA in a sample of full-time workers.
2. Assess whether the time spent sitting, standing and stepping during work hours is associated with cognition and mood.
3. Assess whether the time spent sitting, standing and stepping during weekdays and the weekend is associated with cognition and mood.

4. Assess whether the time spent in light-, moderate- and vigorous-intensity PA during work hours, weekdays and the weekend is associated with cognition and mood.

In line with Aim 2:

1. Measure cerebrovascular function, cognition and mood prior to and following an acute period of prolonged, uninterrupted sitting.
2. Assess whether any observed changes in cerebrovascular function are related to any observed changes in cognition or mood.

In line with Aim 3:

1. Compare the effect of a prolonged, uninterrupted sitting period to breaking up sitting with walking breaks on cerebrovascular function, cognition and mood.
2. Compare two different walking break protocols to establish the most effective PA break protocol to potentially enhance cerebrovascular function.

In line with Aim 4:

1. Engage office workers in an 8-week intervention using a computer-based prompting software designed to reduce workplace sitting.
2. Objectively assess SB and PA levels prior to and after using the prompting software for 8-weeks.
3. Compare the effects of using the prompting-software or a no-software control period on SB and PA levels, cerebrovascular function, cognition and mood.

# 2. Literature Review

**Parts of this review are based on the publication in Exercise and Sport Sciences Reviews, 2017 ‘Sedentary behavior and cardiovascular disease risk: mediating mechanisms.’**

## 2.1. The Cerebrovasculature and Cerebrovascular Function

### 2.1.1. Cerebral Vasculature

At rest, blood flow to the brain accounts for 15% of total cardiac output (Willie et al., 2011). The combination of the brain's high energy demand, accounting for 20% of the body's total resting oxygen consumption (Ainslie and Duffin, 2009), and small energy storage capacity (Willie et al., 2011) mean that the constant delivery and regulation of cerebral blood flow (CBF) is vital. The brain is perfused by four main blood vessels: two internal carotid arteries and two vertebral arteries, with the latter joining to form the basilar artery (Cipolla, 2009; Willie et al., 2014). These vessels subsequently unite to form the Circle of Willis, which in turn branches into three pairs of arteries: the anterior, middle and posterior cerebral arteries, with each pair perfusing the left and right side of the brain (Cipolla, 2009; Querido and Sheel, 2007). The middle cerebral arteries (MCA) and the anterior cerebral arteries (ACA) supply blood to the frontal, temporal and parietal brain regions, whilst the posterior cerebral arteries (PCA) supply the occipital lobe and inferior part of the temporal lobe. These vessels then branch into smaller pial arteries which travel across the brain surface and further divide into penetrating arteries and arterioles which infiltrate the cerebral cortex (Cipolla, 2009; Girouard and Iadecola, 2006; Willie et al., 2014; Figure 2-1).

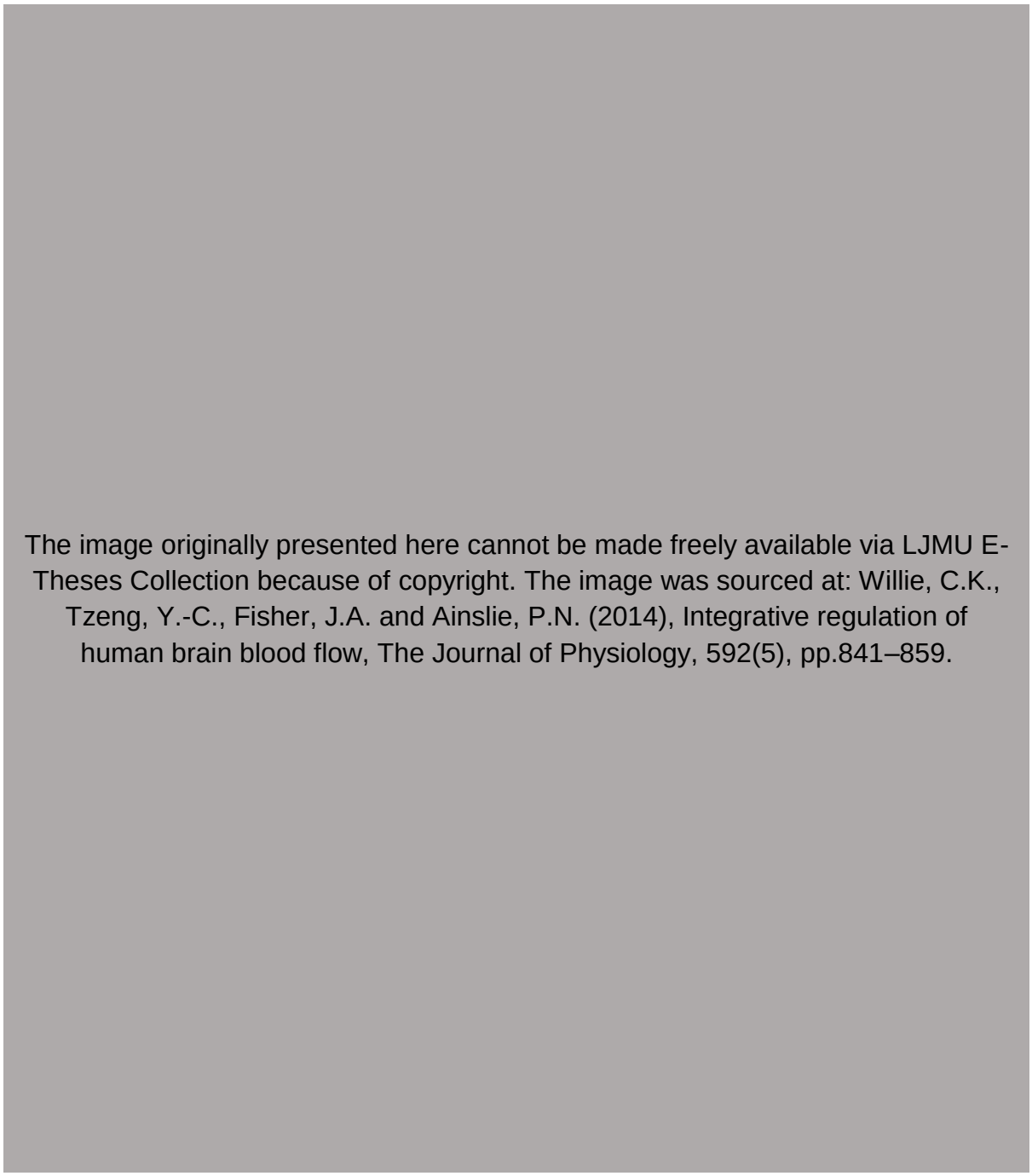



**Figure 2-1:** (a) The cerebral circulation (adapted from Willie et al., 2014). (b) Regions of the cortex supplied by the middle, posterior and anterior cerebral arteries.

### 2.1.2. Cerebral Blood Flow and Function

CBF is determined by cerebral perfusion pressure and cerebrovascular resistance (Ainslie and Duffin, 2009; Tzeng and Ainslie, 2014). Cerebral perfusion pressure is the difference in blood pressure (BP) at the circle of Willis and intracranial pressure, with the latter formed from central venous pressure and the pressures within the cerebrospinal fluid (Ainslie and Duffin, 2009). Cerebrovascular resistance describes the resistive forces acting on blood flow through the brain. Resistance to flow occurs mostly in the cerebral arteries and capillary beds, with increasing vascular tone in turn increasing resistance (Ainslie and Duffin, 2009; Phillips et al., 2016). Regulation of CBF is essential to prevent the risk of ischemic brain injury and damage, but also to prevent hyperperfusion of the cerebral tissues, as excessive blood flow can cause the breakdown of the blood-brain barrier, permitting the transudation of fluid into the interstitium and pericapillary astrocytes. Such changes can underlie the development of hyper-perfusion syndromes including seizures, headaches, encephalopathy and stroke (Tzeng and Ainslie, 2014). The regulation of CBF is an integrative process, with at least four key regulators involved: BP (cerebral autoregulation), chemical factors (cerebrovascular reactivity), cerebral metabolism (neurovascular coupling) and the autonomic nervous system (Willie et al., 2011a, 2012, 2014; Figure 2-2).

**Figure 2-2:** A summary of the main mechanisms contributing to the regulation of cerebral blood flow (CBF). BP- blood pressure; SNA- sympathetic nerve activity; $PCO_2$- partial pressure of carbon dioxide (adapted from Ainslie and Duffin, 2009).

*2.1.2.1. Chemical Factors*

The partial pressure of arterial carbon dioxide ($PaCO_2$) is the main regulator of CBF (Ainslie and Duffin, 2009), with cerebral perfusion exhibiting high sensitivity to changes in $PaCO_2$ levels (Willie et al., 2014). This high $PaCO_2$ sensitivity is unique to the cerebrovasculature (Ainslie et al., 2005) and is evident across the cerebral arterial tree. The pial arterioles are considered the main site of resistance modulation as they are located within the cerebrospinal fluid in the subarachnoid space, meaning they are readily exposed to changes in metabolic conditions (Willie et al., 2014).

The term cerebrovascular carbon dioxide ($CO_2$) reactivity (CVR) provides a measure of the ability of the cerebrovascular bed to dilate or constrict in response to changes in $PaCO_2$ (Ainslie and Duffin, 2009). Increased $PaCO_2$ elevates CBF by around 3-6% per millimetre of mercury change in $CO_2$ above that during normal breathing (Willie et al., 2014). In contrast, CBF declines in response to reductions in $PaCO_2$, with decreases observed between 1-3% per millimetre of mercury decrease in resting $CO_2$ (Willie et al., 2014). This regulation of blood flow functions to maintain cerebral $CO_2$ and in turn keep pH

levels constant (Willie et al., 2012). Furthermore, this serves as a control mechanism for respiration, as within the brainstem are respiratory chemoreceptors sensitive to alterations in pH (Ainslie and Duffin, 2009; Willie et al., 2012). The mechanisms underlying the cerebrovasculature's sensitivity to $CO_2$ are not fully explained (Ainslie and Duffin, 2009; Willie et al., 2014), but likely relate to changes in pH. It is suggested that reductions in blood pH activate potassium channels in the vascular smooth muscle, enacting endothelial cell hyperpolarisation and subsequent smooth muscle dilation. Additionally, shear-stress mediated release of vasodilators, such as nitric oxide (NO) and prostaglandins, may also contribute (Ainslie and Duffin, 2009).

*2.1.2.2. Blood Pressure*

Cerebral autoregulation (CA) is the physiological process that maintains constant CBF despite changes in mean arterial pressure (MAP) (Ainslie and Duffin, 2009; Numan et al., 2014; Querido and Sheel, 2007). The concept of CA was introduced by Lassen (1959), who developed a CA curve based on data gathered from studies examining various patient groups with a range of BP values. This curve showed CBF to be stable across a BP range of 60-150 mmHg (Figure 2-3a), termed static autoregulation (Willie et al., 2014). However, this curve was generated from data acquired as averages over several minutes and compared between different patient groups. Nonetheless, examining within-subject CBF responses to changes in BP is challenging as the baroreflex limits the range of BP values and using other techniques to manipulate BP, such as vasoactive drugs, can confound results. Despite this, recent within-subject data

suggests the static autoregulatory range is smaller than 60-150 mmHg (Willie et al., 2014; Figure 2-3b).

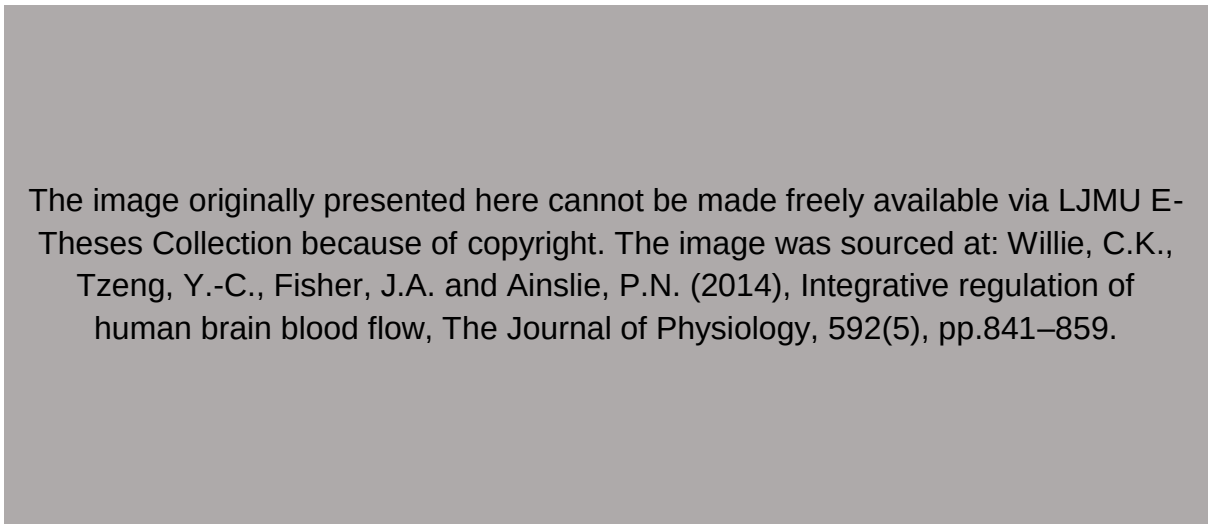



**Figure 2-3:** (a) The classical view of the dynamic autoregulation. (b) A schematic diagram based on contemporary data indicating a small plateau region for dynamic autoregulation (adapted from Willie et al., 2014).

In addition to static autoregulation, advancements in the temporal resolution of data collection technologies has enabled the development of the concept of dynamic autoregulation (Aaslid et al., 1989). Seminal work by Aaslid et al. (1989) demonstrated CBF followed the rapid decline in MAP that occurred during the release of inflated thigh-occlusion cuffs. Dynamic autoregulation therefore describes the transient response of CBF to sudden changes in BP (Panerai, 2009), such as when changing posture (Willie et al., 2014). Whilst static autoregulation maintains CBF during gradual variations in BP occurring over minutes to hours (Ainslie and Duffin, 2009; Numan et al., 2014), dynamic autoregulation refers to how CBF responds to rapid BP alterations occurring within a few seconds (Ainslie and Duffin, 2009). The latter has evolved as a result of being able to measure beat-to-beat CBF and BP, therefore they are

more of an experimental rather than physiological distinction (Willie et al., 2014).

CA occurs by altering cerebrovascular resistance in accordance with BP changes. Increases in BP causes vasoconstriction, whilst vasodilation occurs when BP decreases and together this functions to maintain a consistent blood flow (Gommer et al., 2012). In the main this regulation occurs at pial arteries via alterations in their vascular tone, however the physiological mechanisms underpinning this are unclear (Peterson et al., 2011; Tzeng and Ainslie, 2014). The endothelium and arterial vascular smooth muscle likely contribute via their mechanoreceptor properties and changes in shear stress and stretch responses respectively (Peterson et al., 2011). However, the larger extracranial arteries may also play a role in CA as, due to their compliant nature, it is suggested they mechanically buffer changes in BP based on the arterial Windkessel model (Chan et al., 2011; Willie et al., 2014). Indeed, during increases and decreases of BP via drug infusion the internal carotid artery has been shown to constrict and dilate respectively (Liu et al., 2013), whilst constriction of the internal carotid artery and vertebral artery contribute to a hypotension induced decrease in CBF during lower-body negative pressure (Lewis et al., 2015).

#### *2.1.2.3. Cerebral Metabolism*

Cerebral perfusion is linked to cerebral metabolic activity, whereby local blood flow changes according to the regions of the brain that are activated (Girouard and Iadecola, 2006; Willie et al., 2014). This temporal and regional linkage between neural activity and CBF response is termed neurovascular coupling

(NVC) (Phillips et al., 2016). Structurally, the vascular and nervous systems within the brain are closely linked, creating a neurovascular unit (Girouard and Iadecola, 2006; Willie et al., 2014) supporting this functional relationship. The neurovascular unit is formed of three components: the vascular smooth muscle cell, the neuron and the astrocyte glial cell (Phillips et al., 2016). As arterioles penetrate deep into the cerebral tissue they directly contact astrocytic end feet, linking to the nervous system (Girouard and Iadecola, 2006).

There are many mechanisms suggested to mediate NVC including vasoactive ions, metabolic by-products, and vasoactive factors released in response to neurotransmitters (Girouard and Iadecola, 2006). Increases in extracellular glutamine released from neural synapses are key to the process of NVC. Glutamine can interact with both neural and astrocyte cells to activate signalling cascades leading to vasodilation of local arteries due to the release of vasodilators (Figure 2-4). In neural cells, glutamine stimulates the release of NO via the N-methyl-D-aspartate receptor, while in astrocytes glutamine stimulates metabotropic glutamine receptors leading to the production of prostaglandins and epoxyeicosatrienoic acid (Girouard and Iadecola, 2006; Phillips et al., 2016). Pericytes, small contractile cells that wrap around capillaries, are also suggested to have an important role in NVC due to their closer proximity to neurons compared to arterioles. It is therefore suggested that neural activation first alters pericyte tone on capillaries via glutamine leading to vasodilation (Phillips et al., 2016). Vasoactive ions and factors also likely contribute to NVC. Neural signalling resulting in the generation of action potentials and synaptic transmissions produces potassium and hydrogen ions, which open potassium

channels on smooth muscle cells, causing dilation. Furthermore, during neuronal metabolism the production of adenosine and lactate also elicits vasodilation (Girouard and Iadecola, 2006). Taken together, the NVC response is the result of multiple mediators working to produce the functional link between neural activity and blood flow responses.

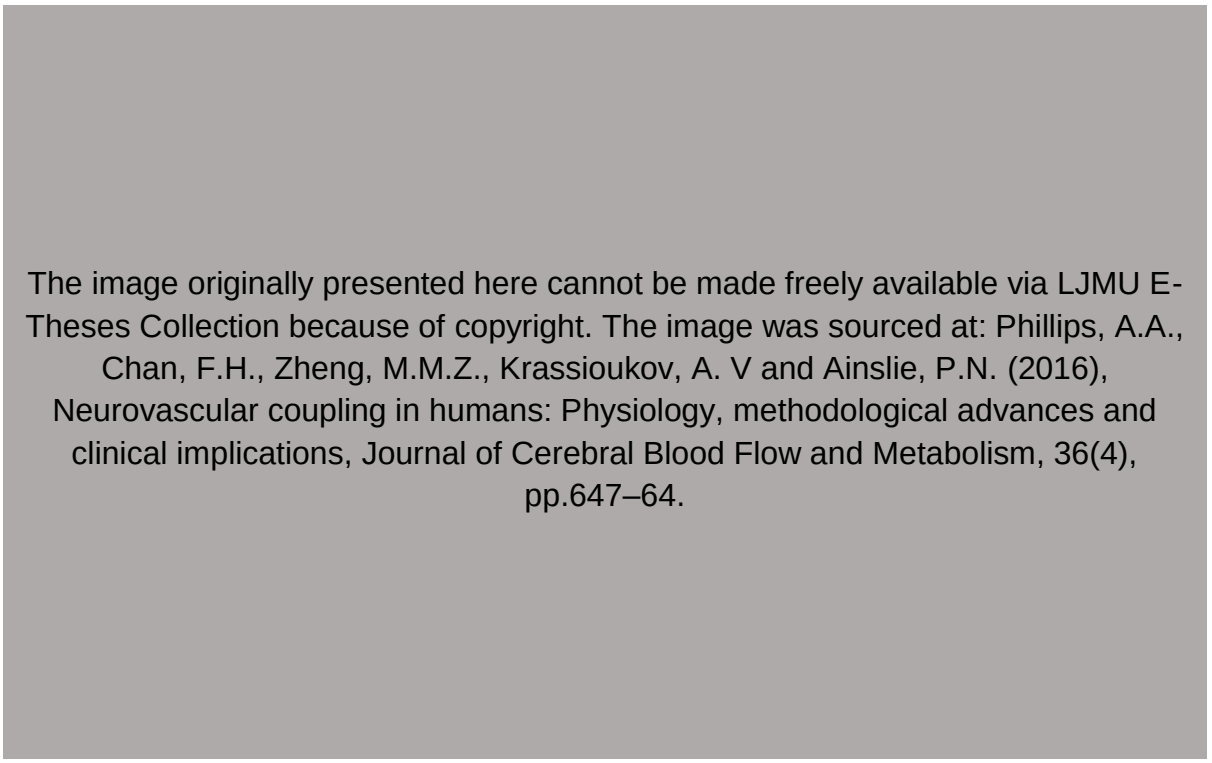



**Figure 2-4:** The primary glutaminergic pathways involved in neurovascular coupling (NVC) (adapted from Phillip et al., 2015).

*2.1.2.4. Autonomic Nervous System*

The cerebrovasculature is extensively innervated by adrenergic (sympathetic) and cholinergic (parasympathetic) fibres (Willie et al., 2014). Alpha-adrenergic receptors located in the extracranial arteries, (internal carotid artery, vertebral artery), intracranial arteries (MCA, PCA, ACA) and small pial arterioles are activated via noradrenaline release causing vasoconstriction. Whilst beta-adrenergic receptors are primarily located in the parenchymal arterioles, activation of which leads to vasodilation (Brassard et al., 2017). Despite this

high innervation, the sympathetic regulation of CBF is controversial (Ainslie and Duffin, 2009; Brassard et al., 2017; Willie et al., 2014). Indeed, it has been suggested that any sympathetic regulation is masked by the other stronger regulatory mechanisms, namely CA and CVR (Ainslie and Duffin, 2009). Furthermore, sympathetic and parasympathetic activity may instead have an indirect influence on CBF, since this neural activity also influences BP and venous tone, which contribute to CBF (Brassard et al., 2017).

However, using various approaches to investigate sympathetic nervous activity, research indicates sympathetic activity may contribute to the regulation of CBF. Indeed, the removal or blockade of ganglia increases CBF (Ter Laan et al., 2013; Willie et al., 2014). Furthermore, reductions in CBF following an orthostatic challenge are suggested to be in part due to sympathetic-mediated vasoconstriction of extracranial or intracranial arteries (Brassard et al., 2017; Tymko et al., 2016). For example, lower body negative pressure reduces MCA and PCA blood flow velocities despite controlling for hypocapnia, which could also cause vasoconstriction (Tymko et al., 2016), however sympathetic activity was not directly assessed. CA is also impaired following the removal of sympathetic activity (Hamner and Tan, 2014; Ter Laan et al., 2013; Zhang, 2004). For example, following pharmaceutical autonomic ganglion blockade, CA failed to attenuate the reduction in CBF that occurred during the BP-lowering Valsalva manoeuvre (Zhang, 2004). More recently, when modelling the relative contributions of sympathetic, cholinergic and myogenic mechanisms to CA, sympathetic activity was identified as the second largest contributor (Hamner and Tan, 2014). The role of the sympathetic nervous system in the response to

alterations in arterial $CO_2$ levels is however less clear with decreases (Zhang et al., 2011) or no change (Ainslie et al., 2005) following sympathetic nervous system modulation. However, these effects may in part be due to concurrent changes in MAP influencing results (Willie et al., 2014). Alternatively, there is a paucity of data examining the cholinergic regulation of CBF, especially in humans (Ainslie and Duffin, 2009). Nonetheless, systemic cholinergic blockade impaired CA in healthy humans, suggesting some contribution (Hamner et al., 2012).

Despite studies supporting a role for sympathetic activity in the control of CBF, this is not universal finding and hence the topic remains controversial (Ainslie and Brassard, 2014; Brassard et al., 2017). Factors adding to this controversy include if regional differences in cerebral circulation responses exist, the influence of perfusion pressure on sympathetic activity and whether brain metabolic activity may blunt the sympathetic vasoconstrictive response (Ainslie and Brassard, 2014). Additionally, differences in the methodologies used to assess CBF and the experimental approaches used to examine sympathetic activity may further contribute to discrepancies (Brassard et al., 2017). For example, in a review of studies that have used alpha-adrenergic blockade to assess CBF regulation, eleven out of the twelve studies concluded sympathetic activity regulates CBF; however in the two studies that used beta-adrenergic blockade the opposite conclusion was drawn (Brassard et al., 2017). Overall, it appears the autonomic nervous system contributes to the regulation of CBF, however the extent of its role is not clear.

In summary, the regulation of cerebrovascular function is a multi-dimensional process that functions to maintain constant CBF, in turn reducing the risk of damage to the brain. This regulation has a critical role in preserving vital brain functions, such as cognition and mood, as will now be discussed.

## 2.2. Cerebrovascular Function, Cognition and Mood

### 2.2.1. Cerebrovascular Function and Cognition

Cognition refers to the mental abilities that facilitate processes such as memory, planning, inhibition, and problem-solving (Gijselaers et al., 2016). Chronically, reduction in CBF is a risk factor for cognitive impairment (Ruitenberg et al., 2005) and is associated with cerebrovascular diseases whose aetiology include a decline in cognitive function, such as Alzheimer's disease and dementia (Sabayan et al., 2012; Schuff et al., 2009; Wolters et al., 2017; Yew and Nation, 2017). In healthy adults, a lower CBF at baseline is associated with a higher risk of dementia (Hazard Ratio: 1.31) and an accelerated rate of cognitive decline over the following seven years (Wolters et al., 2017). Furthermore, Alzheimer's disease patients at baseline with a lower CBF exhibit an increased degree of cognitive impairment over a two-year follow up (Benedictus et al., 2017). In healthy ageing, there is a progressive decline in CBF of around 28–50% from the age of 30 to 70 years (Ogoh and Ainslie, 2009). Concomitantly, cognitive performance is known to reduce with advancing age and may be partly associated with this decline. Indeed, young healthy adults exhibit higher resting CBF and superior cognitive performance compared to older counterparts (Bertsch et al., 2009).

CVR, NVC and CA are also impaired in Alzheimer's disease and dementia patients. Even after controlling for risk factors, MCA reactivity to hypercapnia and hypocapnia is lower in Alzheimer's disease and vascular dementia patients compared to healthy individuals (Glodzik et al., 2013; Vicenzini et al., 2007). Furthermore, a systematic review examining the relationship between CVR and

cognition using magnetic resonance imaging found CVR was consistently lower in these patients groups or in those with cognitive impairment compared to healthy individuals (Catchlove et al., 2018). Alzheimer's patients also exhibit impaired CA as, during BP oscillations the relative change in CBF is higher compared to healthy individuals, indicating less effective damping by CA (den Abeelen et al., 2014). Various studies have also shown that during visual and verbal stimulating tasks, Alzheimer's patients exhibit attenuated CBF in the corresponding cerebral regions, indicating NVC dysfunction (Girouard and Iadecola, 2006). Finally, impaired CVR also predicts future stroke incidence (Markus and Cullinane, 2001; Ogasawara et al., 2002) and transient ischemic attack incident (Markus and Cullinane, 2001), both of which can lead to diminished cognition (Ganzer et al., 2016; Sun et al., 2014).

In addition to clinical populations, impaired CVR can cause cognitive impairment in healthy adults. In older adults, those with a greater CVR were less likely to show cognitive decline over a a six-year follow up (Ruitenberg et al., 2005), whilst in young adults greater CVR was associated with better cognition as assessed by inhibitory control (Guiney et al., 2015). It is suggested impaired CVR may cause diminished cognition due to influence of other CVD risk factors on the cerebrovasculature (Catchlove et al., 2018). Indeed, the Framingham cardiovascular risk profile correlates with CVR (Glodzik et al., 2011), whilst peripheral artery disease patients exhibit impaired CVR and concomitant reductions in cognitive performance (Glodzik et al., 2011).

Collectively these studies indicate that in both healthy and patient groups, optimal functioning of the cerebrovasculature is important to maintain cognitive functions (Catchlove et al., 2018).

### 2.2.2. Cerebrovascular Function and Mood

Mood is defined as a short-term, diluted response to general environmental stimuli (Rothbard and Wilk, 2011). Moods tend to be diffuse, not focused on a specific cause and usually take the form of general positive or negative feelings (Frijda, 1986). Impairments in CBF are associated with mood disorders such as depression (Honda et al., 2014; Nobler et al., 2002; Videbech, 2000) and bipolar disorder (Benabarre et al., 2005; Dev et al., 2015). Patients with major depression have reduced blood flow in specific brain regions (Videbech, 2000) and reduced cerebrovascular reactivity (Neu et al., 2004). Furthermore, Alzheimer's disease patients with higher scores on the Geriatric Depression Scale exhibit significantly greater regional hypoperfusion compared to those with low scores (Honda et al., 2014). Interventions designed to increase cerebrovascular function also enhance mood. In post-menopausal women, fourteen weeks supplementation with resveratrol increased CVR and also mood, which was suggested to be due to an enhanced ability for the cerebral vessels to modulate brain perfusion during times of demand (Evans et al., 2017). Whilst these data indicate a role of cerebrovascular function in clinical mood disorders, relationships between CBF and function with everyday alterations in mood have received little attention and warrants further investigation.

Collectively, impairments in cerebrovascular function are associated with neurodegenerative diseases and subsequently reduced cognitive functioning. Moreover, cerebrovascular function may contribute to changes in mood. It is therefore of great importance to identify risk factors for cerebrovascular dysfunction to in turn prevent disease incidence. Meta-analyses and reviews indicate that physical activity (PA) is associated with decreased cerebrovascular disease risk (Guure et al., 2017; Stephen et al., 2017), however, less is known about the influence of sedentary behaviour (SB) on cerebrovascular function. The focus of the next section of this literature review is therefore to discuss SB as an independent health risk factor and the potential impact of SB on cerebrovascular function, cognition and mood.

## 2.3. Sedentary Behaviour

### 2.3.1. Definition and Prevalence of Sedentary Behaviour

Physical inactivity has long been recognised as detrimental to cardiovascular and metabolic health (Hamilton et al., 2008), however SB has also been identified as an independent health risk factor (Dunstan et al., 2012; Healy et al., 2008). SB describes any waking behaviour in a sitting, reclining or lying posture with an energy expenditure of less than 1.5 metabolic equivalents (METs) (Sedentary Behaviour Research Network, 2012; Tremblay et al., 2017) and includes activities such as workplace sitting, television viewing and computer use (Dunstan et al., 2012). The inclusion of a MET threshold within this definition has recently been challenged as some sitting-based activities, such as driving, exceed this limit (Henson et al., 2016; Mansoubi et al., 2015). Most importantly however, is that the definition of SB is distinct to that of physical inactivity, with the latter describing the failure to meet the minimum guideline of 150 minutes of moderate-intensity activity a week (Henson et al., 2016). It is therefore possible for an individual to be physically active, but also highly sedentary and this behavioural pattern has been termed the active couch potato phenomenon (Owen et al., 2010). Due to developments in transportation, workplace, and daily living technologies, SB is increasing both in the workplace and during everyday life (Dunstan et al., 2012; Owen et al., 2010). Although estimates of the prevalence of SB differs depending on the assessment tool, it is estimated that adults spend 6 to 8 hours per day in SB, which includes sitting, television viewing, screen time and computer use (Young et al., 2016).

### 2.3.2. Workplace Sedentary Behaviour

The workplace has been identified as a key setting where most adults accrue SB as many occupations are computer-based, resulting in predominantly sedentary work (Parry and Straker, 2013; Ryan et al., 2011). Indeed, workplace SB has increased during the past decades, with many light activity jobs now requiring extended sitting periods (Owen et al., 2010). UK office workers spend 60-65% of their work time sitting which is not compensated with increased leisure time PA (Clemes et al., 2014, 2016). During a weekday, English office workers spend 66.2% of their time sedentary, 23.3% of their time standing and 10.5% stepping (Smith et al., 2015). Furthermore, SB at work is accrued in prolonged periods and importantly the manner in which SB is accumulated has important implications for many health risk factors as described in section 2.7. Of their total sitting time, UK office workers spent 67% in sedentary bouts longer than 20 minutes, 52% in bouts longer than 30 minutes and 25% in bouts longer than 55 minutes (Ryan et al., 2011). In acknowledgement of the high prevalence of SB within workplaces, guidelines have been published emphasising the need to reduce this behaviour during the work day. It is suggested that a less sedentary office environment has the potential to enhance workforce health, productivity and profitability by reducing factors such as employee sickness and absenteeism (Buckley et al., 2015).

## 2.4. Sedentary Behaviour as an Independent Health Risk Factor

Associations between prolonged periods of SB and all-cause morbidity and mortality have been observed, which are not due to the lack of engagement in low-, moderate- or vigorous-intensity PA (Biswas et al., 2015). It could be postulated that the association between SB and disease risk and mortality is merely due to a lack of PA which is displaced by sedentary time. To date however the evidence indicates otherwise, in that SB is a risk factor independent of PA levels (Hamilton et al., 2008).

In adults meeting weekly PA guidelines, dose-response associations are observed between television viewing time and several cardiometabolic risk factors including waist circumference, systolic BP and 2-hour plasma glucose (Healy et al., 2008). Moreover, individuals participating in more than seven hours of moderate-to-vigorous PA a week, yet also accruing more than seven hours of daily television viewing time, present a two-fold greater risk of cardiovascular mortality compared with those engaging in seven hours of moderate-to-vigorous PA a week and only one hour of daily television viewing time (Matthews et al., 2012). Whilst these studies only considered television viewing time, a recent systematic review and meta-analysis assessing all forms of SB determined that, after statistical adjustment for PA, sedentary time was independently associated with increased risk for CVD incidence and mortality, in addition to all-cause mortality, cancer mortality and incidence, and type 2 diabetes (Biswas et al., 2015). A limitation to these studies showing associations between SB, morbidity and mortality is that causation cannot be determined. However, using Bradford Hill's causal criteria (Bradford-Hill, 1965),

which assesses if observed epidemiologic associations are causal, it was concluded that there is reasonable evidence for a likely causal relationship between SB and all-cause mortality (Biddle et al., 2016)

Nonetheless, the risk of mortality associated with sitting may be partially attenuated by PA. In a meta-analysis, compared to those sitting for one hour a day, the risk for mortality was 52% higher in physically inactive sedentary adults, whereas this risk was reduced to 34% in active sedentary individuals (Chau et al., 2013). More recent meta-analyses indicate engagement in at least one hour per day of moderate-intensity PA appears to offset the sitting-associated all-cause, CVD and cancer mortality (Ekelund et al., 2016, 2018). However, such high levels of PA greatly exceed current UK guidelines of 150 minutes of moderate-intensity PA a week (Department of Health Physical Activity Health Improvement and Protection, 2011), meaning those who sit for prolonged periods may still have an increased mortality risk. In addition to the engagement in PA reducing sitting-associated mortality, it appears even small movements in the form of fidgeting may also negate the risk. In a cohort of women followed up over 12 years, self-report fidgeting behaviour modified the association between sitting and mortality, independent of PA levels. Sitting for more than seven hours per day was associated with increased all-cause mortality in the low fidgeting group however not in the medium and high fidgeting groups (Hagger-Johnson et al., 2016). Objective and posture-specific assessments of fidgeting are needed to explore the potential protective role of fidgeting further. Collectively, data indicates SB contributes to disease

development and increased mortality risk and that this is largely independent from engagement in PA.

## 2.5. Sedentary Behaviour and Cardiovascular Health

### 2.5.1. Sedentary Behaviour and Cardiovascular Morbidity and Mortality

Evidence is accumulating that SB might be associated with increased cardiovascular-specific and overall mortality. Indeed, there is a substantial body of prospective data on the associations of SB with the risk of developing diabetes mellitus and CVD, as well as with overall mortality (Young et al., 2016).

Early work, using data from Australian Diabetes, Obesity and Lifestyle Studies, showed television viewing time (as a surrogate for SB) was associated with an increased risk of all-cause and CVD-related mortality. Every one-hour increment in television viewing time was associated with an 11% and 18% increased risk of all-cause and CVD mortality respectively. Furthermore, each additional hour of viewing time was associated with an increased CVD mortality risk of 18% (Dunstan et al., 2010). The negative association between SB and mortality persists when total sitting time is assessed, with dose-response relationships observed between all-cause mortality and sitting even among individuals with high levels of PA (van der Ploeg et al., 2012). Furthermore, in healthy women, sitting more than ten hours a day compared with five hours a day was associated with increased CVD risk (Hazard Ratio: 1.18), taking into account PA levels (Chomistek et al., 2013). Finally, a recent systematic review and meta-analysis demonstrated SB was associated with increased CVD incidence (Hazard Ratio: 1.14) and mortality (Hazard Ratio: 1.18) after statistical adjustment for PA (Biswas et al., 2015).

A small number of prospective studies have investigated the association of SB as a risk factor for developing type 2 diabetes mellitus, with most showing a consistent positive association, which has been further confirmed by meta-analyses and systematic reviews (Young et al., 2016). Once again using television viewing as a surrogate for SB, each additional two hours of viewing per day was associated with a relative risk of 1.20 of developing type 2 diabetes mellitus (Grøntved and Hu, 2011). More recently, a meta-analysis demonstrated both television viewing time and total SB were linearly associated with type 2 diabetes mellitus independent of PA (Patterson et al., 2018).

There is also some evidence that SB is associated with cerebrovascular disease, namely stroke incidence. In postmenopausal women over a mean follow-up of 12.2 years, sitting for more than 10 hours per day was associated with a stroke Hazard Ratio of 1.21 compared to those who sat for less than 5 hours per day (Chomistek et al., 2013). Further work assessing healthy males and females demonstrated that those who watched television (surrogate for SB) for more than four hours per day were significantly more likely to have a stroke than those who watched television for two hours per day (Hazard Ratio 1.37) (McDonnell et al., 2016), however snacking behaviours associated with television viewing may also contribute to this risk. It is suggested that prolonged sitting may increase stroke risk through detrimental effects on known stroke risk factors such as glycaemic control, BP and waist circumference (McDonnell et al., 2016), however further research is needed to understand potential mechanisms underlying this association.

### 2.5.2. Sedentary Behaviour and Cardiovascular Risk Factors

The potential mechanisms underlying the relationship between SB and CVD mortality and morbidity are currently unknown but are possibly related to the impact of SB on both traditional and novel CVD risk factors.

#### 2.5.2.1. *Traditional Risk Factors*

Early, large cross-sectional studies using data from the US National Health and Nutrition Examination Survey (2003-2004 and 2005-2006) and Australian Diabetes, Obesity and Lifestyle study (2004-2005) were the first to demonstrate detrimental associations between SB and CVD risk factors. Such studies showed that, in healthy adults, SB is positively associated with body mass index (BMI), serum triglycerides, 2-hour plasma glucose (Healy et al., 2008), waist circumference (Healy et al., 2008; 2011) and inflammatory markers (Healy et al., 2011). Since these seminal studies, further research supports this link between SB and CVD development.

In young and old adults with known risk factors for type 2 diabetes, SB is detrimentally associated with 2-hour plasma glucose, high-density lipoprotein cholesterol and triacylglycerol after adjusting for PA and BMI. Importantly, these associations were stronger compared to total or moderate-to-vigorous PA (Henson et al., 2013). More recently, a systematic review of objective, accelerometer-measured SB demonstrated total SB is negatively associated with insulin sensitivity, whilst some evidence supported an unfavourable association between SB and fasting insulin, insulin resistance (HOMA-IR) and triglyceride levels (Brocklebank et al., 2015). Furthermore, meta-analyses have

demonstrated high levels of SB are associated with a 112% increased risk (relative risk 2.12) of diabetes (Wilmot et al., 2012) and a 73% increased odds of metabolic syndrome (Edwardson et al., 2012) compared to low SB groups, and importantly such conditions are associated with CVD complications. Collectively, such data highlights SB has an important impact on several cardiometabolic health risk factors in both healthy and at-risk young and old populations and alterations in these metabolic parameters may further mediate the heightened CVD risk associated with sitting.

Cross-sectional and prospective observations indicate positive associations between SB and BP. Systolic BP was 2.1 mmHg and 1.5 mmHg lower for middle-aged men and women, respectively, in the lowest quartile of television viewing compared to the highest quartile (Jakes et al., 2003). Importantly, in middle-age individuals, a reduction in systolic BP of 2 mmHg is associated with a 7% lower incidence of death following stroke and a 10% lower incidence from other vascular causes (Lewington et al., 2002). Furthermore, in healthy university graduates the most sedentary subjects had a 48% increased risk of developing hypertension compared to their non-sedentary peers (Hazard Ratio: 1.48), independent of PA levels (Beunza et al., 2007). This relationship extends to populations with heightened CVD risk as more time spent sedentary was associated with higher brachial and central BP in hypertensive patients (Gerage et al., 2015), while in severely obese patients every additional hour of sitting was associated with a 14% higher risk of developing hypertension (King et al., 2016). Although this area of research shows promise, there are very little data

available, therefore the influence of SB on BP in both heathy and high-risk populations warrants further investigation.

Cross-sectional studies frequently find positive associations between SB and body weight (Campbell et al., 2018). Self-report SB is associated with body fat percentage (Wanner et al., 2016), waist circumference and BMI (Stamatakis et al., 2012). However, when SB is objectively monitored associations between waist circumference and BMI are no longer observed (Stamatakis et al., 2012), highlighting the potential limitations of self-report data such as the inaccurate reporting of data or response bias (Prince et al., 2008). More recently, a systematic review and meta-analysis of prospective studies including objective and self-report SB data observed a significant association between SB and waist circumference (Campbell et al., 2018). Furthermore, the odds ratio of becoming overweight or obese during follow-up was 1.33 in the highest compared with lowest category of SB (Campbell et al., 2018). Collectively, data indicates SB may have a small effect on body weight and composition, but whether these observed changes are clinically meaningful is questioned (Campbell et al., 2018).

*2.5.2.2. Novel Risk Factors*

Recent experimental evidence indicates that in addition to changes to traditional CVD risk factors, SB exerts direct and indirect effects on the vascular system itself, leading to increased CVD risk (Carter et al., 2017). Total sedentary time and bouts of sedentary time greater than ten minutes are associated with increased arterial stiffness (García-Hermoso et al., 2015) and carotid intima-

media thickness (García-Hermoso et al., 2015). Furthermore, weekend SB was positively associated with arterial stiffness, even after adjustment for vigorous PA (Huynh et al., 2014).

Endothelial dysfunction is an early marker of atherosclerosis (Bonetti, 2002; Lerman and Zeiher, 2005; Versari et al., 2009) and is associated with increased risk of cardiovascular events such as myocardial infarction, heart failure and stroke (Bonetti, 2002; Lerman and Zeiher, 2005). A single bout of prolonged sitting leads to acute lower limb peripheral conduit artery endothelial dysfunction, likely due to reductions in blood flow and shear stress (Restaino et al., 2015; 2016; Thosar et al., 2014; 2015). Three hours of uninterrupted sitting decreases superficial femoral artery mean shear rate (SR; an estimate of shear stress) and endothelial function (Thosar et al., 2014; 2015), whilst reductions in popliteal artery SR and endothelial function are also observed after six hours of sitting (Restaino et al., 2015). However, preventing lower limb decreases in blood flow and shear stress using limb heating (Restaino et al., 2016) or by small amounts of fidgeting leg movements (Morishima et al., 2016) abolishes the impairment in popliteal artery endothelial function observed in the opposite limb (Morishima et al., 2016; Restaino et al., 2016). This indicates SR contributes to sitting-induced vascular dysfunction. Contrastingly, sitting for up to six hours did not impair brachial artery endothelial function (Restaino et al., 2015; Thosar et al., 2014). This difference may be due to the study protocols completely restricting lower limb motion during sitting but permitting upper limb movements, thereby maintaining blood flow and SR, or that this vessel may

exhibit a greater resilience to SR reductions (Restaino et al., 2015; Thosar et al., 2014; 2015).

The type of shear stress experienced by the endothelium can also affect endothelial function. Antegrade shear stress, caused by a constant smooth laminar blood flow, preserves or enhances endothelial function, whilst retrograde or oscillatory shear, caused by turbulent blood flow at arterial bifurcations, promotes atherosclerosis and inflammation (Chatzizisis et al., 2007; Johnson et al., 2011). Three hours of sitting reduces antegrade shear in the superficial femoral and brachial artery and increases brachial artery oscillatory shear (Thosar et al., 2014). Interestingly, changes in the shear pattern of both vessels occurred over distinct time courses. The reduction in femoral artery antegrade SR was evident after one hour of sitting, coinciding with the reduction in endothelial function. In contrast, in the brachial artery the changes in antegrade and oscillatory SR were observed after three hours of sitting. These data indicate that over a relatively short time scale, uninterrupted sitting elicits negative effects on antegrade and oscillatory shear and, consequently, endothelial function in the lower limbs. However, the negative SB effects on shear patterns in the upper limb occur over a longer period and are not accompanied by endothelial dysfunction (Thosar et al., 2014). Studies of a longer duration are needed to fully examine the effects of sitting-induced alterations in shear patterns on endothelial function.

Increased inflammation may also contribute to the heightened CVD risk associated with sitting, as activation of the inflammatory cascade is a key

process in atherosclerotic plaque development (Willerson and Ridker, 2004) and is associated with CVD incidence (Healy et al., 2011). Indeed, greater SB is associated with increased markers of inflammation (Allison et al., 2012; Healy et al., 2011; Howard et al., 2015; Stamatakis et al., 2012; Yates et al., 2012), although this may in part be mediated by adiposity levels (Healy et al., 2011; Howard et al., 2015). Inflammatory cytokines also activate vascular production of reactive oxygen species (Zhang et al., 2010), which may further explain the association between SB and CVD risk as reactive oxygen species are thought to be an important component in the pathogenesis of CVD (Sugamura, 2011; Taniyama and Griendling, 2003; Zhang et al., 2010). Indeed, the reduction in superficial femoral endothelial function following three hours of sitting was prevented by oral administration of vitamin C, a potent reactive oxygen species scavenger (Thosar et al., 2015). However, the study did not perform additional testing to confirm that vitamin C was indeed responsible for a reduction in oxidative stress. Consequently, these initial findings support the need for further work to focus on a potential role of reactive oxygen species contributing to the impact of prolonged sitting on vascular function and subsequently CVD development.

Overall, there is accumulating evidence that prolonged sitting is associated with increased mortality risk and CVD development which cannot be explained by an absence of PA. This association likely relates to the effect on CVD risk factors and experimental work has begun to explore the mechanisms underlying these associations. However, to date research has focused on peripheral arteries and, despite some evidence that SB may be associated with cerebrovascular

diseases, little experimental work has examined the direct effects of SB on the cerebrovasculature.

## 2.6. Sedentary Behaviour, Cerebrovascular Function, Cognition and Mood

The influence of SB on cerebrovascular structure and function, and how this may in turn influence cognition and mood, has received little scientific attention. Investigating the impact of SB is of critical importance considering the role of cerebrovascular function for cognition, mood and neurodegenerative disease development. Furthermore, due to the high prevalence of workplace SB, any influence on cerebrovascular function, cognition or mood would have important implications for the health, productivity, performance and presenteeism of the workforce (Buckley et al., 2015; Wennberg et al., 2016).

### 2.6.1. Sedentary Behaviour and Cerebrovascular Function

To date, there are no studies specifically assessing the influence of SB on cerebrovascular function (Zlatar et al., 2014). Research exploring differences between physically active or exercise-trained individuals and sedentary individuals does however provide indication that cerebrovascular function is influenced by SB. Endurance-trained men had a 17% greater CBF compared to sedentary counterparts, which was present across more than a 60 year age span (Ainslie et al., 2008). Decreased CBF has also been observed in older sedentary women, who were classified as having a $\dot{V}O_2$max less than 90% of their age-predicted value, compared to older active women, who undertook regular aerobic exercise and had a $\dot{V}O_2$max greater than 90% of their age-predicted value (Brown et al., 2010). Furthermore, when comparing both old and young sedentary individuals who completed no recreational activity outside of everyday living to trained individuals who completed at least 150 minutes of moderate-to-vigorous intensity activity each week, the latter had enhanced CVR

(Bailey et al., 2013). Conversely, there was no difference in CA between Masters athletes and sedentary controls (Aengevaeren et al., 2013). However, in these studies participants were classified as sedentary if they did not complete regular PA or exercise training, or based on their $\dot{V}O_2$max. Yet, physical inactivity and training status are distinct from SB and it is possible an individual may have an active lifestyle but still have a low $\dot{V}O_2$max, thus these studies did not truly assess SB. Interestingly, those with a genetic deposition for Alzheimer's disease have shown increased CBF with longer sedentary time when objectively measured using accelerometery (Zlatar et al., 2014). However, this is suggested to be a compensatory mechanism for the greater metabolic demand for neuronal activity associated with the disease (Zlatar et al., 2014). Consequently, there is a need for future research to establish whether SB is an independent risk factor for impaired cerebrovascular function.

### 2.6.2. Sedentary Behaviour and Cognition

To date only a small number of studies have investigated the influence of SB on cognition and few have explored potential mechanisms. A systematic review concluded SB is negatively associated with cognitive function (Falck et al., 2017). However, of the eight included studies, all were observational in design, all included subjective methods to assess SB (of which some had not been previously validated) and some misclassified SB as a lack of PA (Falck et al., 2017). Consequently, this conclusion should be viewed cautiously.

Mechanistically, it has been suggested that SB may be a risk factor for cognitive decline due to the interaction between brain blood glucose regulation and CBF.

It is proposed that SB leads to impaired glucose regulation, in turn reducing CBF and that over time this may present as a risk factor for cognitive decline and disease development (Wheeler et al., 2017). Acute hyperglycaemia reduces regional CBF and increases insulin to enable glucose clearance. This creates a glucose nadir, which can impair endocrine counter-regulation to subsequent decreases in glucose, exacerbating the hypoglycaemia. Chronically, poor glycaemic control can impair brain structure and function (Geijselaers et al., 2015) by causing pericyte damage and endothelial dysfunction of brain arterioles, resulting in chronic hypoperfusion (Wheeler et al., 2017). Importantly, hypoperfusion of the brain may be both a consequence and a cause of early neurodegeneration in both vascular dementia and Alzheimer's disease (Wheeler et al., 2017). With relation to SB, glycaemic regulation is impaired during prolonged sitting periods (Dempsey et al., 2016; Dunstan et al., 2012; Duvivier et al., 2016; Peddie et al., 2013), suggesting SB could contribute to cognitive impairments.

Recent research examining the effects of SB on structural changes to the brain provides indication that SB does affect cognitive functioning. The atrophic processes that occur during cognitive decline are thought to take place in the medial temporal lobe of the brain which is involved in memory processes. Indeed, medial temporal lobe volume atrophy is associated with memory impairment and Alzheimer's disease (Rusinek et al., 2003). In non-demented middle-aged and older adults, SB was associated with reduced thickness of the medial temporal lobe. Importantly, no association was found between PA and lobe thickness indicating SB is a more significant predictor of changes in brain

structure (Siddarth et al., 2018). Whilst results must be viewed with caution as the study relied on self-report PA and SB levels, it further supports the need to investigate the potential mechanisms mediating relationships between SB and cognition.

### 2.6.3. Sedentary Behaviour and Mood

Long-term studies suggest SB itself may directly influence mood. One week of free-living SB decreased mood in older adults (Edwards and Loprinzi, 2016a). Furthermore, pleasantness was increased after four days of reducing sitting time (Duvivier et al., 2017), while breaking up six hours of uninterrupted sitting with hourly treadmill walking bouts enhanced mood state (Bergouignan et al., 2016). Moreover, these findings have been replicated over a longer exposure to SB. Negative mood score increased following two weeks of free-living SB, independent of changes in objectively measured moderate-to-vigorous PA (Endrighi et al., 2016). The mechanisms underlying the association between SB and mood require further investigation, but may relate to increased inflammation (Endrighi et al., 2016). Longer sitting time is associated with markers of systemic inflammation (Healy et al., 2011; Howard et al., 2015) and interestingly, following increased sitting time, individuals with greater mood disturbance had an elevated inflammatory response to a stress test (Endrighi et al., 2016). This enhanced response was suggested to increase negative mood by upregulating inflammatory signalling pathways and increasing vulnerability to mood disturbances (Endrighi et al., 2016). This is plausible considering inflammation is a critical mediator in the pathophysiology of mood disorders (Rosenblat et al., 2014).

SB is also associated clinical mood disorders, such as depression (Stubbs et al., 2018; Zhai et al., 2015) and anxiety (Teychenne et al., 2015; Vancampfort et al., 2018). A meta-analysis of observational studies demonstrated SB is associated with increased risk of depression, with relative risk of depression 1.25 for those with the highest SB (Zhai et al., 2015). Indeed, adults with depression spend on average 26 more minutes per day in SB than non-depressed individuals, with the highest prevalence of depression in those spending more than eleven hours sedentary (Stubbs et al., 2018). Since depression is associated with cognitive impairment (Stubbs et al., 2018), this further indicates reducing SB could enhance cognition and mood. The risk of anxiety is also elevated as SB increases (Teychenne et al., 2015). Adults with anxiety engage in 24 more minutes per day of SB than non-anxious individuals (Vancampfort et al., 2018). Furthermore, when SB was experimentally increased for one week in young adults, anxiety levels increased (Edwards and Loprinzi, 2016b). A possible explanation for these associations is that SB may displace PA, which has been shown to be beneficial in reducing the risk of mood disorders (Zhai et al., 2015), but further research is required exploring the mechanisms underlying these associations.

Overall, a small body of research has considered the potential impact of SB on cerebrovascular function, cognition and mood; however most have not actually assessed SB and, while mechanisms have been suggested, these have not been explored or tested. Consequently, these areas warrant further research. Collectively this will allow a true picture of the influence on SB on cerebrovascular health, and in turn allow suitable interventions to be designed

to reduce any associated health risks. Indeed, evidence is emerging that breaking up periods of prolonged sedentary time with either short PA bouts or merely standing up can improve cardiometabolic health and CVD risk factors.

## 2.7. Breaking Up Sedentary Behaviour with Physical Activity

Many health promotion and PA interventions aim to reduce SB by targeting moderate-to-vigorous PA, which is unlikely to be achievable within the constraints of a high SB environments such as the workplace (Buckley et al., 2015). Importantly, accumulating evidence suggests that light-intensity PA is beneficially associated with biomarkers of cardiometabolic health and may reduce mortality risk (Füzéki et al., 2017). Collectively this indicates that sedentary individuals should be encouraged to engage in PA of low intensities to confer improvements to health, which is supported by research studies using light-intensity PA to break up SB.

The health benefits of breaking up sitting were first demonstrated in large cross sectional studies using data from the Australian Diabetes, Obesity and Lifestyle Studies which showed the number of interruptions to sedentary time, independent of total sedentary time and time spent in moderate-to-vigorous PA, is beneficial to BMI, serum triglycerides, 2-hour plasma glucose (Healy et al., 2008), waist circumference (Healy et al., 2008; 2011) and inflammatory markers (Healy et al., 2011). More recent cross-sectional data has started to delineate the specific activity-related benefits of breaking up SB. Increasing standing time by two hours per day with a concurrent reduction in the sitting time was associated with reduced fasting glucose, triglycerides and increased high-density lipoprotein cholesterol. While displacing two hours of sitting with stepping was associated with lower BMI, waist circumference, 2-hour plasma glucose, triglycerides and greater high-density lipoprotein cholesterol (Healy et al., 2015). This indicates there may be important differences to cardiometabolic

health markers based on the type of activity used to break up SB. Controlled laboratory-based studies have begun to address the different break modalities and frequencies that can achieve this.

A range of activity types have been shown to be effective at negating the negative consequences of prolonged sitting. Intermittently walking to break up SB enhances glucose metabolism by attenuating postprandial glucose and insulin levels (Bailey and Locke, 2015; Dunstan et al., 2012; Peddie et al., 2013). In obese or overweight participants, two-minute light- or moderate-intensity treadmill walking breaks every twenty minutes over five hours reduced postprandial plasma glucose by 24-30% and serum insulin levels by 23% (Dunstan et al., 2012). Similarly, but in a non-obese population, two-minute walking breaks every twenty minutes lowered postprandial plasma glucose by 16% (Bailey and Locke, 2015), demonstrating metabolic health improvements are not limited to higher risk populations. In healthy adults, breaking up three hours of sitting with five-minute light-intensity treadmill walks every hour prevented the decline in superficial femoral endothelial function that was otherwise observed (Thosar et al., 2015). Femoral artery endothelial function was also maintained in children who completed a ten-minute cycling bout every hour during three hours of sitting (McManus et al., 2015). Finally, in type 2 diabetes patients systolic and diastolic BP were reduced after breaking up sitting with either light-intensity walking breaks or simple resistance activities (Dempsey et al., 2016).

Importantly, the intensity of the PA break does not need to be high to produce metabolic changes. In overweight women, standing for five minutes every thirty minutes lowered postprandial glucose by 28% and insulin by 20% and such improvements were similar to that observed using treadmill walking breaks (Henson et al., 2016). Moreover, breaking up five hours of sitting with either light- or moderate-intensity PA breaks significantly lowered resting systolic BP by 2-3 mmHg and diastolic BP by 2 mmHg in overweight and obese adults (Larsen et al., 2014).

It appears the frequency of the PA bout is of importance; as breaking up sitting with frequent bouts of activity is more effective at enhancing metabolic health markers than a single continuous exercise session (Duvivier et al., 2013; Peddie et al., 2013). Interrupting nine hours of sitting with frequent, short duration treadmill walks lowered postprandial plasma insulin and glucose concentrations to a greater extent than a single thirty-minute walk followed by a prolonged sitting period and crucially, total exercise duration was the same for both conditions. Compared to sitting, postprandial glucose and insulin concentrations were lowered by 37% and 18% respectively with regular breaks, whilst the single PA bout lowered levels by only 4% and 10% (Peddie et al., 2013). Moreover, completing low-intensity standing and walking during the day was more effective at attenuating the impairment in insulin sensitivity following prolonged sitting than a single exercise bout (Duvivier et al., 2013). Taken together these data emphasise the importance of the dispersion of PA throughout prolonged sitting periods.

Longer-term research also supports the beneficial effect of breaking of SB on cardiometabolic health. Five days of alternating between standing and sitting every thirty minutes attenuated postprandial glucose levels, as has been observed in acute studies (Thorp et al., 2014). Interestingly, there was no difference in responses between day one to day five, indicating that longer time periods are required to produce larger cardiometabolic health improvements. Supporting this, when sitting was broken up with two-minute walking bouts every twenty minutes for three days, postprandial glucose was attenuated, but there was no difference in the magnitude of this reduction between day one and day three (Larsen et al., 2015).

### 2.7.1. Breaking Up Sedentary Behaviour and Cognition

It is well established that PA is associated with improvements in cognitive functioning and the maintenance of cognition in later life (Blondell et al., 2014; Hillman et al., 2008; Kramer and Erickson, 2007). Despite this association between PA and cognition, experimental studies assessing the effects of using PA breaks to interrupt sitting on cognition have shown little effect. Interrupting five hours of sitting with three-minute light-intensity walking bouts every thirty minutes had no effect on executive function, episodic memory or inhibition (Wennberg et al., 2016). The intensity of the walking breaks were suggested to be too low to influence cognition; however breaking up sitting with five-minute moderate-intensity walking breaks also had no influence on inhibitory control, attention and cognitive flexibility (Bergouignan et al., 2016). Furthermore, no differences were observed in attention, memory or executive function following four days of a free-living ‘sit’ (walking or standing for less than one hour a day)

strategy compared to a ‘sit less’ (substituting at least seven hours a day of sitting with walking or standing) strategy (Duvivier et al., 2017). Despite this, acute improvements in cognitive performance were observed when breaking up eight hours of sitting with either standing, walking or cycling bouts. A pooled cognitive z-score was significantly higher in all three activity conditions compared to sitting which was suggested to be due to posture-induced increases in arousal, as heightened arousal improves cognition (Mullane et al., 2017). Importantly however, unlike in previous studies (Bergouignan et al., 2016; Duvivier et al., 2017; Wennberg et al., 2016), cognition was assessed during the middle of day rather than at the end, so data is not truly comparable due to differences in SB exposure times.

### 2.7.2. Breaking Up Sedentary Behaviour and Mood

It is well documented that PA and exercise can enhance mood and that this is a primary benefit of PA (Berger and Motl, 2000). Furthermore, breaking up sitting time with PA breaks appears to improve measures of mood (Bergouignan et al., 2016; Duvivier et al., 2017) likely due to the known benefits of exercise for mental well-being (Paluska and Schwenk, 2000; Stathopoulou et al., 2006). Pleasantness increased after four days of following a free-living ‘sit less’ strategy, where participants substituted seven hours of sitting with walking and standing, compared to four days of free-living SB, where participants were instructed to restrict waking and standing to less than one hour per day (Duvivier et al., 2017). Furthermore, compared to six hours of uninterrupted sitting, breaking this time up with hourly treadmill walking bouts enhanced mood state (Bergouignan et al., 2016).

In summary, laboratory-based research studies have shown that breaking up SB with various types of PA can prevent impairments to cardiometabolic health markers. There is also promising evidence that breaking up sitting may improve mood. Consequently, the possibility of applying these strategies to high SB environments, such as the workplace, has been explored.

### 2.7.3. Breaking Up Sedentary Behaviour in the Workplace

Due to the high prevalence of workplace SB, a range of intervention strategies to reduce sitting have been employed targeting this environment. This can include, but is not limited to, changes to the workplace environment and its design such as active workstations, e-health interventions and adapting office layouts to promote more movement; changing workplace organisation policies; and the provision of information about the benefits of reducing SB (Shrestha et al., 2018).

Active workstations are designed to incorporate PA into normally sedentary desk tasks (Torbeyns et al., 2014) and can include treadmill desks, stepping or cycling devices positioned under a desk, or height-adjustable and sit-to-stand workstations (Neuhaus et al., 2014). Recent meta-analysis and reviews have concluded active workstations are an effective intervention strategy to reduce workplace sitting time (Commissaris et al., 2015; Neuhaus et al., 2014; Torbeyns et al., 2014), with a pooled effect of a reduction in 77 minutes of sitting per eight-hour workday (Neuhaus et al., 2014). It appears sit-to-stand desks appear to be driving this reduction in sitting, as when active workstations are reviewed individually, sit-to-stand workstations reduce sitting by 84 to 116

minutes per day however the effects of treadmill desks or cycling desks are inconsistent (Shrestha et al., 2018). This may however reflect the disproportionate research focus given to the different types of active workstation, with most studies examining sit-to-stand desks.

Despite active workstations showing promise as an intervention to reduce workplace sitting, the limitation of this strategy is the cost required by employers to purchase and install them, something which may be beyond the financial restraints of certain workplaces. As such, there is a need examine workplace interventions that are low-cost to consider workplaces with limited financial resources (Shrestha et al., 2018). One such alternative low-cost method to reduce workplace SB is using prompting devices to encourage workers to take a break from sitting. A range of computer (Evans et al., 2012; Gilson et al., 2015; Júdice et al., 2015; Mainsbridge et al., 2014, 2016; Pedersen et al., 2014; Swartz et al., 2014) and mobile phone (Bond et al., 2014; Pellegrini et al., 2015) technologies have been employed, with varying levels of success. A smartphone prompting app encouraging PA breaks resulted in significantly reduced sitting time by 47 minutes from baseline (Bond et al., 2014). Furthermore, computer-based prompts reduced the number and duration of sitting bouts lasting 30 minutes or longer (Swartz et al., 2014). Alternatively a computer programme with a pop-up window reminding workers to take a break did not significantly decrease total sitting time (Evans et al., 2012).

Whilst workplace interventions can reduce workplace sitting, minimal research has focused on their effect on health-related outcomes therefore any potential

influence on health parameters is less clear (Neuhaus et al., 2014; Winkler et al., 2018). The effect of sit-to-stand desk interventions are inconsistent. Office workers using sit-to-stand desks for three months had increased fasting high-density lipoprotein cholesterol concentrations, but no change in total cholesterol, triglycerides or blood glucose concentrations (Alkhajah et al., 2012). Contrastingly, office-based workers who displaced an afternoon of sitting with standing, demonstrated attenuated postprandial blood glucose concentrations alongside elevated energy expenditure (Buckley et al., 2014). A more recent randomised control trial evaluated the impact of using sit-stand workstations for eight weeks on cardiovascular and metabolic health outcomes in university office workers. Significant reductions in sitting time, alongside improvements in total cholesterol levels were observed, whilst there were likely and possible beneficial improvements in brachial artery endothelial function and diastolic BP respectively (Graves et al., 2015). Alternatively, the influence of prompting devices on markers of health is not well researched. However, a computer-based prompting software promoting workers to take PA breaks including walking breaks and desk-based exercises increased work-time PA and improved markers of health. Workers using the software for up to 26 weeks spent an additional eight minutes a day performing PA breaks (Mainsbridge et al., 2014, 2016; Pedersen et al., 2014), which resulted in increased self-report health and wellbeing (Mainsbridge et al., 2016), increased energy expenditure (Pedersen et al., 2014), and reductions in MAP (Mainsbridge et al., 2014).

Alternatively, multi-component interventions appear to be effective at both reducing workplace sitting (Shrestha et al., 2018) and improving health markers.

Such multicomponent interventions combine environmental changes, such as active workstations, with strategies such as organisational support, educational sessions and goal setting. For example, an intervention incorporating organisational, individual, and environmental components reduced prolonged workplace sitting time by 39 minutes and increased stepping time by 12 minutes (Maylor et al., 2018). Interventions of this type have reduced BP (Winkler et al., 2018), triglyceride and cholesterol concentrations (Winkler et al., 2018), and favourably improved body composition (Danquah et al., 2016; Maylor et al., 2018; Winkler et al., 2018).

Collectively, whilst laboratory-based studies have shown breaking up sitting can improve cardiometabolic health markers, there is a lack of research translating these practises into the workplace, where high amounts of SB are accrued. Indeed, a recent Cochrane review of workplace interventions designed to reduce sitting time highlighted the need for future research assessing valid measures of productivity and cardiometabolic health (Shrestha et al., 2018). Research needs to also consider using multi-component approaches and strategies other than active work stations, such as computer prompts, to reduce the costs associated with these interventions.

## 2.8. Summary

Prolonged SB has emerged as risk factor for cardiometabolic and cardiovascular morbidity and mortality, independent of PA levels. Despite this, little research has focused on the influence of SB on cerebrovascular function and health. This is of upmost importance since the regulation of cerebrovascular function is critical for cognitive performance, mood and the prevention of cerebrovascular disease development. The inclusion of PA breaks during prolonged sedentary periods can reduce cardiometabolic and cardiovascular risk factors, however the influence on cerebrovascular function is unknown. Breaking up sitting improves mood and potentially cognition, thus owing to the impact of cerebrovascular function on these variables, changes in cerebrovascular function may be a contributing mechanism (Figure 2-5). Consequently, research is needed assessing the acute and longer-term effects of SB on cerebrovascular function and, as seen in existing research, if using PA breaks during sitting can enhance cerebrovascular function or prevent any impairments. Furthermore, any impact of SB on cognition and mood should be considered. Owing to the high incidence of workplace SB, such information could have important implications for workers' health and productivity.

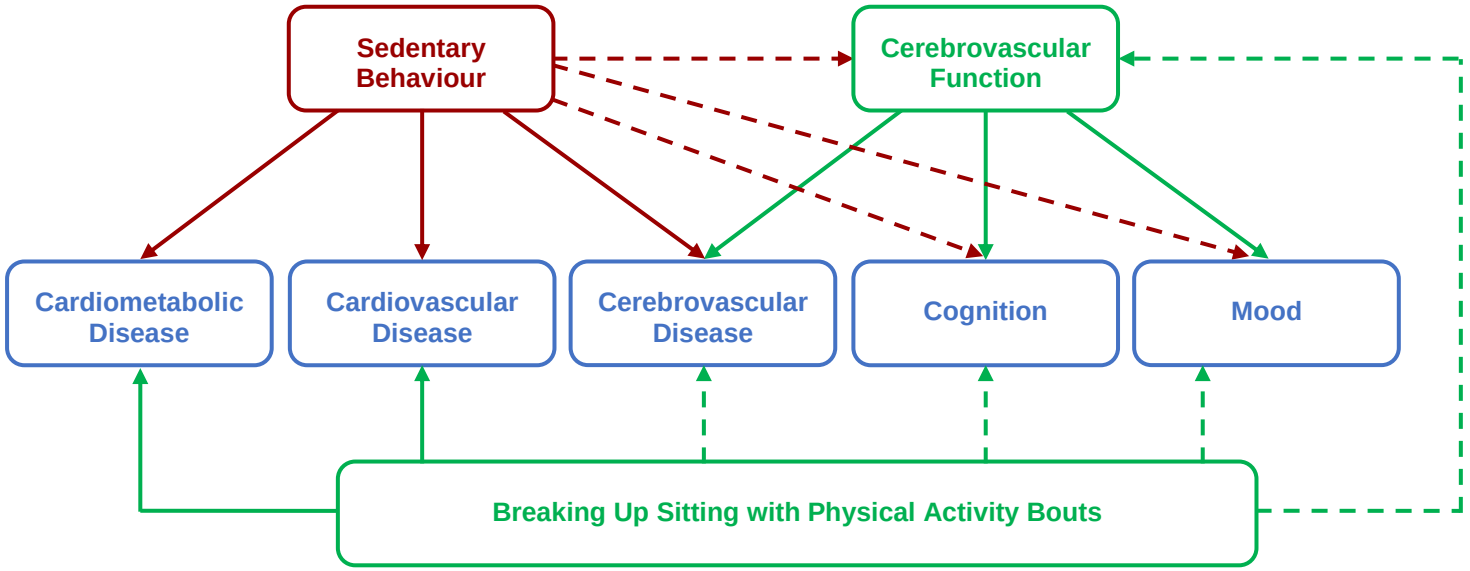



**Figure 2-5:** A summary of the known and potential interactions between sedentary behaviour, cardiometabolic, cardiovascular and cerebrovascular disease; cerebrovascular function; cognition and mood described in this literature review. Dashed lines indicate potential influence; red lines indicate negative influence; green lines indicate positive influence.

# 3. General Methods

The majority of the measurements and protocols undertaken in this thesis were adopted throughout all studies. This general methods chapter therefore describes general information regarding participants, data collection and analyses. The specific experimental design and protocols used for each study are detailed in the respective methods section in each chapter.

## 3.1. Participants

All participants were informed of the procedures and requirements for each study in writing and then written informed consent was obtained prior to inclusion. All participants were screened by the principal researcher prior to testing using an adapted health screening questionnaire based on the Physical Activity Readiness Questionnaire (PAR-Q; Adams, 1999). Participants were screened for exclusion criteria including: use of medication known to influence the cardiovascular and cerebrovascular system, smoker, BMI >35 or <18 $kg \cdot m^{-2}$, use of hormone-based contraception and diagnosis of cerebrovascular, cardiovascular or metabolic disease. Participants were desk-based workers who worked full-time, typically in an office environment.

## 3.2. Experimental Conditions

The experimental protocols in Chapters 5, 6 and 7 were conducted in a temperature controlled (20-22 °C) laboratory at the Research Institute for Sport and Exercise Sciences at Liverpool John Moores University. For multiple laboratory visits, participants attended at the same time of day between 7.00-9.00 am. Prior to experimental visits, participants were instructed to avoid strenuous exercise for 24 hours, complete an overnight fast and a 12-hour

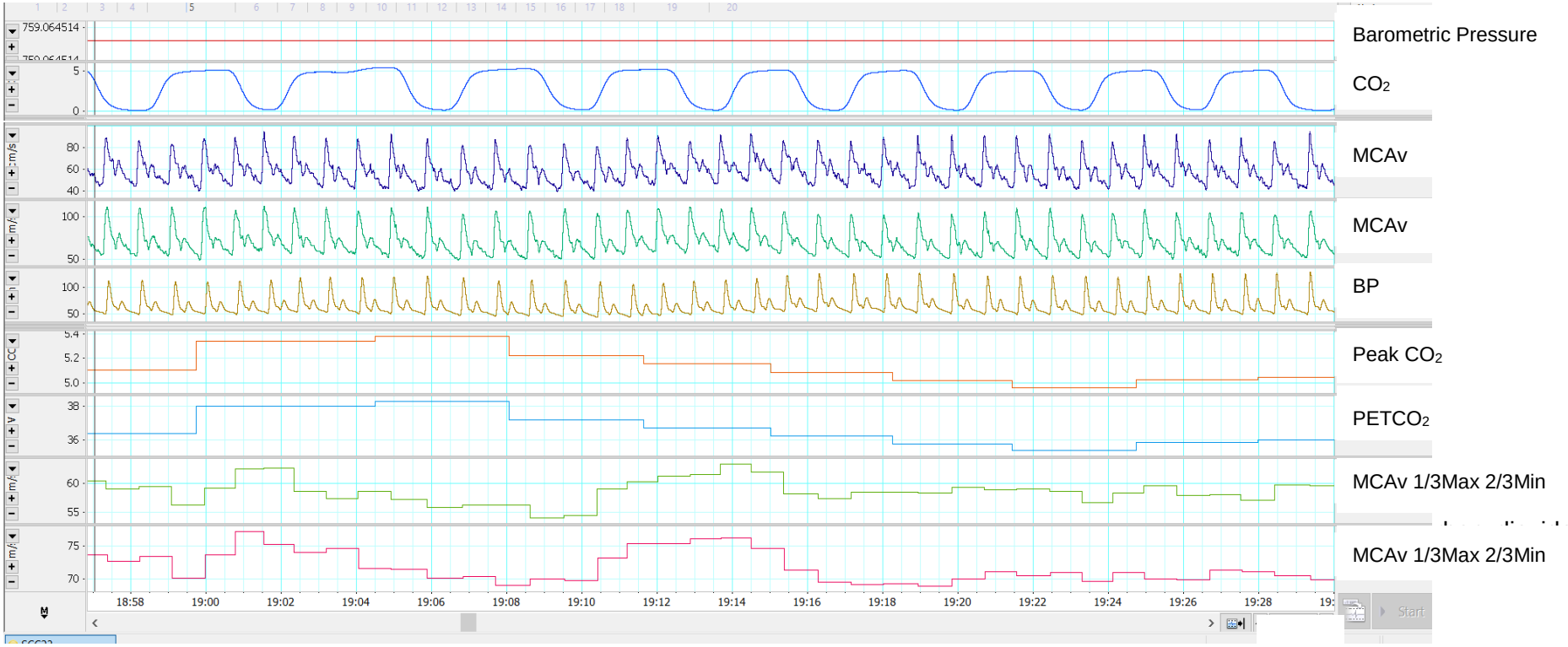
abstinence from caffeine and alcohol. Women were assessed in the follicular phase of the menstrual cycle (days 1-7). For Chapter 4 data collection occurred either at Liverpool John Moores University or at the participants' workplace. In the case of the latter, testing was conducted in a private, quiet room without any external disturbances. All study procedures were approved by the Liverpool John Moores University Ethics Committee and adhered to the Declaration of Helsinki.

## 3.3. Anthropometrics

For each participant, anthropometric measures of stature and body mass were acquired. Stature was measured to the nearest 0.1 cm using a portable stadiometer (SECA, Hamburg, Germany) with the participants' head in the Frankfort Plane. In minimal clothing and without shoes, body mass was measured to the nearest 0.1 kg using an electronic scale (SECA 799, Hamburg, Germany). BMI was subsequently calculated (mass/stature$^2$).

## 3.4. Data Acquisition

Physiological data measurements were continuously acquired at 50 Hz using an analogue-to-digital convertor (PowerLab ML880, ADInstruments, Colorado Springs, Colorado, USA) and displayed in real time on a computer with commercially available software (LabChart Version 7.0, ADInstruments;
Figure 3-1).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19:28
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BP
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PETCO2
MCAv 1/3Max 2/3Min
MCAv 1/3Max 2/3Min

## 3.5. Partial Pressure of End-Tidal Carbon Dioxide

The partial pressure of end-tidal carbon dioxide ($PETCO_2$) is the partial pressure or maximal concentration of $CO_2$ at the end of an exhaled breath, which is expressed as a percentage of $CO_2$ or mmHg. In this thesis, a gas analyser containing an infrared $CO_2$ sensor was used to measure $PETCO_2$ (ML206, ADInstruments, Colorado Springs, Colorado, USA). Expired gases were sampled at the mouth, with an expiratory sample line connecting a mouth piece to the gas analyser. A pump within the gas analyser draws the sample into the transducer and the percentage of inspired and expired $CO_2$ is then measured using an infrared transducer, which works on the principle of absorption spectroscopy. An infrared light is projected through the gas sample and any gas molecules that are the same size as the infrared light wavelength (in this case $CO_2$) are absorbed. Close to the end of the sampling tube there is an optical filter, which absorbs all wavelengths of light except that of $CO_2$. At the end of the sample tube, there is an infrared detector that records the amount of light that was not absorbed by the $CO_2$ molecules or optical filter. The difference in the amount of light projected and absorbed is proportional to the number of $CO_2$ molecules in the sample. Prior to each testing session, the gas analyser was calibrated with known oxygen and $CO_2$ gas concentrations (5% $CO_2$, 21% oxygen and nitrogen balance) from a gas cylinder. During data collection, breath-by-breath $CO_2$ was sampled using the calibrated gas analyser at a flow rate of 200 ml/min. Peak $PETCO_2$ was calculated in LabChart, using the peak cyclic $CO_2$ value for each breath, with correction for the daily barometric pressure.

## 3.6. Continuous Assessment of Blood Pressure: Finger Photoplethysmography

Finger photoplethysmography utilises a finger cuff to provide the continuous non-invasive assessment of finger BP. The use of a finger cuff was first developed by Penaz (1973) and utilises the principle of the 'unloaded arterial wall' (Ogedegbe and Pickering, 2010) and volume-clamp technique (Hodgson and Choate, 2012). Commonly, a Finometer (Finapress Medical Systems B.V.) is used for continuous measurement of finger arterial pressure (Guelen et al., 2003). Compared to BP measured using a mercury sphygmomanometer, reconstructed brachial BP measures obtained using the Finometer are within the American Association for the Advancement of Medical Instrumentation (AAMI) and British Hypertension Society (BHS) validation criteria (Guelen et al., 2003; Schutte et al., 2004).

The Finometer features three main components: a finger cuff containing an inflatable air bladder and infrared plethysmograph; a servo-controller system; and a main unit containing an air pump. Blood flow at the level of the finger is detected using the infrared photoplethysmograph whereby infrared light is emitted into the finger and absorbed by the blood flowing through the artery, with the remaining light signal sensed by a detector (Nijboer et al., 1981). Changes in the amount of blood flowing through the artery due to pulsations therefore cause variations in the intensity of the detected light (Nijboer et al., 1981). Using this information, the artery is 'clamped' at a certain diameter (set point) despite changes in arterial pressure during each cardiac cycle. Increases in the light signal intensity, such as that occurring during systole, are sent as a

signal to the servo-controller system which compares the signal to that of the set point. Differences between this signal and that of the set point are in turn sent to the control system containing the air pump. The control system can increase air delivery to the air bladder at the finger cuff, therefore increasing the cuff pressure and preventing any change in arterial diameter. This therefore keeps the pressure of the cuff equal to that of arterial pressure (Hodgson and Choate, 2012). Consequently, cuff pressure provides an indirect measure of intra-arterial pressure at the finger. This pressure reading is then filtered and reconstructed using an algorithm which corrects for the pressure gradient between the finger and the upper arm, in order to form a reconstructed brachial artery BP (Guelen et al., 2003).

To ensure accurate measurements, defining the correct unloaded diameter of the artery is essential, however this can be influenced by changes in haematocrit, stress and the tone of the smooth muscle in the arterial wall. Consequently, the unloaded diameter is usually not constant during a measurement and has to be verified at intervals (Bogert and van Lieshout, 2005). The Finometer features an inbuilt Physiocal algorithm which allows for this (Wesseling et al., 1995). Physiocal applies a brief period of constant pressure to analyse the plethysmography signal, derive the unloaded diameter of the finger and adjust the finger cuff accordingly (Bogert and van Lieshout, 2005).

### 3.6.1. Assessment of Continuous Blood Pressure

Participants were fitted with a photoplethysmographic cuff on the index or middle finger of the right hand (Finometer model 1, Finapres Medical Systems BV, Amsterdam, The Netherlands). Stature, weight, sex and age were input into the Finometer and the hydrostatic height correction was used to correct for position changes of the hand with respect to heart level (Figure 3-2). The height correction unit comprises of a liquid-filled tube with one end attached to reference component and one end to a transducer. While participants rested in a supine position, the reference component and transducer were both placed on the photoplethysmographic cuff and 'nulled' to 'zero' the transducer to the hydrostatic reference. The transducer was then secured to the cuff and the reference component was attached at heart level. Prior to collecting data, the Finometer was left measuring until the intervals between Physiocal (Wesseling et al., 1995) were greater than 30 seconds, indicating a stable BP reading. Arterial pressure was recorded using BeatScope software, which enables beat-to-beat analysis of the 'raw' arterial pressure waveform, from which height correction can be applied. This enables systolic BP and diastolic BP to be obtained and from this MAP is calculated as: 1/3 systolic BP + 2/3 diastolic BP.

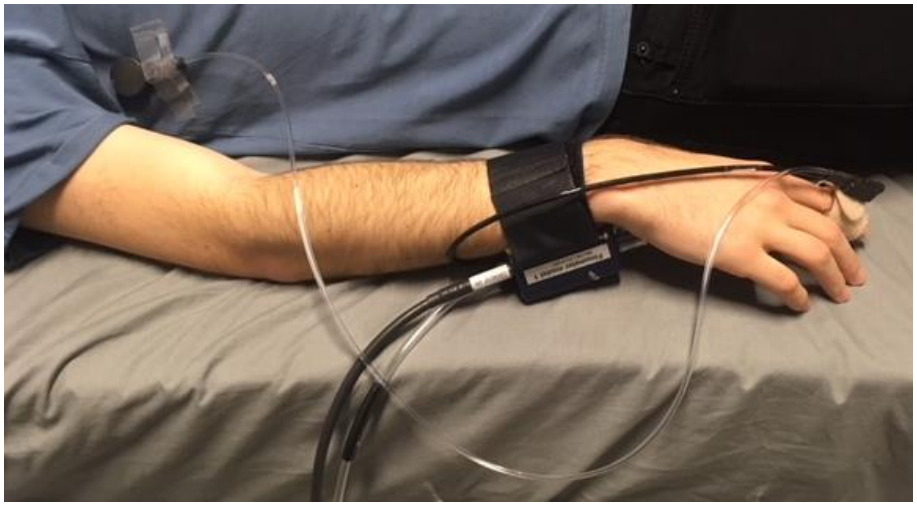

**Figure 3-2:** Continuous measurement of blood pressure using Finometer with hydrostatic height correction.

## 3.7. Cerebrovascular Measurements

### 3.7.1. Cerebral Blood Flow

Most recently the use of transcranial Doppler ultrasound (TCD) has been used for the assessment of CBF (Willie et al., 2011). TCD was first used by Aaslid in 1982 and represents a non-invasive tool that can assess the haemodynamic characteristics of the major cerebral arteries in normal and pathological conditions (Aaslid et al., 1982). Using TCD, the MCA, PCA and ACA can be assessed, in addition to the basilar artery (BA) and the vertebral arteries (VA) (Stroobant and Vingerhoets, 2000; Figure 3-3).

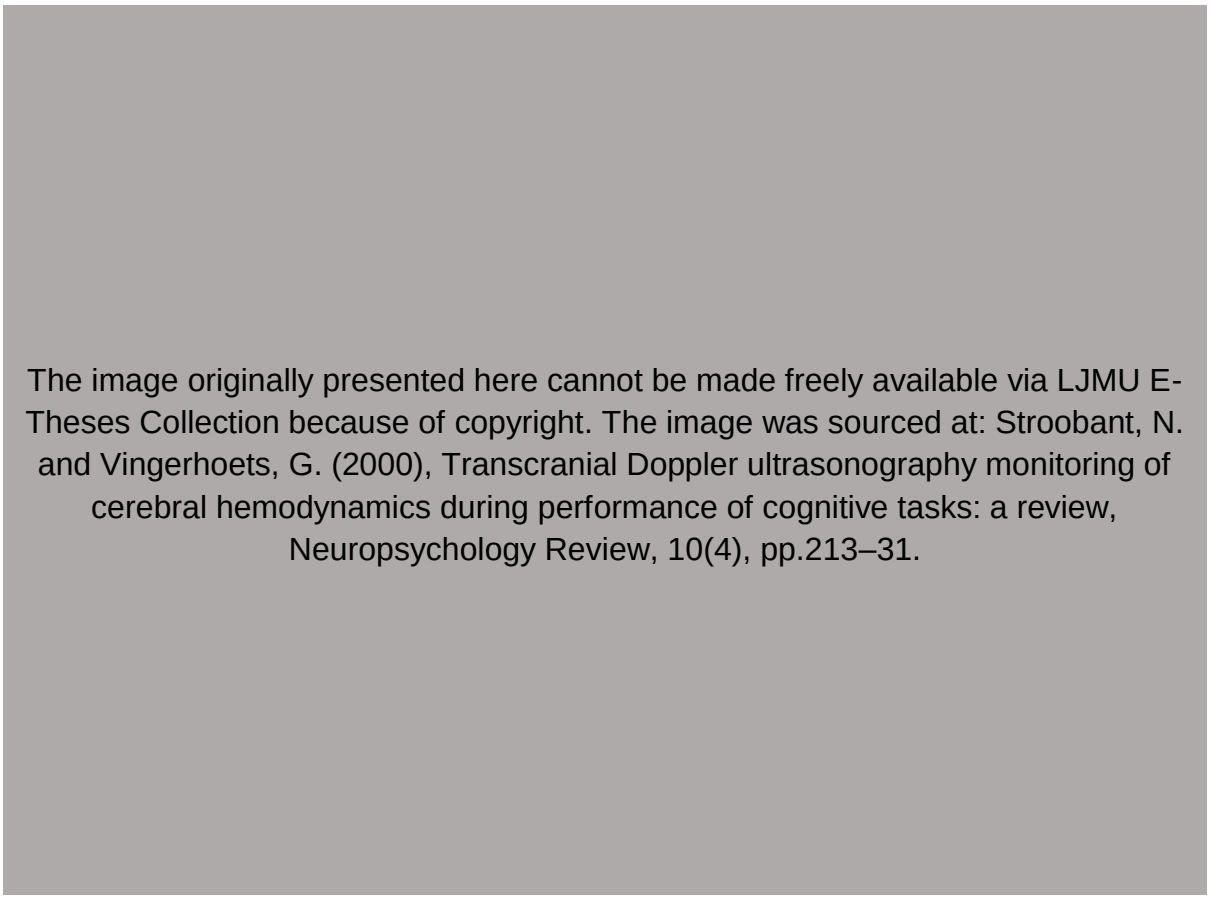


**Figure 3-3:** The middle (MCA), anterior (ACA with A1 and A2 segments) and posterior (PCA with P1 and P2 segments) cerebral arteries that can be assessed using transcranial Doppler ultrasound. The basilar arteries (BA) and vertebral arteries (VA) can also be measured (adapted from Stroobant and Vingerhoets, 2000).

The method relies on the Doppler shift effect, which describes the difference in frequency between an emitted and received signal. TCD ultrasound is pulsed, meaning a pulse of ultrasound is emitted and followed by a period of 'listening'. The time between the pulse emission and receiving the reflected pulse determines the depth at which the Doppler frequency shift is detected (Moppett and Mahajan, 2004). Ultrasonic beams can cross the skull at points known as 'windows' and are reflected by the blood cells of all blood vessels flowing in its path (Moppett and Mahajan, 2004; Stroobant and Vingerhoets, 2000). Once positioned at a window, the Doppler probe emits an ultrasonic wave which passes through the skull and is emitted into the cerebral vessel of interest. The wave contacts the red blood cells within this vessel and is then reflected back to the transducer by these moving red blood cells. The difference between the transmitted signal and the signal received back from the red blood cell is the Doppler shift and is calculated as: $2 \times V \times Ft \times \cos\theta / C$. Where V is the velocity of the reflector (red blood cells), Ft is the transmitted frequency (2 MHz), C is the speed of sound in soft tissue (1,540 $m \cdot s^{-1}$) and $\cos\theta$ is the correction factor based on the angle ($\theta$) of insonation (Moppett and Mahajan, 2004).

A critical factor when using TCD is the angle of insonation. As the transmitted frequency and the speed of sound in soft tissues are constant variables, the Doppler shift frequency depends on the blood flow velocity and the angle of insonation of the TCD probe. The observed velocity is inversely proportional to the cosine of the angle of incidence between the ultrasound beam and the blood vessel (Moppett and Mahajan, 2004). The angle of insonation should therefore be kept constant during measurements to ensure accurate measures.

At an angle of insonation of 15° the cosine of this angle is 0.96, indicating that 96% of the frequency shift within the signal is being transmitted back to the receiver and within this range any error caused by a change in insonation angle is less than 4% (Moppett and Mahajan, 2004). However, at insonation angles >60° there is less than 50% of the signal being received and at 90° no signal will be recorded. For optimal signal quality the angle of insonation should be between 0 and 30 degrees (cosine range of 1.00 to 0.87) (Moppett and Mahajan, 2004); however, as long as the insonation angle is below 60 degrees (cosine of 0.50) the signal is deemed adequate for assessment (Taylor and Holland, 1990). When insonating the MCA, if the middle portion of the temporal window (see Figure 3-5) is used then the insonation angle is virtually inline (<15 degrees) with the probe and this low angle helps create optimal signal quality. The PCA can also be acquired with a low angle of insonation from the anterior portion of the temporal window.

Compared to other techniques utilising tracer methodologies, TCD has a greater temporal resolution, enabling the continuous assessment of changes in blood flow (Willie et al., 2011). Moreover, as a non-invasive technique, this heightens its practicality and usefulness in both clinical and research settings (Stroobant and Vingerhoets, 2000; Willie et al., 2011). A limitation of TCD however, is that it measures cerebral blood flow velocity (CBFv) as opposed to actual CBF (Willie et al., 2011). When assessing other vascular beds, the use of Duplex ultrasound enables the combined measurement of blood velocity and arterial diameter and subsequent formulation of blood flow. To date, this technology is not available for TCD, meaning that CBFv is used as a surrogate

for CBF (Willie et al., 2011). The measurement is thus based on the assumption that the diameter of the cerebral vessel remains constant despite changes in BP or $PaCO_2$ (Ogoh and Ainslie, 2009; Willie et al., 2011).

The validity of TCD to measure CBFv is consequently based on this assumption of a constant arterial diameter. Data from Serrador et al. (2000) demonstrated no change in MCA diameter assessed using magnetic resonance imaging during both hypercapnia (+8 mmHg $PETCO_2$) and hypocapnia (-13 mmHg $PETCO_2$), and this has been widely used as validation of TCD for CBFv assessment (Ainslie and Hoiland, 2014). Indeed, a range of studies have shown the MCA diameter does not change over a range of partial pressures of carbon dioxide or arterial pressures (Moppett and Mahajan, 2004). However, this common assumption has been recently challenged as when using magnetic resonance imaging to measure MCA diameter a ~1.5% and ~7% increase in diameter were observed during elevations of $PETCO_2$ by 7.5 and 5 mmHg respectively (Verbree et al., 2014). Despite this, changes in $PETCO_2$ over a smaller range (±5 mmHg) are suggested to have negligible effect on the discrepancy between blood flow and blood flow velocity (Ainslie and Hoiland, 2014; Figure 3-4). TCD has been shown as a reproducible method during repeated measurements on a single day and on the following day with intra-observer coefficients of variation for mean middle cerebral arteries blood flow velocity (MCAv) of 7.5% and 13.2% respectively (Maeda et al., 1990). Good correlations have also been shown between two TCD measurements, separated by one hour, for peak and mean MCA, PCA and ACA blood flow velocities, with correlation coefficients ranging from 0.78 to 0.96 (Totaro et al.,

1992). Collectively, this supports the use of TCD as a reliable and reproducible technique to examine CBFv, although some limitations must be taken into consideration when planning scientific studies and interpreting data.

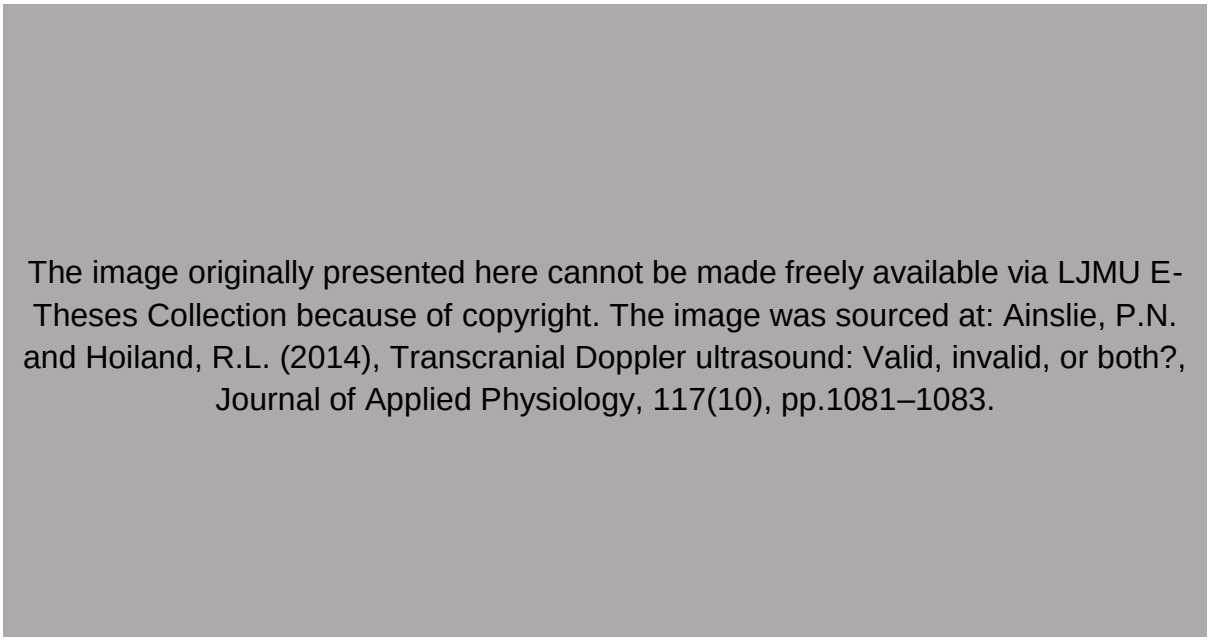


**Figure 3-4:** Previously reported changes in middle cerebral artery (MCA) diameter and the calculated impact on the discrepancy between flow and velocity measures during changes in end-tidal partial pressure of carbon dioxide ($PETCO_2$). Changes in $PETCO_2$ ±5% likely have negligible effect on the discrepancy between flow and velocity (adapted from Ainslie and Hoiland, 2014).

There are three TCD approaches based on the principal acoustic windows: the transtemporal approach, the transocular approach and the suboccipital or foramen magnum approach (Willie et al., 2011). The assessment of the MCA, PCA and ACA can be achieved using one of these three TCD approaches: the transtemporal approach (Stroobant and Vingerhoets, 2000; Willie et al., 2011; Figure 3-5a). This approach features three acoustic windows (anterior, middle and posterior) from which the vessels can be insonated, as these regions of the cranium are thin enough to enable the penetration of the ultrasonic waves. Based on this, the assessment of CBFv with TCD is limited to the major

cerebral vessels meaning local blood flow is not captured and data is limited to global blood flow responses (Willie et al., 2011). The anterior window is located above the anterior process of the zygomatic arch, the posterior window is immediately anterior to the ear above the zygomatic arch, while the middle window is located between the anterior and posterior windows (Willie et al., 2011). The insonation of the Circle of Willis via the anterior window requires the probe to be aimed posteriorly, while for the posterior window the probe is aimed anteriorly. For the medial window, direct medial insonation of the MCA is possible (Figure 3-5b).

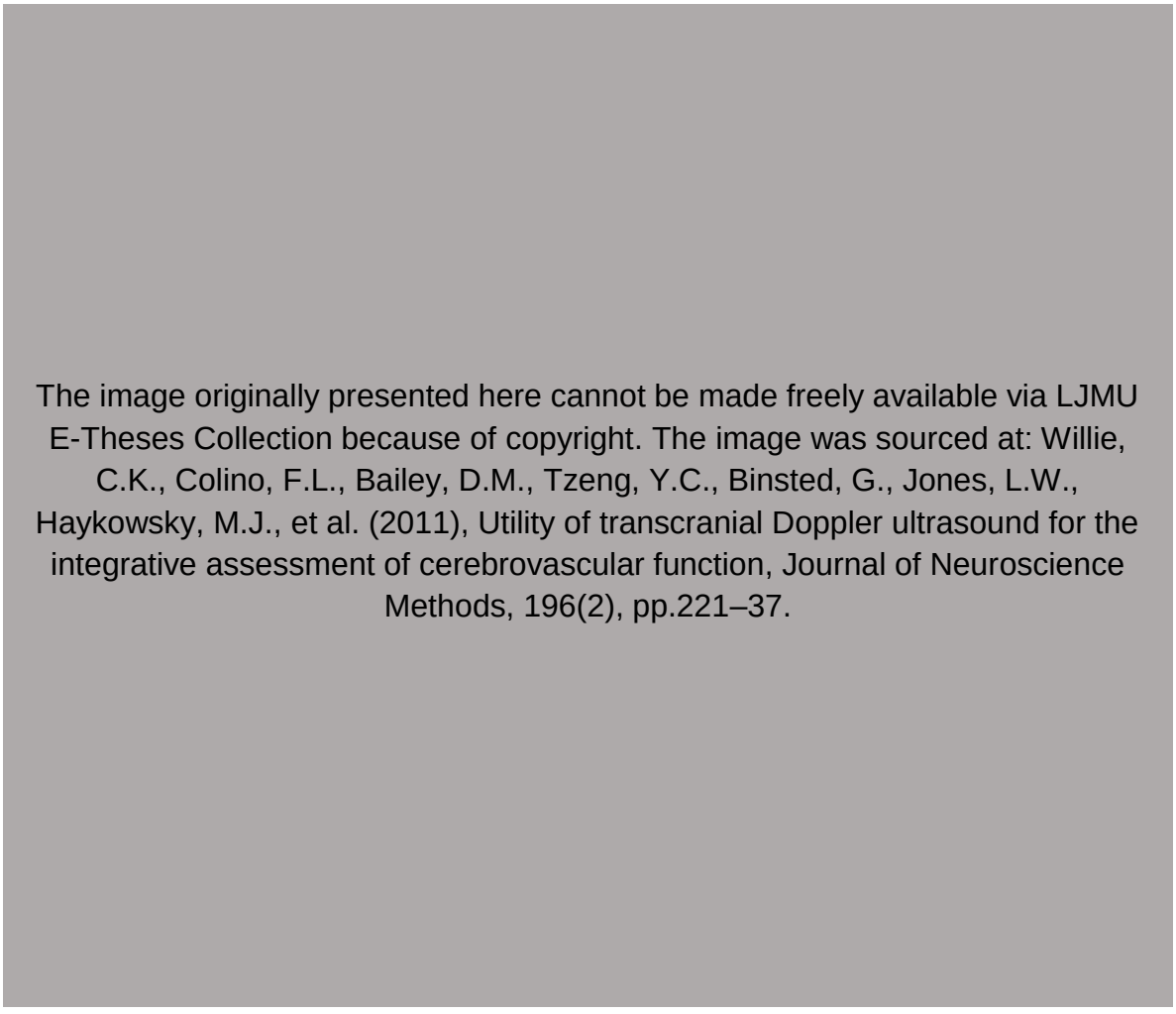


**Figure 3-5:** (a) The three transcranial doppler ultrasound approaches to measuring cerebral blood flow velocity (b) The acoustic windows used from the transtemporal approach: a- anterior; m- middle; p- posterior (adapted from Willie et al., 2011a).

*3.7.1.1. Identification of Cerebral Arteries*

Accurate assessment of CBFv using TCD is reliant on the correct identification of the MCA, PCA and ACA; which requires knowledge of cerebral anatomy and typical blood velocity values and patterns in order to differentiate between the other cerebral vessels (Table 3-1). Parameters used for vessel identification include the depth of the Doppler signal, the direction of blood flow relative to the ultrasound probe, the spatial relationship to the MCA/ACA bifurcation, and the signal response to compression (Willie et al., 2011).

**Table 3-1:** Typical parameters for the identification of cerebral arteries.

| Artery | Window | Depth (mm) | Direction | Mean Flow Velocity ($cm \cdot s^{-1}$) |
|---|---|---|---|---|
| **MCA** | Temporal | 25 to 50 | Toward probe | 55-60 |
| **ACA** | Temporal | 60 to 70 | Away from probe | 50 |
| **PCA** | Temporal | 60 to 70 | Bidirectional | 40-44 |

MCA- middle cerebral artery; ACA- anterior cerebral artery; PCA- posterior cerebral artery. (Modified from Kassab et al., 2007; Willie et al., 2011).

Typically, the MCAs are used to assess CBF and cerebrovascular function as they account for 70-80% of the brain's total perfusion, and also they possess the highest baseline velocity and have the closest proximity to the temporal window (Skow et al., 2013). The MCA is usually viewed at a depth of between 25-50 mm and exhibits the highest velocity, typically around 60 $cm \cdot s^{-1}$ (Panerai, 2009). The direction of flow is towards the ultrasound probe, until the bifurcation with the ACA, which causes forward and backward flow patterns (Panerai, 2009; Willie et al., 2011). Compression of the common carotid artery (CCA) can provide confirmation of the MCA, as a reduction in velocity should occur at the ipsilateral vessel (Panerai, 2009). The vessel is best insonated using the

anterior window as this provides a near-zero insonation angle (Willie et al., 2011). The MCA supplies blood to the frontal, temporal and parietal brain regions. It is assumed measures taken in the MCA are representative of other cerebral vessels (Ainslie and Duffin, 2009); however this has recently been challenged with differences in the reactivity of the posterior and anterior circulations observed (Skow et al., 2013).

The PCA is typically found at a depth of between 60-70 mm and is therefore posterior to, and deeper than, the MCA (Phillips et al., 2016; Willie et al., 2011). Compared to the MCA, blood flow velocity is always smaller in the PCA, with a typical blood flow velocity of 44 $cm \cdot s^{-1}$ (~26% lower than the MCA) (Willie et al., 2011). Insonation of the PCA is best achieved using the anterior window, with the probe directed posteriorly (Phillips et al., 2016). Blood flow for the proximal part of the PCA (P1 segment) is directed towards the probe, while blood flow for the proximal part of the PCA (P2 segment) is directed away from the probe (Phillips et al., 2016; Willie et al., 2011a). The PCAs supply the occipital lobe in addition to the inferior part of the temporal lobe, so identification of the PCA can be confirmed by performing a visual stimulation task (such as opening and closing the eyes) which should elicit a hyperaemic response (Phillips et al., 2016).

Finally, the ACA is found at a depth of between 60-70 mm and has a typical blood flow velocity of 50 $cm \cdot s^{-1}$ (Willie et al., 2011). Blood flow is directed towards the probe and the vessel is best insonated from the posterior window (Willie et al., 2011). The ACAs supply blood to the frontal, temporal and parietal

brain regions. However within research the evaluation of the ACA using TCD is rare, partially because hypoplasia or aplasia (the complete or incomplete underdevelopment of a tissue, respectively) is frequent in the ACA (Kwon and Lee, 2005).

#### *3.7.1.2. Assessment of Cerebral Blood Flow*

MCAv was used as a surrogate measure for CBF as the MCA accounts for 70-80% of the brain's total perfusion (Skow et al., 2013). Following a 20 minute supine rest, resting CBFv of the left and right MCA was measured using continuous bilateral TCD (ST3, Spencer Technologies, Redmond, WA, USA). To identify a vessel, a 2-MHz Doppler probe was positioned over the temporal window, located above the zygomatic arch and was secured using an adjustable headband (Marc 600 Headframe, Spencer Technologies; Figure 3-6). The MCA was identified bilaterally based on the signal depth, peak and mean blood velocity as previously described (Willie et al., 2011). Once optimal signals had been obtained, the transducers were secured into position and the depth, peak and mean blood velocities of each vessel were recorded to ensure within-subject consistency between tests. Additionally, photographs were taken to ensure consistent probe positioning between test visits. The sonographer had a between-day coefficient of variation of 7.8% for the MCAv.

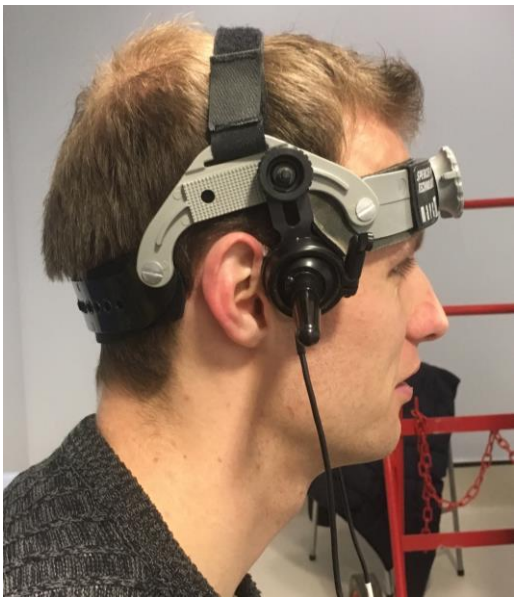

**Figure 3-6:** Measuring cerebral blood flow velocity (CBFv) using transcranial Doppler ultrasound (TCD), with a Doppler probe held in position using an adjustable headband.

Once an MCA was detected, frequency data were captured based on the shift in frequency from the ultrasonic beam emitted from the transducer and the received signal reflected back to the transducer from the red blood cells. This Doppler shift was processed using fast Fourier transformation (FFT) which converts the data from the frequency domain (ultrasonic waves) into the time domain, expressed visually as a velocity trace. FFT analysis provides a visual way of presenting the three-dimensional Doppler data in two dimensions. On the vertical axis velocity (or frequency) is displayed, on the horizontal axis is time, while signal intensity (amplitude) is displayed as the brightness of a point. An envelope curve is then drawn on the visual FFT display and this line corresponds to the maximum velocity of the cardiac cycle (Figure 3-7). This line follows the maximum velocities of every cardiac cycle and it is from this envelope curve that mean velocity of the MCA was calculated (Stroobant and Vingerhoets, 2000).

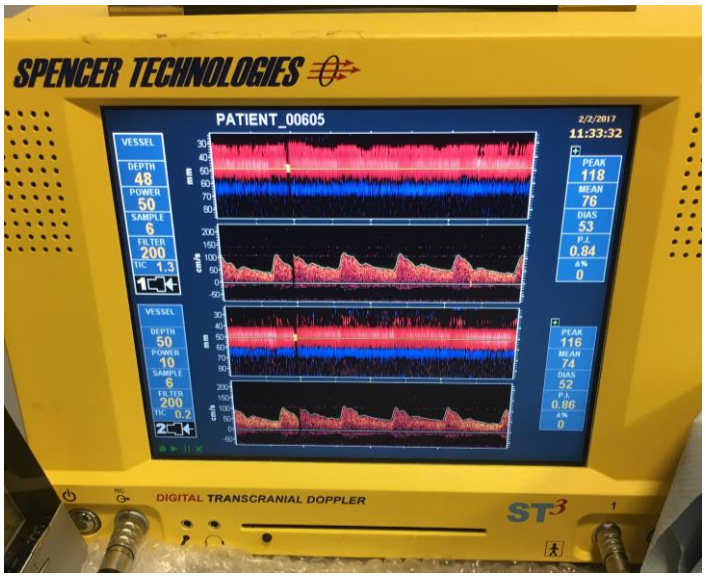


**Figure 3-7:** Example of transcranial Doppler ultrasound (TCD) data collection showing the fast Fourier transformation (FFT) and envelope tracing to obtain mean cerebral blood flow velocity (CBFv) values.

Data were acquired in LabChart where mean blood flow velocity was calculated from the envelope of the velocity tracing using a weighted mean (1/3 maximum + 2/3 minimum) to account for the relative time spent in systolic and diastolic pressures (Skow et al., 2013). When two MCAs were insonated, the mean of these vessels was calculated. In circumstances were only one MCA signal was identified or one had inadequate signal quality, only one value was used. Cerebrovascular conductance (CVC) was then calculated by dividing mean blood flow velocity by MAP (mean blood flow velocity / MAP).

### 3.7.2. Cerebrovascular Function

The complete assessment of cerebrovascular function includes three components: CVR, CA and NVC (Willie et al., 2011).

*3.7.2.1. Cerebrovascular Reactivity*

CVR describes the regulation of CBF in response to changes to a vasoactive stimulus (Fierstra et al., 2013; Regan et al., 2014). Three types of vasoactive stimuli have been used: reduction in MAP, chemical injections and manipulating $PaCO_2$ (Fierstra et al., 2013). In a recent review the latter method was suggested to be the most appropriate due to its ability to be standardised and greater practicality (Fierstra et al., 2013).

Elevations in $PaCO_2$ (hypercapnia) increases CBF via cerebral arteriole vasodilation, while a decline in $PaCO_2$ (hypocapnia) leads to vasoconstriction and a subsequent decrease in CBF; thereby functioning to maintain stable $CO_2$ and pH levels within the cerebral tissues (Ainslie and Duffin, 2009). The subsequent change in arteriole diameter in response to the manipulation of $CO_2$ levels leads to changes in CBF velocity at the main conduit vessels such as the MCA (McDonnell et al., 2013; Skow et al., 2013) and this can in turn be assessed using TCD (Willie et al., 2011). CVR is subsequently calculated as the ratio of change in CBF compared to the change in $PETCO_2$ (Regan et al., 2014; Willie et al., 2011).

The assessment of CVR therefore provides an index of the cerebral vasculature's dilation or constriction in response to the $CO_2$ stimulus (Ainslie and Duffin, 2009; Willie et al., 2011). Typically, $CO_2$ levels are manipulated using either a pharmaceutical stimulus or by ventilatory alterations of $PaCO_2$ (Willie et al., 2011). The latter represents a cheaper and non-invasive technique and is commonly achieved by using either steady state or rebreathing

respiratory tests (Fierstra et al., 2013; Skow et al., 2013). Rebreathing previously exhaled gas is a traditional method to increase $PaCO_2$ (Fierstra et al., 2013). As this exhaled gas has already equilibrated with the blood, the rebreathed portion of the breath does not contribute to alveolar ventilation, therefore there is a steady accumulation of $CO_2$ in the blood. This approach is advantageous since no external $CO_2$ source is required however, the technique has restrictions which limits its usefulness to assess CVR. During rebreathing it is not possible to control the rate at which $CO_2$ increases or to maintain constant oxygen levels, requirements when assessing CVR. Additionally rebreathing does not result in a $CO_2$ plateau since a subject will always produce $CO_2$ therefore continually elevating $PaCO_2$ (Fierstra et al., 2013). Figure 3-8 depicts the typical response observed during a rebreathing CVR protocol, with a continuous rise in $PaCO_2$ and concomitant increase in CBF (assessed at the PCA).



**Figure 3-8:** Representative partial pressure of carbon dioxide ($PCO_2$) and posterior cerebral artery blood flow velocity (PCAv) data during a cerebrovascular reactivity rebreathing protocol. A two minute baseline period (A) is followed by voluntary hyperventilation (B), which lowers both $PETCO_2$ and PCAv. A period of hyperoxic rebreathing follows (C), increasing $PETCO_2$ and PCAv, before a final recovery period (D) where values return to baseline (adapted from Skow et al. 2013).

To overcome the limitations of the CVR rebreathing technique, a specialised rebreathing method which allows changes in $PaCO_2$ but keeps $PaO_2$ constant has been developed, often called the 'Duffin rebreathing test' (Duffin, 2011), which is suitable for CVR measurements using TCD (Fierstra et al., 2013). In this method, a hyperventilation-induced decrease in $PETCO_2$ leads to a decline in CBF which is then followed by a period of hypercapnic breathing via a rebreathing circuit, which elevates CBF (Skow et al., 2013; Willie et al., 2011). This method allows the $PETCO_2$ and $PaCO_2$ to be equivalent, by supplying a flow of gas into the rebreathing circuit equal to the $PETCO_2$ of the previous breath and providing a sufficient supply of oxygen to maintain constant $PaO_2$ (Battisti-Charbonney et al., 2011).

An alternative approach to the rebreathing method is to assess responses in CBF to steady state changes in $PETCO_2$ (Brothers et al., 2014). Using this technique, a brief period of voluntary hyperventilation lowers $PETCO_2$ which is then followed by a participant breathing a predetermined level of higher $CO_2$ content gas for several minutes. Typically a 5 or 7% $CO_2$ gas concentration is used (Fierstra et al., 2013), which causes a gradual stepwise increase in $PETCO_2$ until a plateau is reached (Boulet et al., 2016). A criticism of this approach is that the cerebrovascular vasoconstriction that occurs during the period of hyperventilation may attenuate the vasodilatory response of the vasculature to subsequent $CO_2$ inhalation (Brothers et al., 2014). Figure 3-9 depicts the typical response observed during a steady state CVR protocol, with a rise in $PETCO_2$ and concomitant increase in CBF until a plateau is reached.

**Figure 3-9:** Representative partial pressure of end-tidal carbon dioxide ($PETCO_2$) and cerebral blood flow velocity (CBFv) data during a steady state cerebrovascular reactivity rebreathing protocol of 5% $CO_2$ exposure. CBFv progressively increases to a peak with the increase in $PETCO_2$ and is then maintained at this level (adapted from Ainslie and Duffin, 2009).

*3.7.2.1.1. Assessment of Cerebrovascular Reactivity*

While resting in a supine position, participants were instrumented with a mouth piece (MLA1026, ADInstruments, Colorado Springs, Colorado, USA) with a two-way non-rebreathing valve (MLA1028). A Douglas bag filled with a 5% $CO_2$ mixture and fitted with a three-way valve, enabled the breathing circuit to be alternated between ambient air and the contents of the Douglas bag (Figure 3-10). Breath-by-breath $CO_2$ was sampled using a calibrated gas analyser (ML206, ADInstruments) and peak $PETCO_2$ was calculated in LabChart, using the peak cyclic $CO_2$ value for each breath, with correction for the daily barometric pressure as described in section 3.5.

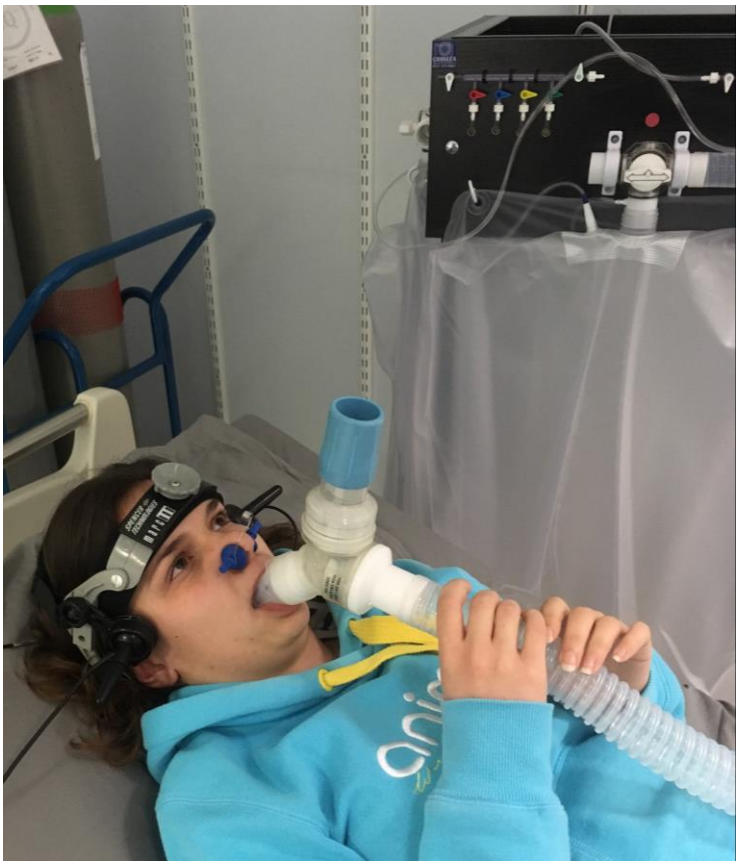

**Figure 3-10:** Assessment of cerebrovascular $CO_2$ reactivity (CVR) using a voluntarily hyperventilation protocol.

MCAv, acquired using TCD as described in section 3.7.1.2, was measured throughout. Baseline $PETCO_2$ and MCAv were acquired for one minute, whilst participants breathed ambient air. Subsequently, participants were coached through a voluntarily hyperventilation protocol for a maximum of three minutes or until $PETCO_2$ was reduced to 20 mmHg (whichever was achieved first). Immediately afterwards the valve on the Douglas bag was switched so that participants would inhale the 5% $CO_2$ mixture. Simultaneously, participants were instructed to return to their normal respiratory rate and breathed from the 5% $CO_2$ mixture for three minutes (Figure 3-11).

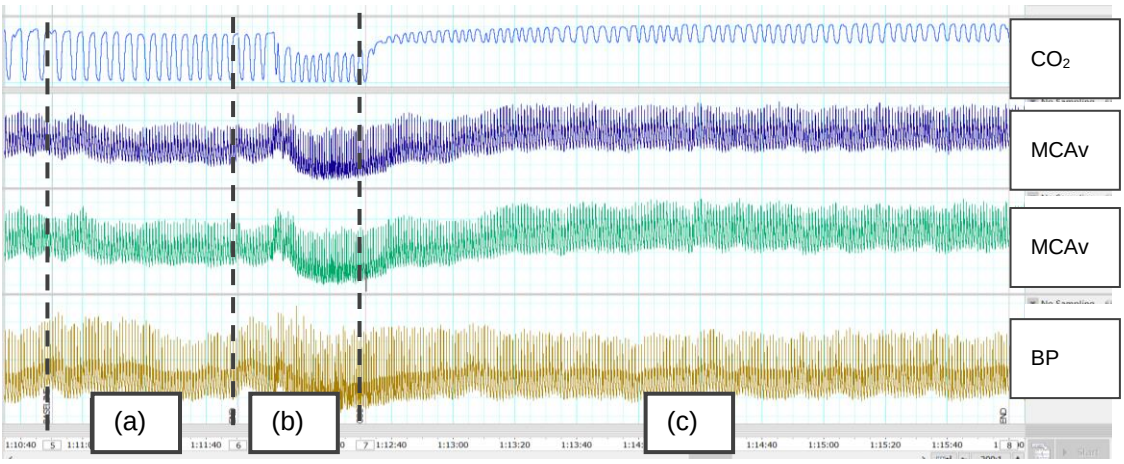


**Figure 3-11:** Representative middle cerebral artery blood flow velocity (MCAv) data in LabChart during the cerebrovascular carbon dioxide reactivity (CVR) assessment. Data shows: (a) one-minute baseline, (b) a voluntarily hyperventilation period and (c) three minutes breathing a 5% carbon dioxide gas mixture. $CO_2$- carbon dioxide; BP- blood pressure.

Data were analysed in LabChart. Baseline $PETCO_2$ and MCAv were calculated as the mean of the one minute prior to hyperventilation, while data during 5% $CO_2$ breathing was collected as ten second averages for the entire three-minute period. Data were then exported to Excel (Microsoft). Absolute and relative MCAv were then plotted against $PETCO_2$ for each 10 seconds of 5% $CO_2$ breathing and quantified by linear regression by adding a linear regression line onto the plotted data. A linear regression line has an equation: $Y = bX + a$, where X is the explanatory variable, Y is the dependent variable, the slope of the line is b, and a is the intercept. The value for the slope of the line (b) was used to represent the rate of change in MCAv per mmHg increase in $PETCO_2$. These slopes were compared statistically and their respective $R^2$ values were reported. Relative MCAv was calculated as the difference between baseline and 5% $CO_2$ MCAv divided by baseline MCAv (([5% $CO_2$ MCAv-baseline MCAv]/ baseline MCAv) x 100%).

Simultaneously, during the baseline and $CO_2$ breathing measurements, arterial diameter and blood flow of the left CCA were acquired using a 10-MHz multi-frequency linear array probe, attached to high-resolution ultrasound machine as described in detail in section 3.10.1. Using ultrasound to assess the dilation of larger extracranial neck vessels during $CO_2$ alterations provides another means to monitor reactivity and vessel dilation not assessable using TCD (Ainslie and Hoiland, 2014; Willie et al., 2012). The extracranial arteries supplying the brain are also sensitive to changes in $CO_2$ levels and therefore contribute to cerebrovascular $CO_2$ regulation. Indeed, increases in $PaCO_2$ causes greater dilation and increased blood flow in the internal carotid artery and vertebral artery compared to the velocity in the MCA and PCA. The difference between blood flow in these intracranial vessels and their respective downstream arteries suggest an underestimation of flow using TCD (Willie et al., 2012). Consequently, at high extremes of $PaCO_2$ this suggests that the MCA dilates, therefore challenging the assumption of using TCD to assess MCAv that diameter remains constant despite changes in $PaCO_2$ (Willie et al., 2012). Consequently, by monitoring the reactivity of the CCA in this thesis, possible changes in MCA diameter undetectable by TCD could be monitored. Indeed, hypercapnia (4.5-6% $CO_2$) causes dilation and increased blood flow of the CCA (Carter et al., 2016), internal carotid and vertebral arteries (Smith et al., 2017).

Images were acquired and optimised in accordance with methodological guidelines (Thomas et al., 2015). To reduce any influence of turbulent flow on vascular responsiveness, the CCA was imaged at least two centimetres below the point of bifurcation. All ultrasound measurements were completed by the

same sonographer, who has a between-day intraobserver coefficient of variation of 3.5% for the CCA, in line with methodological guidelines (Thomas et al., 2015). CCA blood flow and diameter data were analysed as described in detail in section 3.10.1. Baseline $PETCO_2$, CCA blood flow and diameter data were calculated as the mean of the one minute prior to hyperventilation, while data during 5% $CO_2$ breathing was collected as 10 second averages for the entire three-minute period. Data were then exported to Excel (Microsoft). Absolute and relative CCA blood flow and diameter data were then plotted against $PETCO_2$ for each 10 seconds of 5% $CO_2$ breathing and quantified by linear regression by adding a linear regression line onto the plotted data. The value for the slope of the line was used to represent the rate of change in CCA blood flow and diameter per mmHg increase in $PETCO_2$. These slopes were compared statistically and their respective $R^2$ values were reported. Relative CCA blood flow was calculated as the difference between baseline and 5% $CO_2$ CCA blood flow divided by baseline CCA blood flow (([5% $CO_2$ MCAv-baseline CCA blood flow]/ baseline CCA blood flow) x 100%). This same formula was used to calculate relative CCA diameter.

*3.7.2.2. Cerebral Autoregulation*

A large range of methods have been used to examine dynamic CA (Claassen et al., 2016). Dynamic CA can be assessed by inducing rapid fluctuations in BP, such as using suprasystolic thigh cuffs, the use of vasoactive drugs (The Oxford Technique) or completing squat to stand manoeuvres (Claassen et al., 2016; Willie et al., 2011). The latter represents a simple but also ecological method that is typical of daily activities (Claassen et al., 2009; Sorond et al., 2009) and

can produce substantial BP fluctuations, as merely moving from sitting to standing or to a squat position can induce around a 35 mmHg change in BP (Willie et al., 2011).

Different frequencies of squat-stand manoeuvres have been utilised: 0.0025 Hz (20 second squat with 20 seconds standing), 0.05 Hz (10 second squat with 10 seconds standing), and 0.1 Hz (5 second squat with 5 seconds standing) (Claassen et al., 2009), with each protocol typically performed for 5 minutes in duration (Claassen et al., 2009). These squat-stand manoeuvres create oscillations in both BP and CBF (van Beek et al., 2008), which can be captured due to the high temporal resolution of photoplethysmography and TCD respectively (van Beek et al., 2008). Figure 3-12 shows typical BP and CBFv responses observed during two different frequency squat-stand manoeuvres. CA is more effective at low compared to high frequencies, therefore these squatting protocols are recommended as they are performed at a low frequency, but result in high amplitude signals (Claassen et al., 2009). This subsequently results in a greater coherence value, making the statistical computation of CA using transfer function analysis (TFA) more reliable (Claassen et al., 2009).

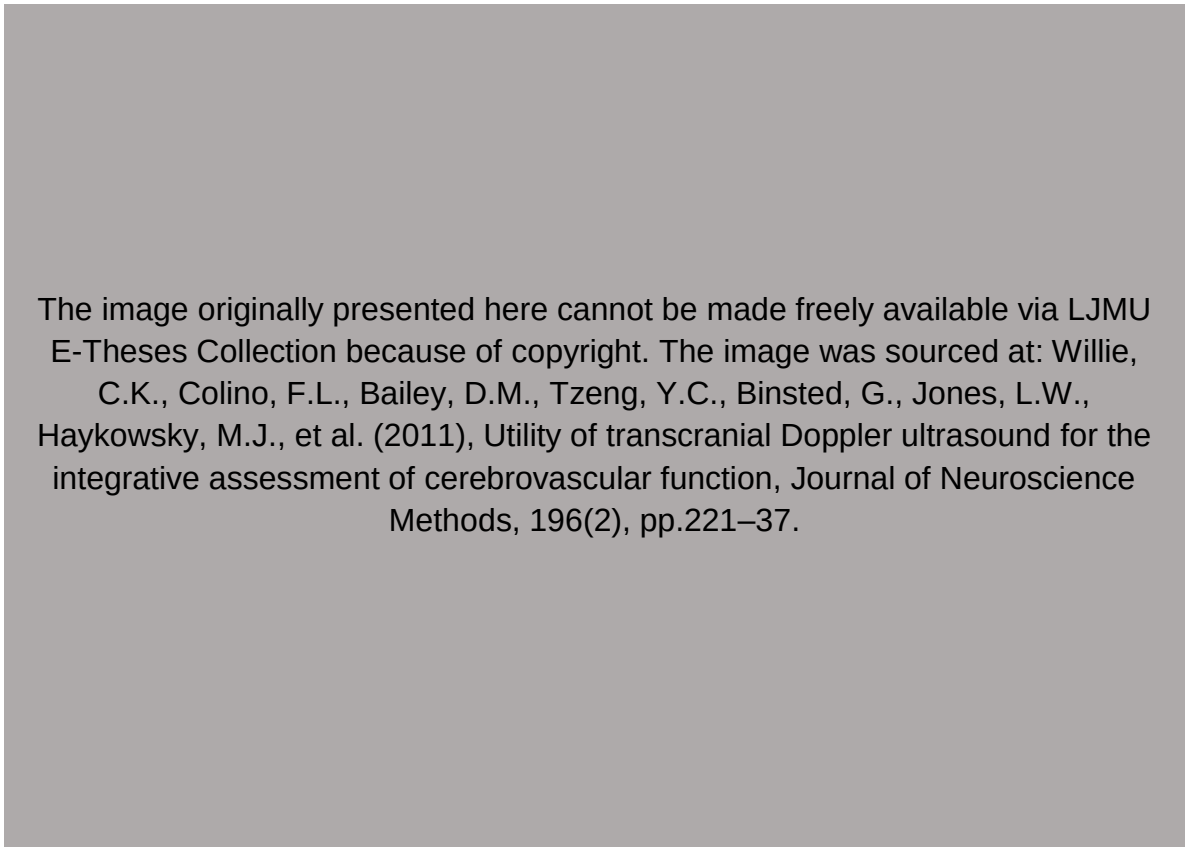


**Figure 3-12:** Representative mean arterial pressure (MAP) and middle cerebral artery blood velocity (MCAv) data at rest (Baseline) and during (A) a 5 second repeated squat-stand manoeuvre (0.05 Hz) and (B) a 10 second repeated squat-stand manoeuvre (0.1 Hz) (adapted from Willie et al., 2011).

There is no gold standard method to quantify CA (Claassen et al., 2016), however a popular approach is to use TFA (Claassen et al., 2009, 2016). TFA views CA as a linear control system (Claassen et al., 2016). In a linear control system, sinusoids at the input are transformed into sinusoids at output of the same frequency, however with a different amplitude and shifted in time (Claassen et al., 2016). In the case of CA, BP is the input and CBF the output, with CA as the regulator between the two (van Beek et al., 2008). The change in amplitude is referred to as the gain, whilst the shift in time is described as the phase shift (Claassen et al., 2016). The calculation of gain and phase is computationally straightforward, however to ensure the statistical reliability of

these values a coherence function is used (Claassen et al., 2016). Coherence tests the linearity of the relationship between input and output and can be used to indicate whether data is reliable (van Beek et al., 2008; Claassen et al., 2016). A coherence threshold is set based on the 95% confidence limit (i.e. 5% critical value) of the null hypothesis that input and output are not related, theoretically corresponding to zero coherence (Claassen et al., 2016). This threshold also accounts for the number of independent observations used in the calculation (degrees of freedom). In order to improve the standardisation of TFA within research, a set of coherence thresholds have been published, which researchers are advised to adhere to and, if data are below this coherence level it should be rejected (Claassen et al., 2016).

TFA therefore describes CA in three parameters: gain, phase and coherence (van Beek et al., 2008). Gain describes the damping effect of CA on the magnitude of BP oscillations, or simply how the changes in BP are transmitted into CBF (Claassen et al., 2009). Therefore, gain provides a measure of the efficiency of the regulator, namely CA (van Beek et al., 2008). Low values of gain are indicative of efficient autoregulation as it indicates that oscillations in CBF in response to changes in BP are buffered by active changes in cerebrovascular resistance and/or by increases in steady-state cerebrovascular resistance (Claassen et al., 2009). Increased gain consequently corresponds to a reduced efficiency of CA (van Beek et al., 2008). Phase describes the synchronicity of two waveforms. Waveforms that are in synchrony are referred to as ‘in phase’, while if these waveforms are displaced from each other it describes a phase shift. Oscillations in CBF and BP are not in sync, as changes

in CBF recover faster than BP. Due to this, oscillations in CBF appear to lead oscillations in BP and this displacement of waveforms is referred to as phase lead (van Beek et al., 2008). During normal autoregulation, CBFv usually precedes BP by 40–60°, with impaired autoregulation assumed when the phase is 0° or near 0° (Müller and Osterreich, 2014). The phase shift is considered a surrogate measure for the time delay of the autoregulatory response, with increases in phase indicating a more efficient CA (van Beek et al., 2008). Coherence tests the linearity of the relation between BP and CBF, with a coherence value approaching one indicating a linear relationship (van Beek et al., 2008). Assessment of coherence is essential to determine the validity and reliability of estimates of gain and phase (Claassen et al., 2016).

*3.7.2.2.1. Assessment of Cerebral Autoregulation*

Participants completed a series of squat-stand tests, differing in the pace and number of the squats completed, in order to induce oscillations in BP (Figure 3-13). This involved repeated cycles of either: the 0.05 Hz protocol (10 seconds squat with 10 seconds standing), or the 0.1 Hz protocol (5 seconds squat with 5 seconds standing) all for a duration of five minutes (Claassen et al., 2009). Participants were coached by the researcher to ensure correct timing and were advised to keep their squat technique consistent throughout all tests. MCAv, $PETCO_2$ and BP were continuously assessed during each squat-stand protocol and acquired in LabChart (Figure 3-13).

**Figure 3-13:** Assessment of cerebral autoregulation (CA) using a squat-stand protocol.

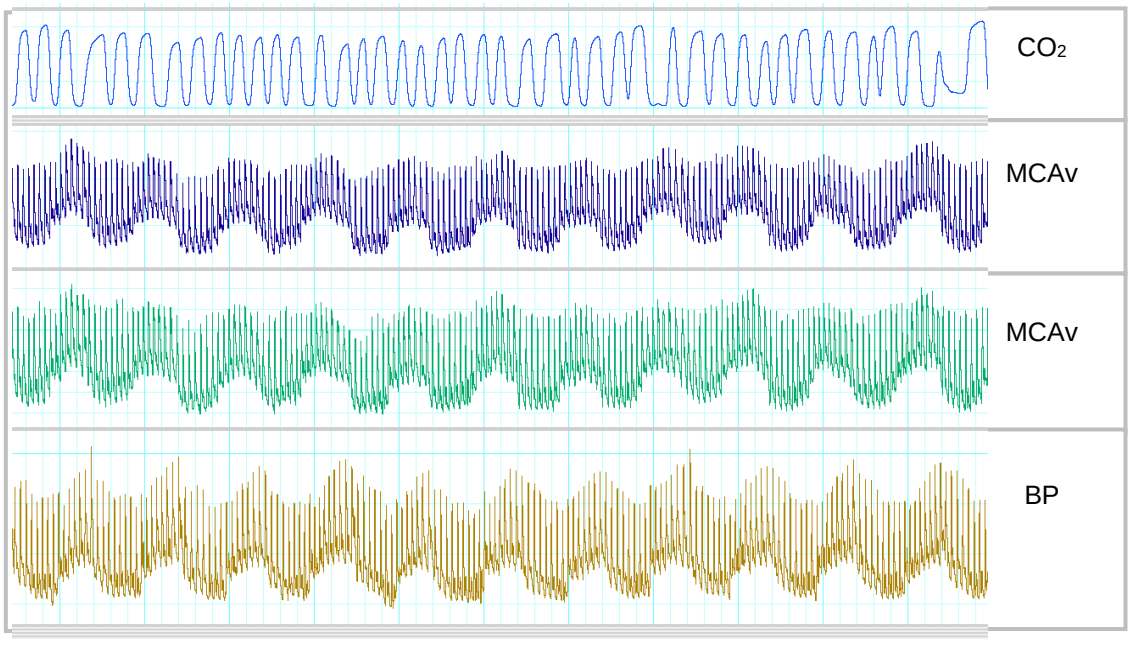


**Figure 3-14:** Representative middle cerebral artery blood flow velocity (MCAv), blood pressure (BP) and carbon dioxide ($CO_2$) data in LabChart during the squat-stand protocol.

Data were analysed using TFA in accordance with standardised guidelines (Claassen et al., 2016). First, data were analysed in LabChart. For each five-minute squat-stand protocol, data were screened to ensure it were free from excessive noise or artefact. Linear interpolation was used to replace short periods of strong artefact (up to three heart beats), data with longer segments of artefact were excluded. Following this, beat-to-beat data were extracted (the mean CBFv, BP and $PETCO_2$ for each heart beat) and exported to Excel (Microsoft) to be reformatted to a compatible format for analysis using the recommended MATLAB (MathWorks-Inc., Natick, MA) code, as provided by the Cerebral Autoregulation Research Network (Claassen et al., 2016). Data were then ran through the MATLAB code. The code first performs a spline interpolation of the data to create equidistant time intervals between the data points (a requirement of TFA). Data were then resampled at a reduced rate to smooth the data and reduce the noise in the signal, with Welch's algorithm applied to smooth the data and improve the precision of the estimates. Furthermore, data were windowed using the recommended Hanning window to prevent spectral leakage. This divides the data into five successive windows that overlap by 50%.

FFT is then used to obtain estimates of auto- and cross-spectra. The subsequent output produces values of gain, phase and coherence for each of the three frequency domains: very low frequency (VLF: 0.02-0.07 Hz), low frequency (LF: 0.07-0.2 Hz) and high frequency (HF: 0.2-0.5 Hz). TFA is a frequency-dependent phenomenon and these domains are within the frequency range CA is thought to operate. CA is viewed as a high-pass filter as the

regulation of CBF is effective in the LF range of BP oscillations, but not in the HF range due to the time delay in initiating cerebrovascular adaptations to the changes in perfusion pressure. CA therefore allows rapid BP changes to be transmitted to CBF, whereas slow BP changes are filtered (van Beek et al., 2008). As a consequence, the three frequency ranges have different responses and are likely controlled by different mechanisms (Zhang et al., 1998). Coherence values were used to accept the validity of gain and phase estimates, with cut-off values for inclusion set at 0.4 in accordance with published guidelines (Claassen et al., 2016). Analyses yielding coherence values lower than this cut-off value were excluded. Data that showed evidence of phase-wrap around (negative values for phase in the VLF and LF) were also excluded. As recommended, gain was normalised to control for possible baseline differences in BP and MCAv between conditions, therefore normalised gain was used for the interpretation of data (van Beek et al., 2008; Claassen et al., 2016).

*3.7.2.3. Neurovascular Coupling*

Despite the inability for TCD to measure blood flow to specific brain regions, its high temporal resolution means it provides a suitable assessment method for NVC (Phillips et al., 2016; Willie et al., 2011; 2014). NVC can be assessed using the presentation of sensory, motor or emotional stimuli or by carrying out cognitive tasks, all of which evoke neural activity, which subsequently mediates a stimulatory effect on CBF, termed functional TCD (Phillips et al., 2016). This can include visual stimulatory tasks, such as reading, opening and closing the eyes, or identifiying a light source (Phillips et al., 2016; Willie et al., 2011), all of which cause increases in neural activity and, subsequently, CBF (Willie et al.,

2011). For example, 40 second cycles of reading printed text caused an absolute increase in the PCA blood flow velocity (PCAv) of ~8 $cm \cdot s^{-1}$ (Willie et al., 2011). Indeed, the response varies between the cerebral vessels and is dependent on the region of the brain the vessels supply. Typically in healthy individuals a 10-20% increase in PCAv occurs, whilst there is a 5-8% increase in the MCAv (Phillips et al., 2016; Figure 3-15).

Recently the publication of new guidelines has aimed to standardise the assessment of NVC using a simple visual stimulation task (Phillips et al., 2016). The visual stimuli involves a period of eyes-open whilst viewing a bright visual stimuli followed by a period of eyes shut. This is combined with the continuous assessment of the CBFv of the PCA and MCA. Recommendations state 5-10 cycles should be repeated whereby one cycle consists of a 20-30s eyes-closed period followed by a 20-30s eyes open period (Phillips et al., 2016).



**Figure 3-15:** Example of neurovascular coupling (NVC) in the (a) posterior cerebral artery (PCA) and (b) middle cerebral artery (MCA) using an established standardized eyes-open/eyes-closed protocol. Grey lines indicate healthy control group, black bars indicate high-level spinal cord injured group. Thick black bar indicates 30-s of eyes-open reading, being immediately preceded by eyes-closed. Smaller boxes represent 5-s bins which were averaged (adapted from Phillips et al., 2016).

*3.7.2.3.1. Assessment of Neurovascular Coupling*

While rested in a supine postion, participants completed a visual stimulation task in accordance with recently published guidelines (Phillips et al., 2016). The visual stimuli involved a period of eyes-open whilst viewing a bright visual stimulation screen, followed by a period of eyes shut. First, participants completed two minutes of eyes closed followed by two minutes of eyes open to act as a baseline for each respective condition. Participants subsequently performed five visual stimuli cycles whereby one cycle consisted of 30 seconds of eyes-closed followed by 30 seconds of eyes-open (Figure 3-16). PCAv and MCAv were measured continuously using TCD as described in 3.7.1.2. The side (left or right) that each vessel was located was kept consistent for any repeated measures. $PETCO_2$ and BP were continuously assessed during the procedure as described in sections 3.5 and 3.6.1 respectively.

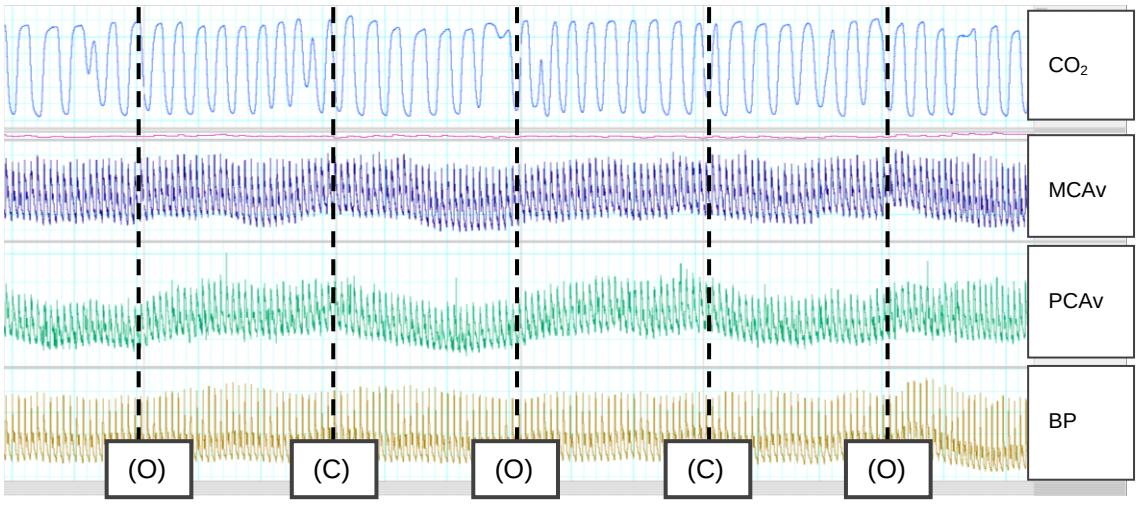


**Figure 3-16:** Representative posterior cerebral artery blood flow velocity (PCAv), middle cerebral artery blood flow velocity (MCAv), blood pressure (BP) and carbon dioxide ($CO_2$) data in LabChart during an eyes-open (O), eyes-closed (C) protocol to assess neurovascular coupling (NVC).

Data were analysed using automated software in accordance with recommended guidelines (Phillips et al., 2016). First, data were analysed in LabChart. Data were inspected and interpolation was used to replace short periods of strong artefact (for example if signal noise occurred due to participant movement), while data with longer segments of artefact were excluded. Following this, beat-to-beat data were extracted ($PETCO_2$; systolic, diastolic, and mean arterial pressure; peak, minimum, and mean MCAv and PCAv) and exported to Excel (Microsoft). Data were then reformatted to a compatible configuration for analysis using the recommended automated MATLAB (MathWorks-Inc., Natick, MA) code. Data were then ran through the MATLAB code. The software automatically combines all cycles from one participant into one average contour for each outcome measure for an eyes-closed and eyes-open period (Figure 3-17). The absolute and percentage change in PCAv and MCAv from pre-visual stimulation and the time to this peak blood flow response were used to quantify the NVC response.

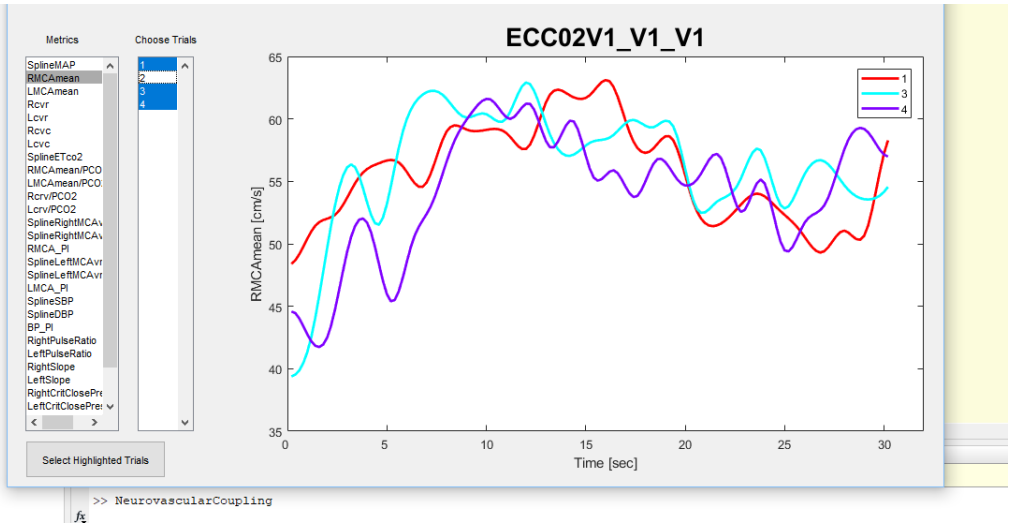


**Figure 3-17:** Example of neurovascular coupling (NVC) analyses completed in MATLAB showing the mean posterior cerebral artery blood flow velocity (PCAv) response during a thirty-second eyes-open cycle.

## 3.8. Cognition

A battery of computer-based cognitive function tests were completed assessing three cognitive components: the Stroop Colour-Word Test to measure executive function, the Attention Network Test (ANT) to assess attention, and the N-Back Task to assess working memory. All tests were conducted using E-Prime software (Version 2.0 Professional, Psychology Software Tools, Pittsburgh, PA) which logs participants' responses and variables such as reaction time (RT) and response accuracy. The E-Prime software was loaded onto a computer and participants completed the tests while seated in a silent room, with only the experimenter present. Therefore, there were no audible or visual distractions during testing. Prior to each test, participants were provided with written instructions and given the opportunity to ask questions. For each test, participants were told to respond as quickly and accurately as possible to each stimulus.

### 3.8.1. Stroop Colour-Word Test

The Stroop Colour-Word Test originated from the research of Stroop in his studies of attention and interference (Stroop, 1935). It is now a widely used test of inhibitory processing and executive function (Cothran and Larsen, 2008; Homack and Riccio, 2004). Due to the development of many variations of the Stroop Test, there is no recognised standard version (Homack and Riccio, 2004). Despite this, the basic model of the test remains, whereby performance on a basic task is compared with performance on a similar task but where a habitual response is supressed by an incongruent interference (Van der Elst et al., 2006). The time taken to complete the latter task is greater compared to the

basic task, and this is described as Stroop Interference or the Stroop Interference Effect (Van der Elst et al., 2006). The Stroop Interference Effect is viewed as a measure of an individual's cognitive flexibility and executive functioning ability (Van der Elst et al., 2006; Homack and Riccio, 2004).

*3.8.1.1. Administration of the Stroop Colour-Word Test*

In a standardised order, participants completed the three tasks that form the Stroop Colour-Word Test: the Word Task, the Colour Task and the Colour-Word Task (Homack and Riccio, 2004). For each task, participants were instructed to name the colour of the ink in which the text was written and to respond as quickly and accurately as possible by pressing on the keyboard the letter that corresponded to that colour. In the Word Task participants were presented with the words 'red', 'blue', 'yellow' or 'green' in a congruous ink colour (e.g. the word 'red' was written in red ink). In the Colour Task a series of four letter X's were displayed (XXXX) in either red, blue, yellow or green ink. For the Colour-Word Task the names of these four colours were presented in an incongruent ink colour (e.g. the word 'red' was written in blue ink).

Participants were instructed to place their left and right middle and index fingers on the letters on the keyboard corresponding to the four colour options and to keep them there throughout all tasks. A practise task of 16 trials was given to ensure participants were familiarised with the test protocol. Following this, the three tasks were completed. Each task was formed of 32 trials, therefore totalling 98 trials across the entire test. A break was given in between each task. For each trial first a fixation (*) was displayed in the centre of the screen for 250 ms. Following this, the stimulus was presented. The stimulus was

presented in the centre of a white screen, in Arial font, size 18. The stimulus remained on the screen for a maximum time of 10000 ms, during which the participant was required to make their response, if not a 'no response' was logged. Following this a visual feedback screen was displayed, informing the participant whether their response was correct.

For each task, the percentage of correct responses was determined and the mean RT for correct responses calculated. An Interference Score was calculated by subtracting the mean time needed to complete the Colour and Word tasks from the time needed to complete the Colour-Word task (Interference = Colour-Word task – [(Word task + Colour task) / 2] (Valentijn et al., 2005).

### 3.8.2. Attention Network Test

The Attention Network Test (ANT) was developed by Fan et al. (2002) to assess three different attentional networks: alerting, orientating and executive control. These networks are suggested to be functionally and anatomically independent but together form the human attentional system (Fan et al., 2002, 2005). The alerting network refers to achieving and maintaining an alert and vigilant state, the orientation network describes the selection of information from sensory input, while executive control is the ability to resolve conflict between expectation, stimulus and response (Fan et al., 2002; Macleod et al., 2010). Importantly, the validity of the test has been confirmed as neuroimaging research has shown regions of the brain associated with these networks are activated while completing the test (Fan et al., 2005). Furthermore, the ANT is

viewed as having good face validity, as it is formed from two well established measures of attention (Macleod et al., 2010): the cued reaction time task (Posner, 1990) and the flanker task (Eriksen and Eriksen, 1974).

*3.8.2.1. Administration of the Attention Network Test*

Participants were presented with a stimulus in the form of a central arrow and were required to indicate the direction (left or right) of this arrow by clicking with the computer mouse in the corresponding direction. The central arrow was flanked by one of three different types of flankers: two arrows each side pointing in the same direction as the central arrow (congruent condition), two arrows each side pointing in the opposite direction of the central arrow (incongruent condition), or two straight lines each side of the central arrow (neutral condition). Prior to the presentation of the stimuli, participants were shown one of four cue (*) types: a central cue, a double cue, a spatial cue, or no cue. The central and double cues indicated to the participant that the stimuli would be presented soon, while the spatial cue additionally provided an indication of where the stimuli would be presented. The ‘no cue’ provided participants with none of this information. For each trial, first a fixation (+) was displayed in the centre of the screen for 400 ms. Following this, one of the four different cue types were presented for 100 ms. A second fixation was displayed for 400 ms, followed by one of the stimuli types (Figure 3-18). The fixation, cue and stimulus were presented in the centre of a white screen, in Arial font, size 18. The stimulus remained on the screen for a maximum time of 1,700 ms, during which the participant was required to make their response, if not a ‘no response’ was logged.

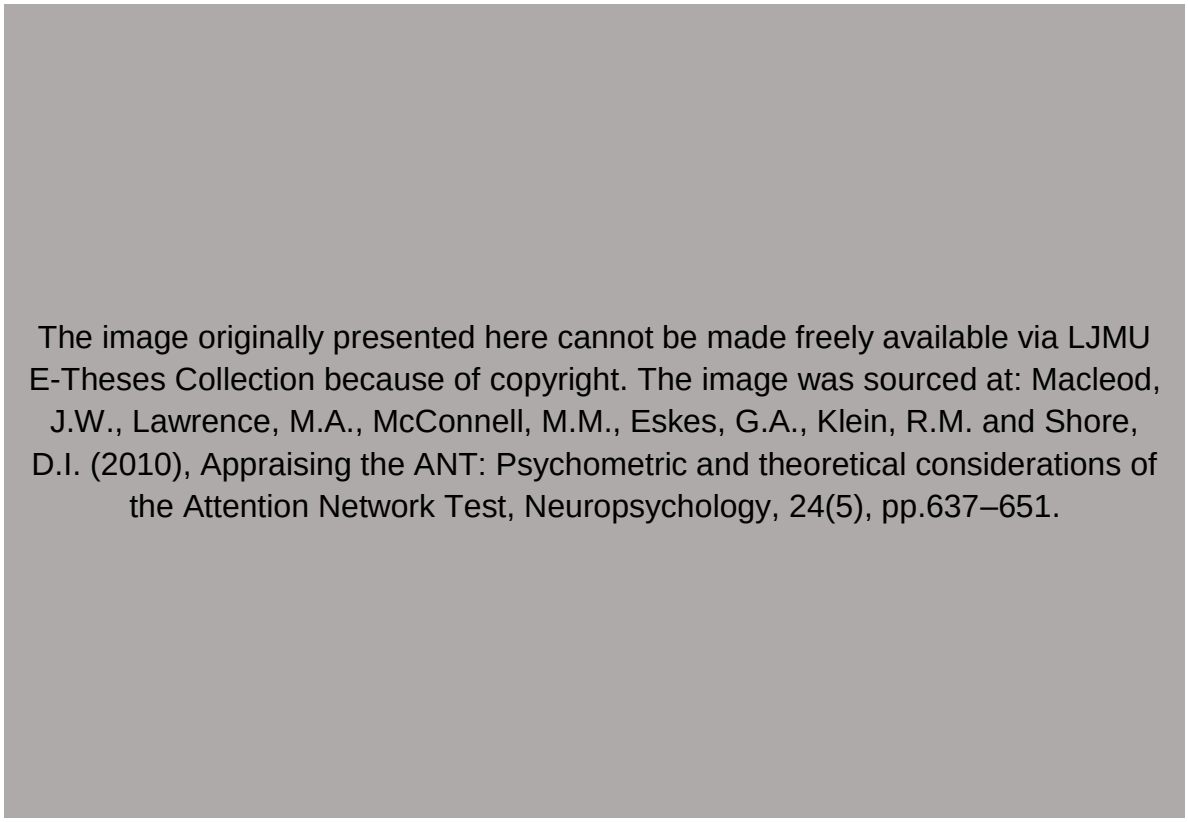


**Figure 3-18:** The Attention Network Test (ANT) experimental protocol. The sequence of events in one trial is shown in the left column, and all possible stimuli associated with each event are presented in the right column (adapted from Macleod et al., 2010).

Participants were told to respond as quickly and accurately as possible to each stimulus and instructed to keep their hand on the computer mouse throughout the task. A practise task of 24 trials with feedback was given to ensure participants understood the test protocol. Following this, participants completed three experimental blocks of trials, without any feedback given. Each block consisted of 96 trials. A break was given in between each experimental block.

The efficiency of the three attentional networks was assessed by determining how the alerting cues, spatial cues and flankers influenced response times (Fan et al., 2002). Mean RT for correct trials was calculated as a function of cue or flanker condition for each participant. To calculate the effect of an alerting cue on response times, the mean RT of the double cue trials was subtracted from

the mean RT of the no cue trials (Fan et al., 2002). To calculate the orienting effect, the mean RT of the spatial cue trials was subtracted from the mean of the central cue trials (Fan et al., 2002). The executive function effect was calculated as the mean RT of the congruent flanker conditions subtracted from the mean RT of the incongruent flanker conditions (Fan et al., 2002).

### 3.8.3. N-Back Task

The N-Back Task is widely used and popular test of working memory (Conway et al., 2005) that was developed from the work of Kirchner (1958). It is a type of working memory span task, which is recognised as a reliable and valid measure of working memory (Conway et al., 2005). The N-Back Task itself is viewed as having face validity to assess working memory (Kane et al., 2007).

#### *3.8.3.1. Administration of N-Back Task*

Participants were required to complete four separate test conditions: zero-back, one-back, two-back and three-back. The condition was manipulated to alter the work memory task demand (Jaeggi et al., 2010). For all conditions a series of letters were presented on the screen and the participants had to respond whether this letter was a target or a non-target. In the zero-back condition participants had to respond each time a specified target letter (‘x’) was presented. In the one-back condition, the target was any letter identical to the letter that immediately preceded it. In the two-back condition, the target was any letter that was identical to the one presented two letters back. Whilst in the three-back condition, the target was any letter that was identical to the one presented three letters back (Figure 3-19).

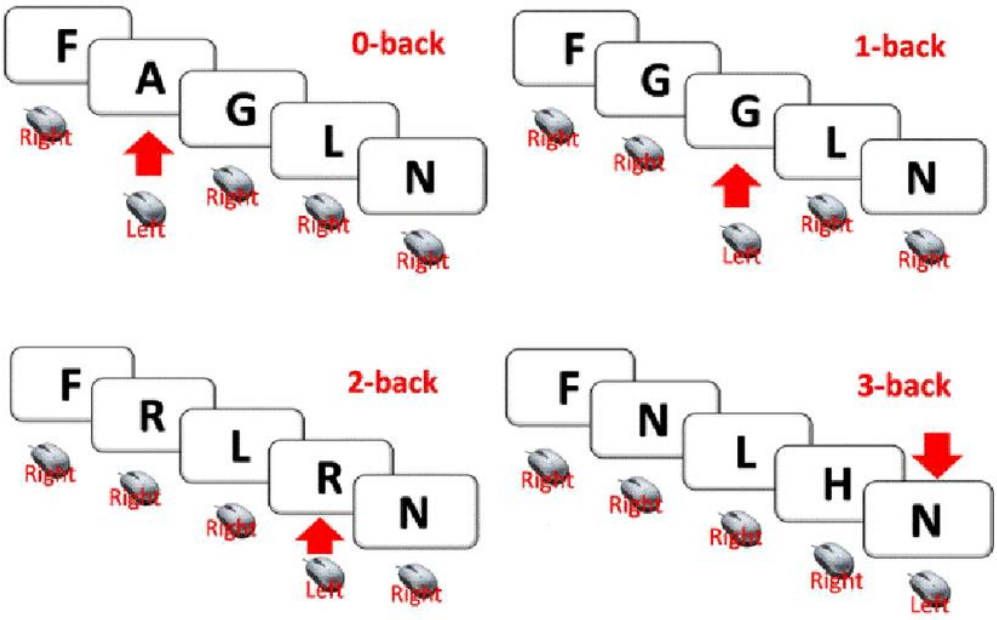


**Figure 3-19:** N-Back Task protocol.

To log their response, participants were required to click with the computer mouse either left for a target letter or right for a non-target letter. Participants were instructed to keep their hand on the mouse throughout the task. Each letter was presented in the center of a white screen, in Arial font, size 18 until the participant made their response. A blank screen was displayed for 3,000 ms in between each letter. For each condition, a practise task of 20 trials was given to ensure participants understood the test protocol. Following this, participants completed two experimental blocks. Each block consisted of 30 trials, of which 33% of the letters displayed were targets and the first three trials were never a target. A break was given in between each experimental block and between test conditions. For each condition, the percentage of correct responses was determined and the mean RT for correct responses calculated. Typically, as the working memory demand increases in each condition, so in turn does RT and the number of errors (Jaeggi et al., 2010).

## 3.9. Mood

### 3.9.1. Positive and Negative Affect Schedule

The Positive and Negative Affect Schedule (PANAS) is a 20-item self-report measure of affect developed by Watson et al. (1988). PANAS provides two overall scores that represent positive and negative affect. Positive affect refers to the extent to which an individual feels enthusiastic, alert and active; whilst negative affect reflects subjective distress and unpleasurable engagement (Watson et al., 1988). PANAS is formed of two 10-item mood scales. Individuals are required to rate on a 5-point scale the extent to which they have experienced each particular emotion within a specified time period (for example that day or over the last week). The scale points are: 1 ‘very slightly or not at all’, 2 ‘a little’, 3 ‘moderately’, 4 ‘quite a bit’ and 5 ‘extremely’ (Crawford and Henry, 2004; Watson et al., 1988). Values are then totalled to give separate positive and negative affect scores ranging from 10-50.

The PANAS is a valid and reliable measurement tool (Crawford and Henry, 2004; Watson et al., 1988). Initial work showed good reliability results, when participants were asked to respond with consideration to several specific time periods. For the positive affect items, the Cronbach’s alpha coefficient was 0.86 to 0.90; while for the negative affect items it ranged from 0.84 to 0.87 (Watson et al., 1988). More recent work supports these finding as Cronbach’s alpha coefficients were 0.89 for positive affect and 0.85 for negative affect (Crawford and Henry, 2004). Furthermore, over an 8-week time period, test-retest correlations were 0.47-0.68 for positive affect items and 0.39-0.71 for negative effect items (Watson et al., 1988). The correlations results were found to

increase as the time frame is which participants were required to recall also increased (e.g. at that moment in time compared to over the past week). However, this is expected as longer periods of recall lead to the averaging of emotions over a greater time period and stability of responses rises with increasing time over which feelings are accumulated (Watson et al., 1988).

### 3.9.2. Bond-Lader Mood Rating Scale

The Bond-Lader Mood Rating Scale (Lader and Bond, 1998) is a visual analogue scale that assesses three mood factors. The scale is formed of 12 individual visual analogue scales featuring bipolar end-points for different mood dimensions. Each scale is formed of a 100 mm line anchored at each end by antonyms. Individuals are required to mark on each scale where they feel at that moment. Each scale is scored from 0 to 100 based on the position the mark from the negative mood dimension. These scores are then combined to form three mood factors: alert, calm and contented; with each mood factor calculated as an average of the scores from the relevant mood scales (Lader and Bond, 1998).

## 3.10. Endothelial Function: Flow-Mediated Dilation

Flow-mediated dilation (FMD) provides a non-invasive assessment of endothelial function by measuring a vessel's vasodilator capacity (Corretti et al., 2002; Harris et al., 2010; Thijssen et al., 2011). The technique was introduced by Celermajer et al. (1992) and is now the most widely used *in vivo* method within clinical research (Greyling et al., 2016; Thijssen et al., 2011). This is largely due to the independent prognostic information FMD can provide, as FMD is predictive of cardiovascular events in both asymptomatic individuals and those with cardiovascular diseases (Thijssen et al., 2011). Meta-analyses have shown that brachial FMD is inversely associated with CVD incidence (Inaba et al., 2010; Ras et al., 2013) and that a 1% decrease in FMD is associated with a 13% higher risk of a future cardiovascular event (Inaba et al., 2010). The principle of FMD utilises a rapid increase in blood flow and subsequently shear stress to stimulate vasodilation (Pyke and Tschakovsky, 2007). The method involves the placement of a cuff around a limb which is then inflated for five minutes to occlude this vascular bed. The deflation of this cuff results in a rapid re-introduction of blood flow (reactive hyperaemia), elevating shear stress, which in turn promotes arterial vasodilation (Corretti et al., 2002; Thijssen et al., 2011; Figure 3-20).

The physiological basis of the FMD assessment is that peripheral conduit arteries regulate their vascular tone in response to blood flow (Tousoulis et al., 2005). Elevations in flow subsequently increase the shear stress against the endothelium of the vessel wall, which functions as a stimulus to enact a signalling cascade involving the hyperpolarisation of the endothelial cell via

opening of ion channels (Moens et al., 2005). This in turn increases calcium entry which activates endothelial NO synthase leading to the formation of NO and subsequent vessel dilation (Corretti et al., 2002; Moens et al., 2005). Endothelial dysfunction presents as impaired ability for vascular dilation, and thus attenuated FMD (Corretti et al., 2002; Moens et al., 2005; Thijssen et al., 2011).

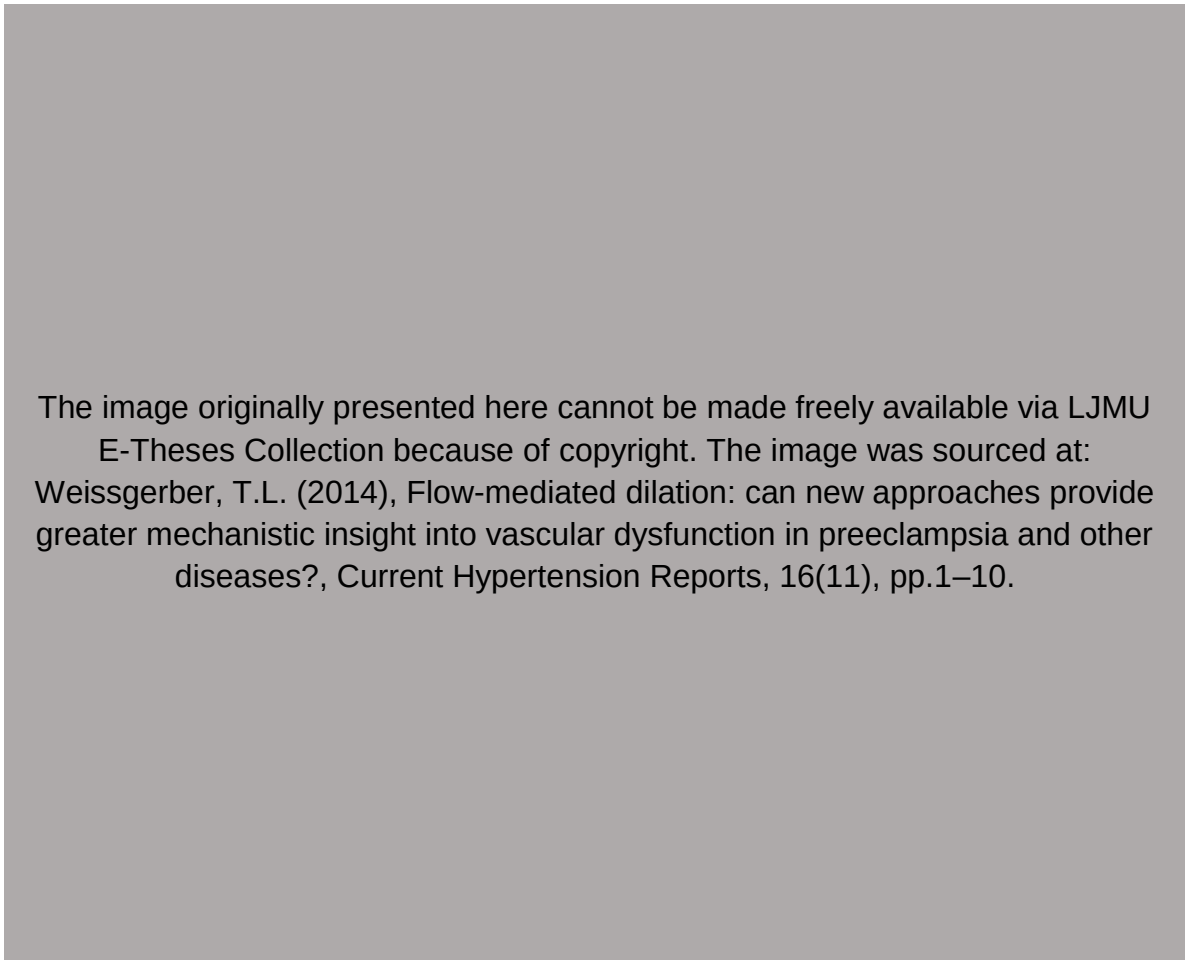


**Figure 3-20:** The flow-mediation dilation (FMD) protocol including a one minute baseline measurement, five minutes of distal cuff occlusion and three minutes of reactive hypereamia (adapted from Weissgerber, 2014).

### 3.10.1. Assessment of Endothelial Function

The use of Doppler ultrasound has become the prominent method to assess endothelial function using the FMD technique, mainly due to its non-invasive protocol (Harris et al., 2010). The use of Duplex mode ultrasound is preferred as this enables two-dimensional imaging of the vessel of interest to assess vessel diameter (B-mode), whilst Doppler is used for the determination of blood velocity (Harris et al., 2010). Using a high-resolution ultrasound probe, a longitudinal image of the vessel is acquired, enabling the borders of the vessel to be determined.

Assessment of brachial and femoral artery endothelial function was performed according to published guidelines (Thijssen et al., 2011). A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA, USA) was positioned either around the right thigh, above the patella or around the left forearm with the proximal border adjacent to the medial epicondyle (Harris et al., 2010; Thijssen et al., 2011). To image the vessels a 10-MHz multi-frequency linear array probe, attached to high resolution ultrasound machine (T3000; Terason, Burlington, MA, USA) was used. Images were acquired proximal to the occlusion cuff (Figure 3-21). For the acquisition of arterial diameters, ultrasound parameters were adjusted to optimise the B-mode image of the lumen-arterial wall interface. Once a satisfactory image was obtained, the probe was held consistently in this position. Arterial blood flow velocity was simultaneously assessed via Doppler ultrasound using the same ultrasound machine with a consistent insonation angle of 60° for each assessment (Figure 3-22). Baseline arterial diameter and blood flow velocity were recorded for one minute.

Following this, the cuff was inflated to 220 mmHg for five minutes to induce local ischemia. After cuff deflation, arterial diameter and blood flow velocity recordings were continued for a further three minutes.

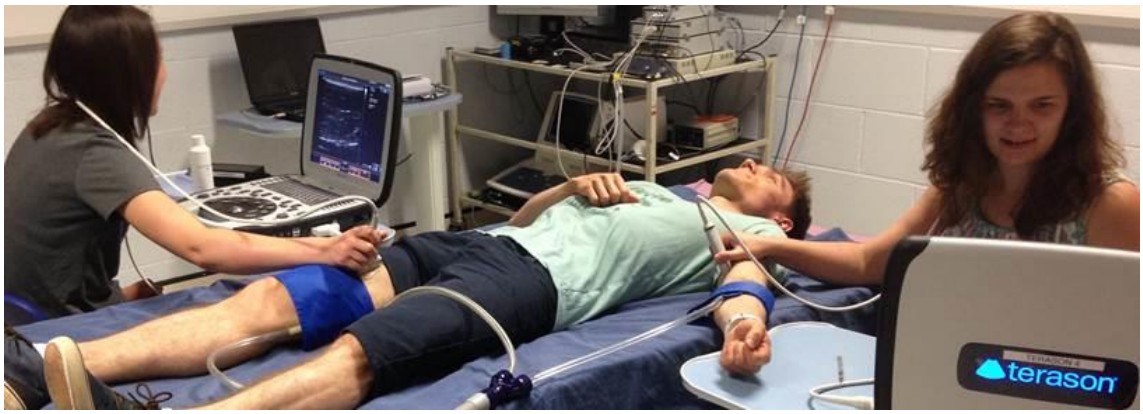


**Figure 3-21:** Assessment of brachial and femoral artery endothelial function using the flow-mediated dilation (FMD) method.

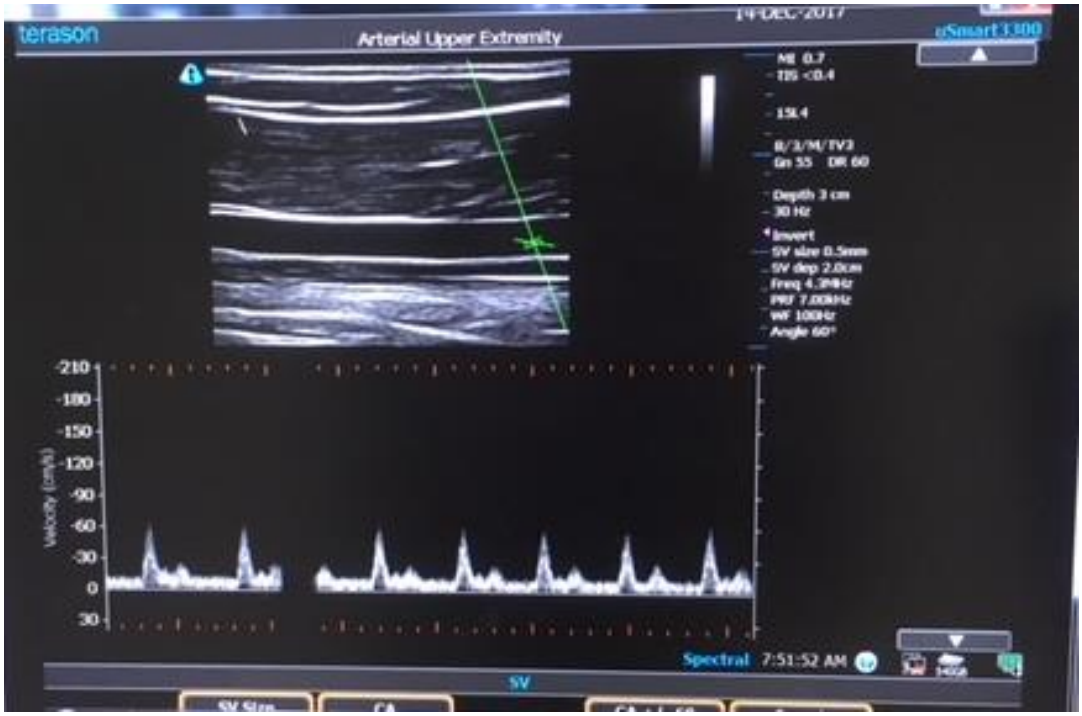


**Figure 3-22:** Acquisition of brachial arterial diameter using B-mode imaging and simultaneous arterial blood flow using Doppler ultrasound.

FMD data were analysed using custom designed automatic edge-detection and wall-tracking software, a reproducible and valid method (Green et al., 2002; Woodman et al., 2001) which is largely independent of investigator bias (Woodman et al., 2001). The software enables regions of interest (ROI) to be selected from the initial frame of each data file for the B-mode image and Doppler waveform. To analyse arterial diameter, a ROI is selected based on the clarity of the B-mode image and the distinction between the arterial wall-lumen interface. Within this ROI a pixel-density algorithm automatically identifies the angle-corrected near and far-wall e-lines for every pixel column (Black et al., 2008). A second ROI is selected to encompass the Doppler waveform, which then automatically detects the peak of the waveform (Figure 3-23; Black et al., 2008). Each frame is then subsequently analysed at a rate of 30 Hz, enabling synchronised arterial diameter, blood velocity, blood flow (the product of arterial cross sectional area and blood velocity) and SR (four times blood velocity divided by arterial diameter) data to be acquired (Figure 3-24; Black et al., 2008).

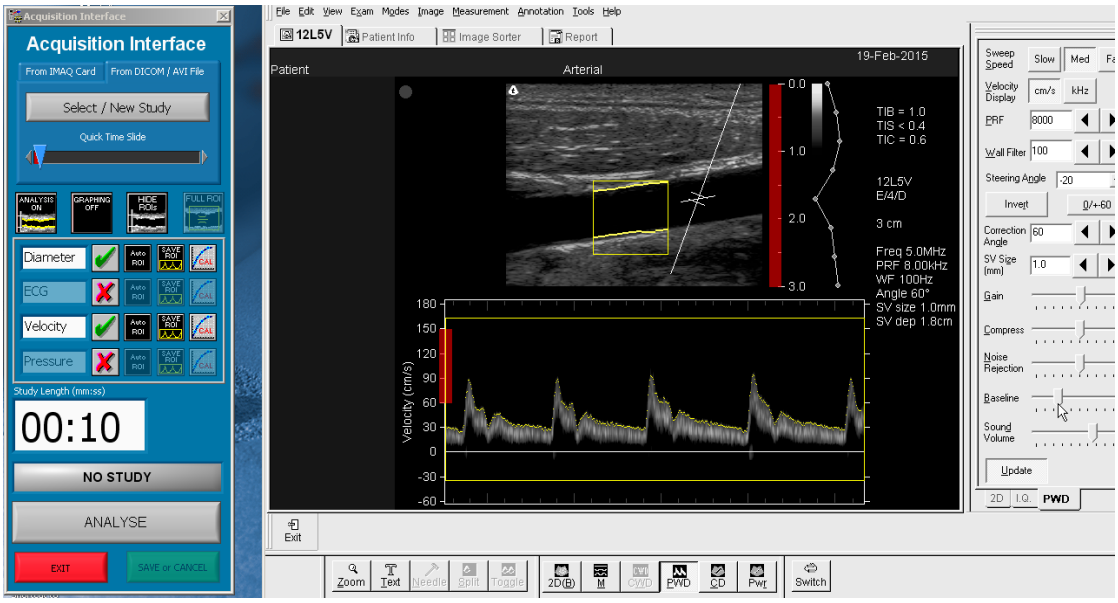


**Figure 3-23:** Analysis of flow-mediated dilation (FMD) data using custom designed automatic edge-detection and wall-tracking software. The yellow boxes represent regions of interest (ROI) that have been selected to identify the arterial wall-lumen interface and the Doppler waveform.

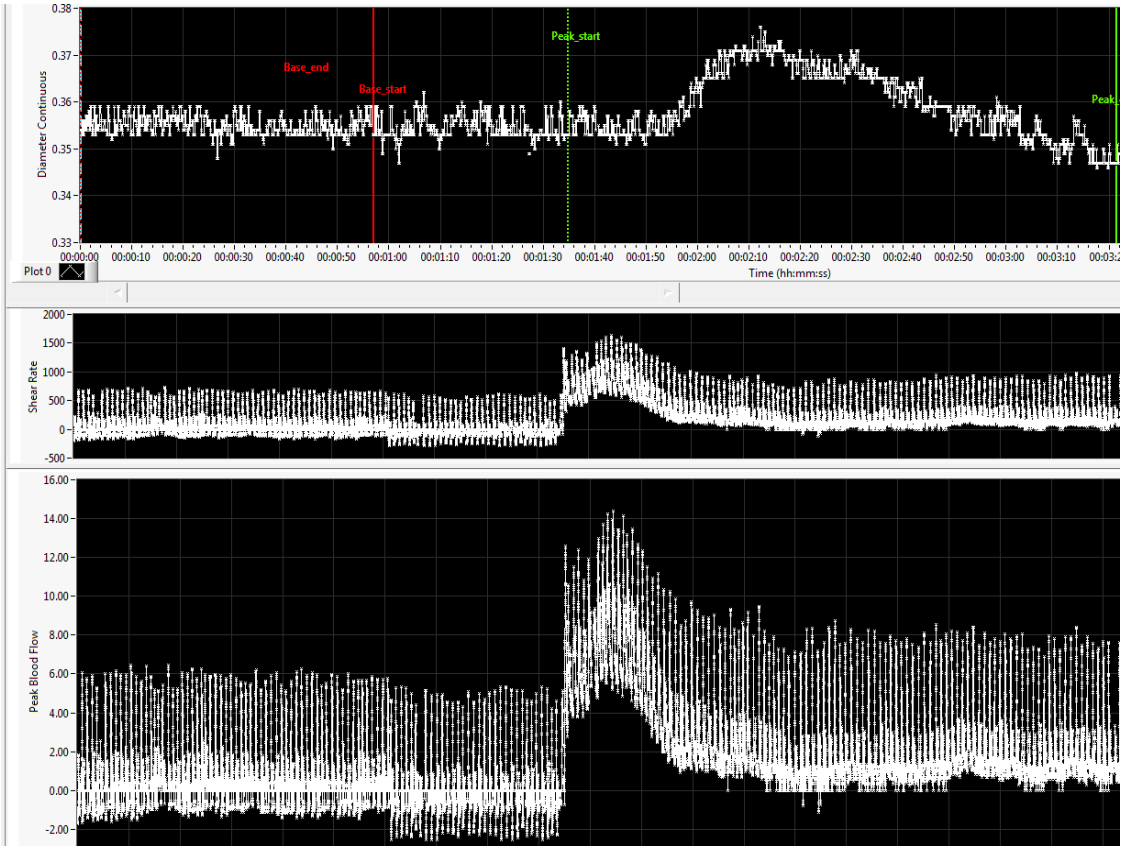


**Figure 3-24:** Output from the analysis of flow-mediated dilation (FMD) data using custom designed automatic edge-detection and wall-tracking software. The top box provides continuous arterial diameter data, the middle box provides shear rate (SR) data, the lower box provides blood flow data (calculated from diameter and velocity).

The validity and reproducibility of this analysis software has been previously shown using three validation studies (Woodman et al., 2001). Firstly, compared to phantom arteries with known diameters, there was only a 0.011 mm difference between the manual measurement and that using the software. The reproducibility of the analysis software was also greater compared to a traditional manual calliper method; with intra-observer coefficients of variations significantly lower for FMD using the software analysis (6.7% vs. 24.8%). Furthermore, the between-visit reproducibility of FMD using the software analysis was shown to be 14.7% (Woodman et al., 2001).

Baseline arterial diameter, blood flow and SR were determined as the mean of the data acquired one minute prior to cuff inflation. Following cuff deflation, peak vessel diameter was automatically calculated using the custom designed software. This process involves an algorithm which identifies the maximum bracket of data using a moving window smoothing function (Black et al., 2008). The median value of 100 consecutive samples is calculated, before the window shifts to the next bracket of data, which has a 20% overlap with the preceding bracket. The maximum value from all median values is automatically determined as peak vessel diameter. From these data, FMD was calculated as the percentage increase in arterial diameter from baseline arterial diameter, and is an observer independent calculation (Black et al., 2008).

## 3.11. Sedentary Behaviour

In this thesis, SB was measured both subjectively, using questionnaires, and objectively, using an inclinometer.

### 3.11.1. Subjective Measurement: International Physical Activity Questionnaire

The International Physical Activity Questionnaire (IPAQ) was developed by a consensus group of PA assessment experts under the premise of creating a valid and reliable questionnaire to measure daily, health enhancing PA (Hagströmer et al., 2006). The IPAQ considers PA across four domains: during transportation, at work, during household and gardening tasks, and during leisure time; and two version of the IPAQ can be used: the short form or the long form. Despite being primarily designed to assess PA levels across multiple domains, the questionnaire also includes items considering sitting time. In both the short and long versions of the IPAQ participants are instructed to recall the time they have spent sitting at work, at home, while doing course work, and during leisure time. They are asked to estimate the total number of hours and minutes per day they spend sitting for a weekday and for a weekend day. The sitting items on the IPAQ have been shown to be a valid and reliable assessment of SB (Rosenberg et al., 2008). IPAQ reported total sitting time was significantly correlated to objectively measured SB using accelerometry both for the long (r=0.33) and short (r=0.34) forms of the questionnaire (Rosenberg et al., 2008).

### 3.11.2. Subjective Measurement: Workforce Sitting Questionnaire

The Workforce Sitting Questionnaire (WSQ; Chau et al., 2011) allows the assessment of domain-specific sedentary time. The questionnaire was adapted from the Marshall Sitting Questionnaire (Marshall et al., 2010) which found that sitting time across all domains was more reliably and validly recalled for weekdays than weekend days (Chau et al., 2011; Marshall et al., 2010). It was suggested this was due to difficulty recalling time spent in less structured activities, such as at a weekend, compared to weekday structured routines, such as work (Marshall et al., 2010). The WSQ is version of the Marshall Sitting Questionnaire designed for a working population and therefore specifically asks participants to recall their SB on a work day and a non-work day. Participants report their time spent sitting while: travelling, at work, watching television, using a computer at home, and doing other leisure activities. Participants are required to recall this for a typical workday and a non-workday over the last seven days. Total sitting time on a workday and on a non-workday is defined as the sum of all domains for each respective day. Participants also report the number of days they were at work over the last seven days, which is used to calculate average total sitting time per work day and non-work day (Chau et al., 2011). The WSQ has been shown to be a valid and reliable measure of SB (Chau et al., 2011). The validity of the questionnaire was compared to objectively measured sitting time using accelerometery over one week and showed sufficient criterion validity (r=0.45), whilst the test-retest reliability was good (Intraclass Correlation Coefficient (ICC)=0.63) (Chau et al., 2011). The questionnaire has been previously used in other research assessing workplace sitting time (Chau et al., 2014; De Cocker et al., 2014).

### 3.11.3. Objective Measurement: activPAL

The activPAL is a small activity monitor (5×3.5×0.7 cm) weighing 20 g which is worn on the anterior mid-line of the right thigh (Figure 3-25). It is attached using a waterproof dressing, enabling it to be worn continuously throughout an assessment period (Ryan et al., 2006). The activPAL contains a tri-axial accelerometer which responds to gravitational acceleration in addition to acceleration due to segmental movement (Grant et al., 2006; Ryan et al., 2006). Consequently, when the device is attached to the thigh, the monitor uses the position of the thigh to infer posture and in turn classify between sitting/lying, standing or walking (Grant et al., 2006; Ryan et al., 2006). The monitor can also determine step count and step cadence during walking activities, and sit-to-stand transitions. Data is collected at a sampling frequency of 20 Hz and a proprietary algorithm is used in manufacturer specific software to convert signals into measures of body posture in 15-second epochs (Kim et al., 2015).

The activPAL has been shown to be a valid and reliable measure of sedentary time (Grant et al., 2006). An initial validation study compared the time spent during sitting, standing, walking and while completing different activities of daily living between data from activPAL monitor and a synchronised video recording (Grant et al., 2006). The ICC for inter-observer reliability was >0.97 for all activities and postures, while inter-device reliability ICC was >0.99 for all postures and activities except walking (ICC=0.79) (Grant et al., 2006). Bland-Altman plots also demonstrated good agreement between the activPAL and direct observation for the time spent during each activity (Grant et al., 2006).

More recent research has further confirmed the validity of the activPAL device to measure SB when compared to direct observation (Kim et al., 2015; Kozey-Keadle et al., 2011). In particular, it has been shown that the activPAL is able to accurately detect purposeful reductions in sitting time (Kozey-Keadle et al., 2011), important for behaviour change research. Due to its reliability and validity, alongside is unobtrusive design, simple application and no need for user calibration, the activPAL has been widely used in SB research (Grant et al., 2006). Additionally, the activPAL monitor has been used to specifically examine SB within a workplace environment (Healy et al., 2013; Ryan et al., 2011; Smith et al., 2015).

**(a)** **(b)**

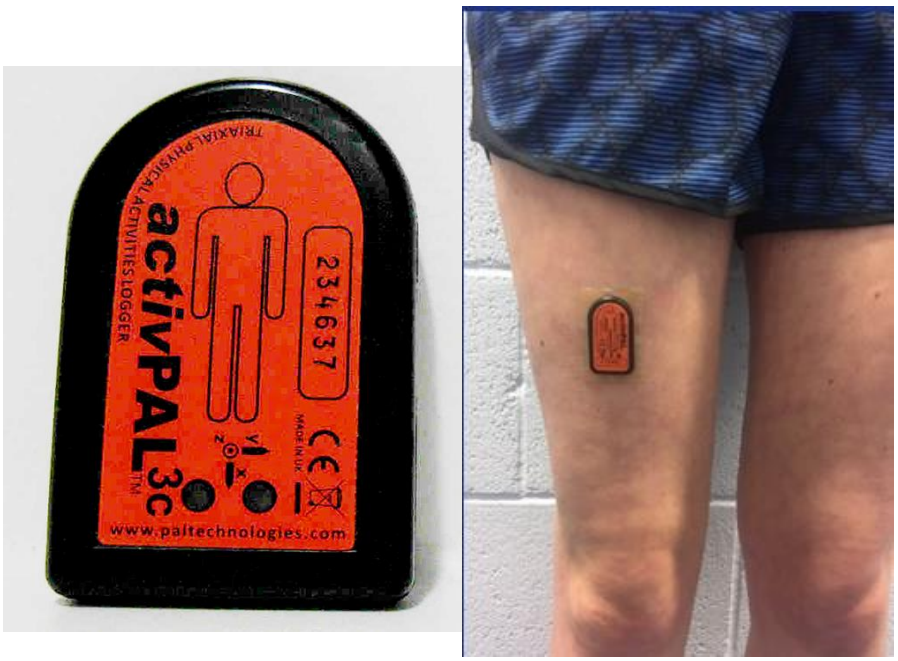


**Figure 3-25:** (a) The activPAL monitor used to objectively measure sedentary behaviour (SB). (b) The activPAL monitor positioned on a participant's leg.

*3.11.3.1. Assessment of Sedentary Behaviour Using activPAL*

For each participant, the activPAL was initialised, waterproofed and secured onto the anterior mid-line of their right thigh. The activPAL was waterproofed using a small flexible sleeve to cover the monitor and then secured to the leg by the principal researcher using a waterproof medical grade adhesive dressing (Tegaderm). Waterproofing the device permitted participants to wear the monitor continuously for the entire assessment period, which can increase wear time compliance (Edwardson et al., 2016). Additional waterproof dressings and attachment instructions were given to participants in case the monitor became detached during the assessment period to allow for reattachment, or they were advised to contact the principal researcher. Participants were instructed to wear the activPAL monitor continuously over five working days and two weekend days (i.e. Saturday and Sunday). Monitoring for seven days is recommended to produce valid data (Edwardson et al., 2016). To delineate between workplace and leisure time SB, participants were given a log book to record the time they started and finished work each day. Additionally, participants recorded the time they woke up and went to bed on each day to allow for only waking hours to be included in analyses. Participants were provided with written and verbal instructions regarding how to wear the device and use the log book.

Data were downloaded using activPAL software (version 7.2.32) and saved in 24-hour periods in 15 second epochs. Data were then exported into Excel for analyses. First, the seven-day monitoring period was divided into individual Excel worksheets, one for each day of the week. Based on a participants' log book, these days were then identified as either a work day or a weekend day.

For each day, total step count, sit-to-stand transitions, stand-to-sit transitions, and the time spent sedentary, upright and stepping were calculated for waking hours, defined using the participants' logbook. For each variable, this involved summing the values in the cell reference range from the self-reported wake up time to the self-reported bed time. Time spent sedentary, upright and stepping were then calculated as a percentage of total wake time. After analysing all seven days, mean values were determined for each variable to represent a working day and a weekend day. Participants' log book recording of their working hours were used to determine the time spent at work. An identical process was used to calculate the same variables, however instead using the cell reference range that corresponded to the self-reported time a participant began work and the self-reported time they finished work. Time spent sedentary, upright and stepping were then calculated as a percentage of total work hours. After analysing all work days, mean values were determined for each variable to represent a working day.

## 3.12. Work Productivity

This thesis included tests of work productivity, which although were unvalidated methods to assess work performance domains, they were included as ecologically important measures representative of an office workers' tasks.

### 3.12.1. Typing Performance

Participants were required to copy nontechnical material of moderate difficulty (syllabic intensity of 1.3) using specialized, split-screen display typing software (Typemaster Pro, Typemaster Finland, Helsinki, Finland). The top window contained the text to be copied, which was typed by participants and appeared in the bottom window (Figure 3-26). All scripts used were of equal syllabic intensity. Participants were instructed to type as fast and as accurately as they could for two minutes and errors could only be corrected while typing the same word. Gross and net typing speed (words per minute) and accuracy were subsequently calculated.

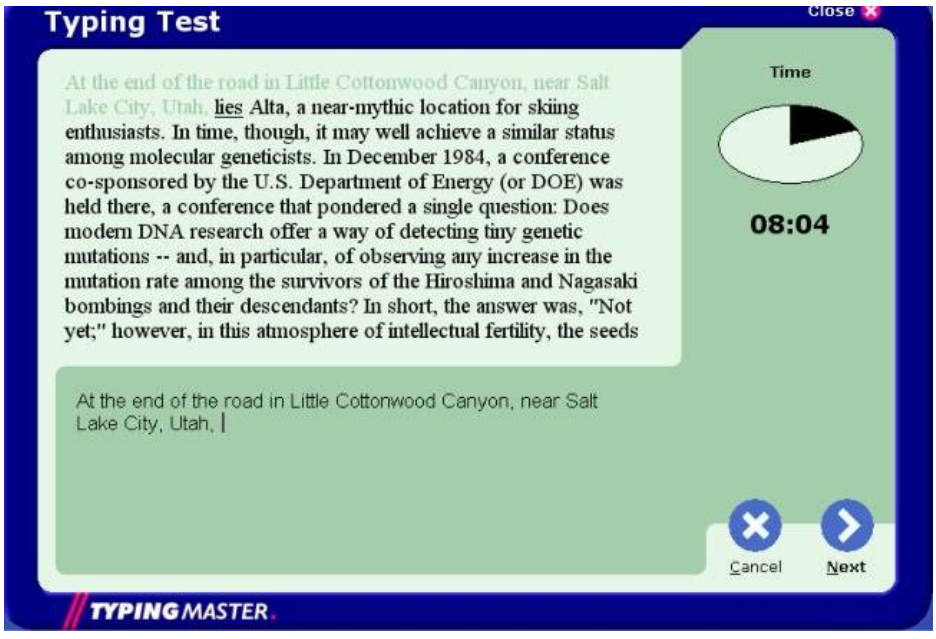


**Figure 3-26:** Screenshot of the Typemaster Pro software used for the typing performance test.

### 3.12.2. Reading and Correcting Task

Participants silently read a generic text from a screen for three minutes. The text featured one character rotations (e.g. “hlelo” instead of “hello”) after approximately every 50 words and participants were required to identify and correct these character rotations. The numbers of characters read and percentage of missed character rotations were recorded.

### 3.12.3. Mouse Dexterity

Participants completed a Random Circles test (Hillcrest Freespace MotionStudio Version 3.4.0) which is based on Fitts' Law (Fitts, 1954). Fitts’ Law predicts that the time required to rapidly move to a target area is a function of the ratio between the distance to the target and the width of the target (MacKenzie, 1989). A total of 100 dots of different sizes appeared randomly on the computer screen which participants were required to click on as fast as possible (Figure 3-27). RT (the interval between the appearance of a dot and the participant clicking the dot) and a performance score (combining accuracy and movement speed) were recorded.

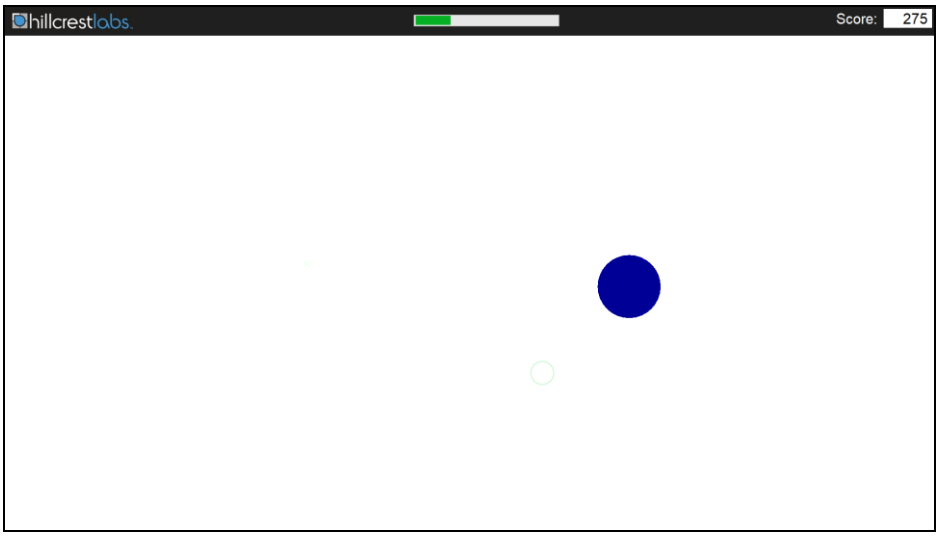


**Figure 3-27:** Screenshot of the Random Circles test to assess mouse dexterity.

# 4. Relationship between workplace sedentary behaviour, cognition and mood in healthy workers

## 4.1. Introduction

The importance of workplace physical inactivity was first demonstrated with the observation that active bus conductors had lower CVD incidence compared to 'inactive', or as they would now be classified, sedentary, bus drivers (Morris and Crawford, 1958). The workplace has since been identified as a key setting where adults accrue high amounts of SB, with office workers spending 65–75% of their work hours sitting, typically in prolonged bouts (Buckley et al., 2015). Importantly, a significant proportion of the week is spent at work, thus exposing workers to high levels of SB. Considering this, recent guidelines suggest reducing workplace SB could improve employee health and wellbeing, as well as their productivity (Buckley et al., 2015). However, there is little evidence from workplace intervention studies to support these recommendations; a fact that has been recently criticised (Stamatakis et al., 2018).

Cognition is related to work performance due to its influence on workers' ability to learn and execute the skills needed to carry out work-specific tasks (Fisher et al., 2017). Indeed, cognitive ability is negatively associated with counterproductive work behaviours (Dilchert et al., 2007) and employees with greater cognitive capabilities perform more work tasks (Morgeson et al., 2005). Pertinently, associations between cognition and SB have been observed. Cross-sectional and prospective studies in older adults indicate that SB is negatively associated with cognition (Edwards and Loprinzi, 2017a, 2017b; Falck et al., 2017). However, such research excludes the working-age population, an important and potentially at risk cohort since some aspects of cognitive performance start declining from the age of 20 years (Salthouse,

2009). Additionally, mood influences work productivity (Kaplan et al., 2009; Shockley et al., 2012), as workers in a positive mood are more efficient and effective within their job roles (Miner and Glomb, 2010; Rothbard and Wilk, 2011). Furthermore, positive affect is positively related to task performance and negatively related to counterproductive work behaviours, with opposite associations observed for negative affect (Kaplan et al., 2009; Shockley et al., 2012). Mood decreases following up to two weeks of experimentally increasing free-living SB (Edwards and Loprinzi, 2016a; Endrighi et al., 2016), however whether SB accrued specifically during working hours contributes to this mood disturbance is unknown.

Although less sedentary individuals may have increased cognitive performance and mood, it is not known what has displaced SB to achieve these benefits, for example standing or stepping. Importantly, guidelines to reduce sitting in the workplace recommend progressing towards two hours of standing and light-activity during working hours (Buckley et al., 2015). However, the recommendation of light-intensity PA and standing is based on previous research showing improved blood glucose and insulin concentrations when breaking up prolonged sitting (Bailey and Locke, 2015; Dunstan et al., 2012; Thorp et al., 2014). Consequently, whether light-intensity PA can have beneficial effect on factors influencing work productivity, such as cognition and mood, is unknown. Accordingly, this study aimed to firstly assess the relationship between cognition, mood and objectively measured time spent sitting, stepping or standing whilst at work as well as during a weekday and a weekend. Secondly, this study aimed to assess the relationship between

cognition, mood and light-, moderate-, and vigorous-intensity PA at work and during a weekday and weekend. It was hypothesised that greater time spent sitting at work would be associated with impaired cognition and mood and, based on current workplace guidelines (Buckley et al., 2015), it was hypothesised that light-intensity PA at work would be positively associated with cognition and mood.

## 4.2. Methods

### 4.2.1. Participants

Eighty-four healthy, full-time workers (33 male) volunteered and written informed consent was obtained prior to inclusion. Participants were screened prior to testing for exclusion criteria including: use of medication, smoker, BMI >35 or <18 $kg \cdot m^{-2}$ and diagnosis of cerebrovascular, cardiovascular or metabolic disease. Study procedures were approved by the Liverpool John Moores University Ethics Committee and adhered to the Declaration of Helsinki. From the originally recruited sample size, nine participants were excluded due to incomplete data (either incomplete log books or non-valid wear time for the activity monitors), thus the final sample size used for analyses was seventy-five.

### 4.2.2. Study Design and Procedures

Data collection occurred either at Liverpool John Moores University or at the participants' workplace in a private, quiet room without any external disturbances. Participants completed two test visits. During visit one, participants were fitted with two activity monitors and given a wear-time log-book to complete. Following this, participants continuously wore the monitors for the next seven consecutive days. The second visit occurred between 7.00-9.00 am the day after participants finished wearing the monitors. During this visit participants completed a battery of computer-based cognitive performance tests and two mood questionnaires (Figure 4-1).

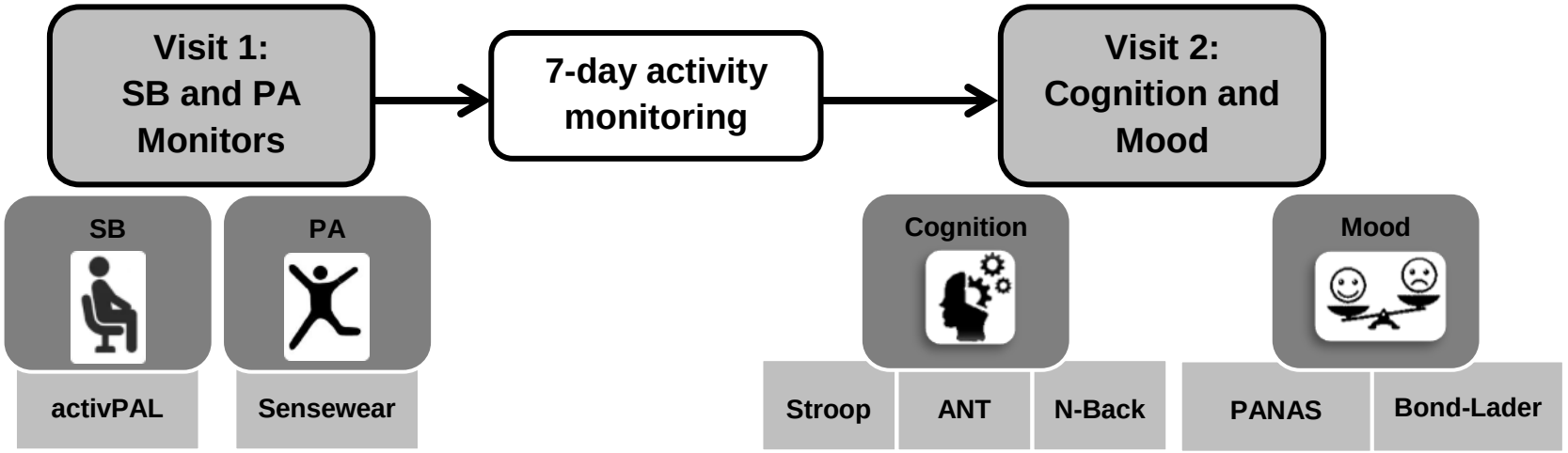


**Figure 4-1:** Study design. SB- sedentary behaviour; PA- physical activity; ANT- attention network test; PANAS- positive and negative affect schedule.

### 4.2.3. Measurements

The measurement of SB, cognition and mood are described in detail in Chapter 3. General Methods, hence here only specific features of this study are outlined.

#### *4.2.3.1. Physical Activity*

PA was assessed using the SenseWear Pro 3 (BodyMedia, Inc., Pittsburgh, PA, USA), a multisensory body monitor that is a valid method to assess energy expenditure and in turn PA (Casiraghi et al., 2013). Each armband was initialised based on participants' stature, body weight, sex and age. Participants then wore the armband around the upper right arm, in accordance with manufacturer guidelines. Participants were instructed to wear the armband continuously for seven days, only removing for showering or other water-based activities. Data were downloaded from the armband and analysed using SenseWear professional software (version 7.0, BodyMedia, Inc.), which uses algorithms developed by the manufacturer to determine MET values for one minute epochs. For each day data were considered valid if the monitor was worn ≥10 hours per day and if wear time corresponded with the participant's self-report wear time diary. Based on this criteria, a participants' data was used in analyses if three weekdays and two weekend days were considered valid (Scheers et al., 2012). These data were then exported to Excel (Microsoft) for further analyses. For each day, the time spent in different categories of PA was determined based on recognised METs values: light-intensity PA 1.5-3.0 METs, moderate-intensity PA 3.1-6.0 METs and vigorous-intensity PA >6.0 METs (Ainsworth et al., 2011). Minute-by-minute data for each category were then summed to determine the total time spent in each intensity of activity per day for waking hours. These values were then summed to calculate total PA per day for

waking hours. Data were then expressed as a percentage of waking hours. Mean values were then determined for each variable for a weekday (WEEK) and a weekend day (WEEKEND). Participant log-book recording of their working hours were used to determine time spent at work. The same variables were then calculated for daily working hours using these self-report times (WORK). Total values for the week (TOTAL) were calculated using a weighted mean to account for the disproportionate time spend in weekdays compared to weekend days across a week (WEEK x 0.71 + WEEKEND x 0.29). Variables for leisure time during the weekday (WEEKLEISURE) were calculated by subtracted WORK data from WEEK data, therefore removing any activity during the time spent at work.

*4.2.3.2. Sedentary Behaviour*

SB was assessed using the activPAL, as described in detail in section 3.11.3.1, hence here only specific features of this study are outlined. Data were considered a valid day if the monitor was worn ≥10 hours per day and if wear time corresponded with the participant's self-report wear time diary. The latter was achieved by visually inspecting the activPAL graphical data and event file outputs following analyses to assess if self-report wake up and bed time corresponded with activPAL data. When assessing working hours, it was required that the monitor was worn for 100% of work time. Furthermore, data were only included if a valid activPAL wear day had a corresponding valid SenseWear wear day, thus both SB and PA data were valid for the same day (i.e. if the participant only wore one of the monitors this day was excluded).

Based on these criteria, activPAL data was only included if there were a minimum of three valid weekdays and two valid weekend days.

For each day, the time spent in sitting, standing and stepping were determined using the method described in section 3.11.3.1. Data were then expressed as a percentage of waking hours (%SIT, %STAND, %STEP). Mean values were then determined for each variable for a weekday (WEEK) and a weekend day (WEEKEND). Participant log-book recording of their working hours were used to determine time spent at work. The same variables were then calculated for daily working hours using these self-report times (WORK). Total values for the week (TOTAL) were calculated using a weighted mean to account for the disproportionate time spend in weekdays compared to weekend days across a week (WEEK x 0.71 + WEEKEND x 0.29). Variables for leisure time during the weekday (WEEKLEISURE) were calculated by subtracted WORK data from WEEK data, therefore removing any activity during the time spent at work.

### 4.2.4. Statistical Analyses

Data were analysed using statistical software (SPSS Version 23.0, IBM Corporation, Somers, NY, USA), with significance accepted as $p<0.05$. Results are presented as means±standard deviation (SD). Data were assessed for normal distribution using Shapiro-Wilk tests. Pearson's bivariate correlation (parametric data) and Spearman's correlation (non-parametric data) were used to assess the relationship between %SIT, %STAND and %STEP during weekdays, the weekend and during work hours and all cognition and mood variables. Owing to the age-related changes in cognition (Salthouse, 2009) and

mood (Stanley and Isaacowitz, 2011), bivariate correlations were ran between age and all cognition and mood outcomes. Where significant correlations were observed, age was subsequently used as a covariate in subsequent analyses. To assess the independent influence of %SIT, %STAND and %STEP on any relationships, partial correlations were conducted with covariate control for the percentage of time spent in all other activity categories across all domains. To assess if the intensity of PA was associated with cognition and mood, correlation analyses were conducted between light-, moderate- and vigorous-intensity PA and all cognitive performance and mood variables with covariate control for all other activity categories across all domains. Finally, activity monitor wear time was used as a covariate in all analyses to account for differences in wear time between participants.

## 4.3. Results

Participants were a mean age of 33.6±10.4 years, with a body mass of 71.8±14.2 kg, stature of 169.3±9.4 cm and a body mass index of 25.0±3.8 $kg \cdot m^{-2}$. Mean sitting, standing, stepping and PA time for work hours, weekdays and weekends are shown in Table 4-1. During work hours participants on average spent 66.1±14.3% of their time sitting, 23.0±10.8% of their time standing and 10.9±6.5% of their time stepping. During weekdays participants on average spent 61.0±10.1% of their waking hours sitting, 26.1±7.6% of their waking hours standing and 13.0±4.7% of their waking hours stepping. During weekends participants on average spent 56.2±14.5% of their waking hours sitting, 30.2±10.6% of waking hours standing and 13.6±5.5% of their waking hours stepping. Age was significantly associated with the orienting network score ($r=-0.401$, $p<0.01$) and the executive control score ($r=0.273$, $p=0.019$), therefore age was used as a covariate for the analyses of these cognitive outcomes. No other significant associations were observed between age, cognition and mood ($p>0.05$).

**Table 4-1:** Objectively measured mean sitting, standing, stepping and physical activity (PA) time of participants (n=75; mean±SD).

| | Time | % of Waking Wear Time |
|---|---|---|
| **Work Hours** | | |
| Sitting Time (minutes) | 325.6±86.3 | 66.1±14.3 |
| Standing Time (minutes) | 117.9±62.4 | 23.0±10.8 |
| Stepping Time (minutes) | 55.0±36.4 | 10.9±6.5 |
| Light-Intensity PA (minutes) | 142.2±59.3 | 28.7±10.9 |
| Moderate-Intensity PA (minutes) | 40.9±35.2 | 8.2±6.3 |
| Vigorous-Intensity PA (minutes) | 1.8±3.5 | 0.4±0.7 |
| MVPA (minutes) | 42.7±36.0 | 8.6±6.4 |
| Total PA (minutes) | 184.9±80.0 | 37.3±13.9 |
| **Weekdays** | | |
| Sitting Time (minutes) | 581.5±98.2 | 61.0±10.1 |
| Standing Time (minutes) | 250.1±78.7 | 26.1±7.6 |
| Stepping Time (minutes) | 124.6±47.9 | 13.0±4.7 |
| Light-Intensity PA (minutes) | 286.4±83.8 | 31.6±8.6 |
| Moderate-Intensity PA (minutes) | 94.1±58.0 | 10.3±6.2 |
| Vigorous-Intensity PA (minutes) | 9.8±10.6 | 1.1±1.1 |
| MVPA (minutes) | 103.9±62.6 | 11.4±6.7 |
| Total PA (minutes) | 390.3±114.8 | 43.0±11.6 |
| **Weekends** | | |
| Sitting Time (minutes) | 500.8±125.3 | 56.2±14.5 |
| Standing Time (minutes) | 272.6±99.9 | 30.2±10.6 |
| Stepping Time (minutes) | 123.1±54.1 | 13.6±5.5 |
| Light-Intensity PA (minutes) | 304.2±106.3 | 36.7±11.8 |
| Moderate-Intensity PA (minutes) | 90.0±66.0 | 11.0±8.2 |
| Vigorous-Intensity PA (minutes) | 8.0±13.9 | 0.9±1.6 |
| MVPA (minutes) | 98.0±72.9 | 11.9±8.9 |
| Total PA (minutes) | 402.2±132.9 | 48.6±14.2 |
| **Whole Week** | | |
| Sitting Time (minutes) | 556.2±88.3 | 59.7±9.4 |
| Standing Time (minutes) | 255.6±72.5 | 27.2±7.1 |
| Stepping Time (minutes) | 123.7±43.0 | 13.2±4.3 |
| Light-Intensity PA (minutes) | 283.0±87.4 | 32.8±8.0 |
| Moderate-Intensity PA (minutes) | 90.3±54.4 | 10.5±6.1 |
| Vigorous-Intensity PA (minutes) | 9.0±10.0 | 1.0±1.1 |
| MVPA (minutes) | 99.3±59.5 | 11.5±6.5 |
| Total PA (minutes) | 387.5±108.6 | 44.4±10.7 |

PA- physical activity; MVPA- moderate-to-vigorous physical activity.

### 4.3.1. Work Hours

Table 4-2 presents correlation analyses between WORK%SIT, WORK%STAND and WORK%STEP and all cognition and mood variables, with covariate control for all other activity categories across all domains. WORK%SIT was negatively associated with the calm mood state (p=0.027). A negative association was observed between WORK%STAND and the alerting network score (p=0.033), indicating shorter RTs with increased time spent standing. Positive correlations were observed between WORK%STEP and positive affect (p=0.023), and the calm (p=0.010) and content (p=0.022) mood states. No other significant associations were observed between variables in this domain (p>0.05).

Table 4-3 presents correlation analyses between WORK light-, moderate- and vigorous-intensity PA and total PA and all cognition and mood variables with covariate control for all other activity categories across all domains. WORK moderate-intensity PA was positively associated with accuracy on the two back (p=0.006) and three back (p=0.009) trials on the N-Back task. WORK TOTAL PA was positively associated with executive control score (p=0.043), indicating longer RTs with increased time spent engaging in PA. No other significant associations were observed between variables in this domain (p>0.05).

**Table 4-2:** Associations between cognition, mood, and the percentage of time spent sitting (SIT), standing (STAND) and stepping (STEP) during work hours, with statistical covariate control for the percentage of time spent in all other activity categories across all domains.

| | **SIT (% of time) controlling for all other activity categories[$]** | | **STAND (% of time) controlling for all other activity categories[+]** | | **STEP (% of time) controlling for all other activity categories[^]** | |
|---|---|---|---|---|---|---|
| | r-value | *p-value* | r-value | *p-value* | *r-value* | *p-value* |
| **Stroop Colour-Word Test** | | | | | | |
| Interference Score (ms) | 0.209 | *0.090* | -0.211 | *0.087* | *-0.157* | *0.222* |
| **Attention Network Test** | | | | | | |
| Alerting Network (ms) | 0.224 | *0.068* | -0.261 | *0.033** | *-0.119* | *0.356* |
| Orientating Network (ms) | -0.171 | *0.172* | 0.133 | *0.290* | *0.055* | *0.675* |
| Executive Control (ms) | -0.018 | *0.885* | 0.041 | *0.744* | *-0.122* | *0.355* |
| **N-Back Task** | | | | | | |
| One Back Accuracy (%) | 0.067 | *0.594* | -0.046 | *0.717* | *-0.116* | *0.372* |
| One Back RT (ms) | 0.084 | *0.505* | -0.138 | *0.268* | *0.023* | *0.861* |
| Two Back Accuracy (%) | 0.111 | *0.377* | -0.079 | *0.530* | *-0.045* | *0.732* |
| Two Back RT (ms) | 0.017 | *0.891* | 0.019 | *0.878* | *-0.067* | *0.607* |
| Three Back Accuracy (%) | 0.132 | *0.291* | -0.131 | *0.295* | *-0.118* | *0.364* |
| Three Back RT (ms) | 0.014 | *0.912* | -0.037 | *0.770* | *0.016* | *0.904* |
| **Mood** | | | | | | |
| Positive Affect | -0.198 | *0.108* | 0.129 | *0.298* | *0.288* | *0.023** |
| Negative Affect | -0.049 | *0.691* | 0.132 | *0.286* | *-0.166* | *0.197* |
| Alert | -0.109 | *0.453* | 0.020 | *0.890* | *0.238* | *0.116* |
| Calm | -0.312 | *0.027** | 0.245 | *0.087* | *0.378* | *0.010** |
| Content | -0.240 | *0.093* | 0.122 | *0.400* | *0.340* | *0.022** |

RT- reaction time. * Significant association ($p<0.05$).

[$]Controlling for monitor wear time, WEEKLEISURE%SIT, WEEKEND%SIT, TOTAL%STEP, TOTAL%STAND and TOTAL%PA

[+]Controlling for monitor wear time, WEEKLEISURE%STAND, WEEKEND%STAND, TOTAL%STEP, TOTAL%SIT and TOTAL%PA

^Controlling for monitor wear time, WEEKLEISURE%STEP, WEEKEND%STEP, TOTAL%STAND, TOTAL%SIT, WEEKLEISURE%Total PA and WEEKEND% Total PA

**Table 4-3:** Associations between cognition, mood, and the percentage of time spent in light-, moderate-, vigorous-intensity and total physical activity (PA) during work hours, with statistical covariate control for the percentage of time spent in all other activity categories across all domains.

| | **Light-intensity PA (% of time) controlling for all other activity categories[$]** | | **Moderate-intensity PA (% of time) controlling for all other activity categories[+]** | | **Vigorous-intensity PA (% of time) controlling for all other activity categories[^]** | | **Total PA (% of time) controlling for all other activity categories[#]** | |
|---|---|---|---|---|---|---|---|---|
| | r-value | *p-value* | r-value | *p-value* | *r-value* | *p-value* | r-value | *p-value* |
| **Stroop Colour-Word Test** | | | | | | | | |
| Interference Score (ms) | 0.038 | *0.765* | 0.004 | *0.976* | *0.149* | *0.244* | 0.052 | *0.683* |
| **Attention Network Test** | | | | | | | | |
| Alerting Network (ms) | -0.064 | *0.616* | 0.071 | *0.581* | *0.094* | *0.462* | -0.017 | *0.893* |
| Orientating Network (ms) | 0.080 | *0.542* | 0.006 | *0.961* | *0.142* | *0.274* | 0.104 | *0.416* |
| Executive Control (ms) | 0.219 | *0.090* | 0.162 | *0.213* | *0.043* | *0.742* | 0.255 | *0.043** |
| **N-Back Task** | | | | | | | | |
| One Back Accuracy (%) | -0.176 | *0.172* | 0.015 | *0.909* | *-0.211* | *0.099* | -0.130 | *0.307* |
| One Back RT (ms) | 0.062 | *0.632* | 0.075 | *0.564* | *0.094* | *0.466* | 0.052 | *0.684* |
| Two Back Accuracy (%) | 0.008 | *0.953* | 0.346 | *0.006** | *-0.049* | *0.703* | 0.178 | *0.159* |
| Two Back RT (ms) | 0.054 | *0.674* | -0.104 | *0.419* | *-0.019* | *0.883* | -0.023 | *0.858* |
| Three Back Accuracy (%) | 0.002 | *0.989* | 0.327 | *0.009** | *0.112* | *0.345* | 0.123 | *0.331* |
| Three Back RT (ms) | -0.033 | *0.798* | -0.070 | *0.590* | *-0.003* | *0.983* | -0.100 | *0.431* |
| **Mood** | | | | | | | | |
| Positive Affect | 0.147 | *0.250* | 0.132 | *0.304* | *-0.012* | *0.926* | 0.221 | *0.077* |
| Negative Affect | 0.005 | *0.967* | -0.044 | *0.732* | *-0.089* | *0.488* | -0.026 | *0.840* |
| Alert | 0.018 | *0.906* | 0.044 | *0.772* | *0.114* | *0.452* | 0.124 | *0.400* |
| Calm | -0.045 | *0.764* | -0.273 | *0.067* | *0.033* | *0.830* | -0.028 | *0.848* |
| Content | -0.102 | *0.501* | -0.174 | *0.247* | *-0.002* | *0.990* | 0.080 | *0.591* |

RT- reaction time. * Significant association ($p<0.05$).

[$]Controlling for monitor wear time, WEEKLEISURE%Light-PA, WEEKEND%Light-PA, TOTAL%Moderate-PA, TOTAL%Vigorous-PA, TOTAL%SIT and TOTAL%STAND

[+] Controlling for monitor wear time, WEEKLEISURE%Moderate-PA, WEEKEND%Moderate-PA, TOTAL%Light-PA, TOTAL%Vigorous-PA, TOTAL%SIT and TOTAL%STAND

[^] Controlling for monitor wear time, WEEKLEISURE%Vigorous-PA, WEEKEND%Vigorous-PA, TOTAL%Light-PA, TOTAL%Moderate-PA, TOTAL%SIT and TOTAL%STAND

[#] Controlling for monitor wear time, WEEKLEISURE%Total PA, WEEKEND%Total PA, TOTAL%SIT and TOTAL%STAND

### 4.3.2. Weekday

Table 4-4 presents correlation analyses between WEEK%SIT, WEEK%STAND and WEEK%STEP and all cognition and mood variables with covariate control for all other activity categories across all domains. WEEK%STEP was positively associated with positive affect (p=0.034) and the calm mood state (p=0.008). A negative association was observed between WEEK%STEP and the executive control score (p=0.041), indicating shorter RTs with increased time spent stepping. No other significant associations were observed between variables in this domain (p>0.05).

Table 4-3 presents correlation analyses between WEEK light-, moderate- and vigorous-intensity PA and total PA and all cognition and mood variables with covariate control for all other activity categories across all domains. WEEK light-intensity PA was positively associated with the orienting network score (p=0.024), suggesting longer RTs with increased time spent in light-intensity PA. WEEK moderate-intensity PA was negatively associated with the Stroop interference score (p=0.021), indicating shorter RTs with increased time spend in this intensity of PA, and positively associated with accuracy on the two back trial of the N-Back task (p=0.001). No other significant associations were observed between variables in this domain (p>0.05).

**Table 4-4:** Associations between cognition, mood, and the percentage of time spent sitting (SIT), standing (STAND) and stepping (STEP) during weekdays, with statistical covariate control for the percentage of time spent in all other activity categories across all domains.

| | **SIT (% of time) controlling for all other activity categories[$]** | | **STAND (% of time) controlling for all other activity categories[+]** | | **STEP (% of time) controlling for all other activity categories[^]** | |
|---|---|---|---|---|---|---|
| | r-value | *p-value* | r-value | *p-value* | *r-value* | *p-value* |
| **Stroop Colour-Word Test** | | | | | | |
| Interference Score (ms) | 0.643 | *0.057* | -0.103 | *0.404* | *-0.115* | *0.366* |
| **Attention Network Test** | | | | | | |
| Alerting Network (ms) | 0.116 | *0.344* | -0.116 | *0.345* | *-0.149* | *0.239* |
| Orientating Network (ms) | -0.104 | *0.404* | 0.071 | *0.569* | *0.221* | *0.085* |
| Executive Control (ms) | 0.063 | *0.613* | -0.026 | *0.836* | *-0.260* | *0.041** |
| **N-Back Task** | | | | | | |
| One Back Accuracy (%) | 0.127 | *0.306* | -0.128 | *0.302* | *-0.034* | *0.798* |
| One Back RT (ms) | 0.202 | *0.102* | -0.235 | *0.056* | *-0.135* | *0.290* |
| Two Back Accuracy (%) | 0.078 | *0.529* | -0.052 | *0.679* | *-0.074* | *0.562* |
| Two Back RT (ms) | 0.005 | *0.968* | 0.035 | *0.776* | *-0.081* | *0.526* |
| Three Back Accuracy (%) | 0.132 | *0.286* | -0.123 | *0.322* | *-0.039* | *0.763* |
| Three Back RT (ms) | -0.070 | *0.576* | 0.065 | *0.599* | *0.043* | *0.740* |
| **Mood** | | | | | | |
| Positive Affect | -0.151 | *0.220* | 0.042 | *0.734* | *0.265* | *0.034** |
| Negative Affect | -0.049 | *0.690* | 0.174 | *0.155* | *-0.132* | *0.300* |
| Alert | -0.067 | *0.638* | 0.007 | *0.959* | *0.175* | *0.240* |
| Calm | -0.240 | *0.090* | 0.185 | *0.193* | *0.385* | *0.008** |
| Content | -0.233 | *0.099* | 0.075 | *0.603* | *0.237* | *0.109* |

RT- reaction time. * Significant association ($p<0.05$).

[$]Controlling for monitor wear time, WEEKEND%SIT, TOTAL%STEP, TOTAL%STAND and TOTAL%PA

[+]Controlling for monitor wear time, WEEKEND%STAND, TOTAL%STEP, TOTAL%SIT and TOTAL%PA

^Controlling for monitor wear time, WEEKEND%STEP, TOTAL%STAND, TOTAL%SIT and WEEKEND%Total PA

**Table 4-5:** Associations between cognition, mood, and the percentage of time spent in light-, moderate-, vigorous-intensity and total physical activity (PA) during weekdays, with statistical covariate control for the percentage of time spent in all other activity categories across all domains.

| | **Light-intensity PA (% of time) controlling for all other activity categories[$]** | | **Moderate-intensity PA (% of time) controlling for all other activity categories[+]** | | **Vigorous-intensity PA (% of time) controlling for all other activity categories[^]** | | **Total PA (% of time) controlling for all other activity categories[#]** | |
|---|---|---|---|---|---|---|---|---|
| | r-value | *p-value* | r-value | *p-value* | *r-value* | *p-value* | r-value | *p-value* |
| **Stroop Colour-Word Test** | | | | | | | | |
| Interference Score (ms) | -0.073 | *0.567* | -0.289 | *0.021** | 0.051 | *0.690* | -0.173 | *0.164* |
| **Attention Network Test** | | | | | | | | |
| Alerting Network (ms) | 0.046 | *0.716* | 0.085 | *0.505* | 0.149 | *0.238* | 0.061 | *0.627* |
| Orientating Network (ms) | 0.286 | *0.024** | 0.009 | *0.942* | 0.091 | *0.481* | 0.219 | *0.082* |
| Executive Control (ms) | 0.134 | *0.300* | -0.233 | *0.069* | 0.147 | *0.256* | 0.120 | *0.344* |
| **N-Back Task** | | | | | | | | |
| One Back Accuracy (%) | -0.051 | *0.693* | 0.198 | *0.119* | 0.033 | *0.794* | 0.080 | *0.527* |
| One Back RT (ms) | -0.008 | *0.952* | -0.063 | *0.623* | -0.019 | *0.881* | -0.068 | *0.593* |
| Two Back Accuracy (%) | -0.079 | *0.536* | 0.423 | *0.001** | -0.134 | *0.297* | 0.111 | *0.378* |
| Two Back RT (ms) | 0.104 | *0.416* | 0.001 | *0.991* | 0.113 | *0.380* | 0.046 | *0.716* |
| Three Back Accuracy (%) | 0.113 | *0.379* | 0.151 | *0.237* | 0.050 | *0.698* | 0.163 | *0.194* |
| Three Back RT (ms) | 0.032 | *0.805* | 0.157 | *0.219* | 0.039 | *0.764* | 0.057 | *0.654* |
| **Mood** | | | | | | | | |
| Positive Affect | 0.104 | *0.413* | 0.234 | *0.062* | -0.071 | *0.575* | 0.214 | *0.084* |
| Negative Affect | 0.090 | *0.479* | -0.046 | *0.718* | 0.068 | *0.592* | 0.096 | *0.445* |
| Alert | -0.082 | *0.584* | 0.055 | *0.711* | 0.044 | *0.769* | 0.029 | *0.841* |
| Calm | -0.143 | *0.339* | -0.054 | *0.718* | -0.134 | *0.368* | -0.085 | *0.562* |
| Content | -0.190 | *0.202* | 0.034 | *0.818* | -0.079 | *0.596* | -0.185 | *0.203* |

RT- reaction time. * Significant association ($p<0.05$).

[$]Controlling for monitor wear time, WEEKEND%Light-PA, TOTAL%Moderate-PA, TOTAL%Vigorous-PA, TOTAL%SIT and TOTAL%STAND

[+] Controlling for monitor wear time, WEEKEND%Moderate-PA, TOTAL%Light-PA, TOTAL%Vigorous-PA, TOTAL%SIT and TOTAL%STAND

^ Controlling for monitor wear time, WEEKEND%Vigorous-PA, TOTAL%Light-PA, TOTAL%Moderate-PA, TOTAL%SIT and TOTAL%STAND

[#] Controlling for monitor wear time, WEEKEND%Total PA, TOTAL%SIT and TOTAL%STAND

### 4.3.3. Weekend

Table 4-6 presents correlation analyses between WEEKEND%SIT, WEEKEND%STAND and WEEKEND%STEP and all cognition and mood variables, with covariate control for all other activity categories across all domains. No significant associations were observed between any variables in this domain ($p>0.05$).

Table 4-7 presents correlation analyses between WEEKEND light-, moderate- and vigorous-intensity PA and total PA and all cognition and mood variables with covariate control for all other activity categories across all domains. WEEKEND light-intensity PA was positively associated with accuracy on the one back trial of the N-Back task ($p=0.024$), while WEEKEND moderate-intensity PA was negatively associated with RT on the one back trial ($p=0.025$), suggesting shorter RTs with increased time spent in this intensity of PA. No other significant associations were observed between variables in this domain ($p>0.05$).

**Table 4-6:** Associations between cognition, mood, and the percentage of time spent sitting (SIT), standing (STAND) and stepping (STEP) during weekends, with statistical covariate control for the percentage of time spent in all other activity categories across all domains.

| | **SIT (% of time) controlling for all other activity categories[$]** | | **STAND (% of time) controlling for all other activity categories[+]** | | **STEP (% of time) controlling for all other activity categories[^]** | |
|---|---|---|---|---|---|---|
| | r-value | *p-value* | r-value | *p-value* | *r-value* | *p-value* |
| **Stroop Colour-Word Test** | | | | | | |
| Interference Score (ms) | 0.069 | *0.577* | -0.082 | *0.508* | *-0.014* | *0.908* |
| **Attention Network Test** | | | | | | |
| Alerting Network (ms) | -0.051 | *0.682* | -0.050 | *0.688* | *0.046* | *0.711* |
| Orientating Network (ms) | -0.074 | *0.554* | 0.102 | *0.414* | *-0.007* | *0.958* |
| Executive Control (ms) | 0.115 | *0.358* | -0.150 | *0.230* | *-0.018* | *0.889* |
| **N-Back Task** | | | | | | |
| One Back Accuracy (%) | -0.123 | *0.320* | 0.141 | *0.254* | *0.073* | *0.555* |
| One Back RT (ms) | -0.109 | *0.381* | 0.132 | *0.288* | *0.029* | *0.814* |
| Two Back Accuracy (%) | -0.093 | *0.452* | 0.031 | *0.801* | *0.084* | *0.498* |
| Two Back RT (ms) | -0.002 | *0.984* | -0.004 | *0.976* | *-0.071* | *0.567* |
| Three Back Accuracy (%) | -0.091 | *0.466* | 0.062 | *0.616* | *0.069* | *0.578* |
| Three Back RT (ms) | -0.131 | *0.290* | 0.166 | *0.178* | *0.016* | *0.901* |
| **Mood** | | | | | | |
| Positive Affect | -0.015 | *0.901* | 0.072 | *0.561* | *-0.024* | *0.847* |
| Negative Affect | 0.004 | *0.972* | -0.050 | *0.685* | *0.033* | *0.792* |
| Alert | 0.040 | *0.778* | -0.010 | *0.945* | *-0.016* | *0.910* |
| Calm | 0.058 | *0.688* | -0.090 | *0.529* | *0.004* | *0.975* |
| Content | -0.194 | *0.172* | 0.197 | *0.167* | *0.129* | *0.366* |

RT- reaction time.

[$]Controlling for monitor wear time, WEEK%SIT, TOTAL%STAND, TOTAL%STEP and TOTAL%PA

[+]Controlling for monitor wear time, WEEK%STAND, TOTAL%SIT, TOTAL%STEP and TOTAL%PA

^Controlling for monitor wear time, WEEK%STEP, TOTAL%SIT, TOTAL%STAND and WEEK%PA

**Table 4-7:** Associations between cognition, mood, and the percentage of time spent in light-, moderate-, vigorous-intensity and total physical activity (PA) during weekends, with statistical covariate control for the percentage of time spent in all other activity categories across all domains.

| | **Light-intensity PA (% of time) controlling for all other activity categories[$]** | | **Moderate-intensity PA (% of time) controlling for all other activity categories[+]** | | **Vigorous-intensity PA (% of time) controlling for all other activity categories[^]** | | **Total PA (% of time) controlling for all other activity categories[#]** | |
|---|---|---|---|---|---|---|---|---|
| | r-value | *p-value* | r-value | *p-value* | *r-value* | *p-value* | r-value | *p-value* |
| **Stroop Colour-Word Test** | | | | | | | | |
| Interference Score (ms) | -0.001 | *0.993* | 0.207 | *0.101* | -0.015 | *0.907* | 0.088 | *0.483* |
| **Attention Network Test** | | | | | | | | |
| Alerting Network (ms) | 0.125 | *0.324* | -0.022 | *0.860* | -0.181 | *0.153* | 0.098 | *0.432* |
| Orientating Network (ms) | -0.062 | *0.631* | 0.058 | *0.656* | -0.139 | *0.281* | -0.032 | *0.799* |
| Executive Control (ms) | -0.054 | *0.677* | 0.187 | *0.145* | 0.095 | *0.464* | 0.089 | *0.487* |
| **N-Back Task** | | | | | | | | |
| One Back Accuracy (%) | 0.284 | *0.024** | 0.014 | *0.914* | -0.013 | *0.919* | 0.162 | *0.197* |
| One Back RT (ms) | 0.148 | *0.248* | -0.283 | *0.025** | 0.160 | *0.211* | -0.019 | *0.881* |
| Two Back Accuracy (%) | -0.122 | *0.342* | -0.144 | *0.259* | -0.120 | *0.348* | -0.063 | *0.616* |
| Two Back RT (ms) | 0.106 | *0.408* | -0.105 | *0.415* | -0.056 | *0.664* | 0.129 | *0.306* |
| Three Back Accuracy (%) | -0.099 | *0.440* | -0.039 | *0.763* | -0.014 | *0.911* | -0.203 | *0.106* |
| Three Back RT (ms) | -0.104 | *0.416* | -0.168 | *0.189* | -0.082 | *0.522* | -0.132 | *0.295* |
| **Mood** | | | | | | | | |
| Positive Affect | -0.061 | *0.631* | -0.109 | *0.391* | 0.016 | *0.902* | -0.078 | *0.535* |
| Negative Affect | -0.028 | *0.827* | 0.005 | *0.968* | 0.103 | *0.416* | -0.013 | *0.915* |
| Alert | 0.108 | *0.469* | 0.076 | *0.611* | -0.107 | *0.476* | 0.091 | *0.533* |
| Calm | 0.103 | *0.492* | 0.209 | *0.158* | -0.006 | *0.970* | 0.134 | *0.360* |
| Content | 0.065 | *0.665* | 0.057 | *0.705* | -0.137 | *0.358* | 0.096 | *0.513* |

RT- reaction time. * Significant association ($p<0.05$).

[$]Controlling for monitor wear time, WEEK%Light-PA, TOTAL%Moderate-PA, TOTAL%Vigorous-PA, TOTAL%SIT and TOTAL%STAND

[+] Controlling for monitor wear time, WEEK%Moderate-PA, TOTAL%Light-PA, TOTAL%Vigorous-PA, TOTAL%SIT and TOTAL%STAND

^ Controlling for monitor wear time, WEEK%Vigorous-PA, TOTAL%Light-PA, TOTAL%Moderate-PA, TOTAL%SIT and TOTAL%STAND

[#] Controlling for monitor wear time, WEEK%Total PA, TOTAL%SIT and TOTAL%STAND

## 4.4. Discussion

This study assessed, for the first time, whether sitting during work hours is associated with cognition and mood. Interestingly, we found that the time spent sitting during work hours was negatively associated with the calm mood state. Furthermore, time spent at work in a non-sedentary posture, namely standing or stepping, was associated with improved aspects of cognition and mood. Moderate-intensity PA was the only intensity of PA to be positively associated with cognitive outcomes during work hours. This relationship also persisted across weekdays and weekends, suggesting daily moderate-intensity PA in all domains may be important for cognitive performance. Collectively, these findings indicate reducing sitting during work hours and encouraging PA may contribute to heightened productivity, which further research should explore experimentally.

A less sedentary workplace has been suggested to be more productive (Buckley et al., 2015), and cognition and mood likely play a role in employee productivity. In support of this statement, we found that the percentage of time spent sitting during work hours was negatively associated with the calm mood state, indicating the more time spent sitting during work hours, workers were less calm. Calmness can be used to assess psychological stress reactivity; the magnitude of an individual's response to a stressor (Klaperski et al., 2013). Those that are less calm may therefore exhibit a heightened stress response. Importantly, chronic work stress is related to increased risk of CVD morbidity and mortality (Chandola et al., 2006; Kivimäki et al., 2002; Kivimäki and Kawachi, 2015) and mental health conditions (Harvey et al., 2017). In the long

term, sitting at work may therefore have negative implications for employee health and well-being through its effect on mood, which further research should explore.

It has been recommended that sitting during work hours should be replaced with standing or light-intensity PA and that adopting this approach may improve workers' productivity (Buckley et al., 2015), however there is no previous evidence to support that using these modalities to break up sitting can improve factors that contribute to work productivity. Our results show that the time spent standing at work was negatively associated with the alerting network attentional network score, suggesting improved RTs, and therefore attention, with more time spent standing. Additionally, moderate-intensity PA was positively associated with aspects of working memory. Collectively, this indicates that replacing sitting at work with either standing or PA, could improve aspects of workers' cognitive performance. Additionally, the time spent stepping during work hours was positively associated with positive affect and the calm and content mood states. Importantly, workers who are in a positive mood have enhanced work performance and productivity (Miner and Glomb, 2010; Rothbard and Wilk, 2011), indicating being more active during work hours could be beneficial for the output of the workforce. The known benefit of PA on mood (Biddle, 2016) may contribute to these associations. However, standing during work hours was not associated with mood indicating that standing may not be a sufficient intensity to enhance mood and that ambulation is required. In support, in healthy adults, breaking up sitting with walking but not standing improved

postprandial glycemia, possibly due to the lower-intensity PA stimulus (Bailey and Locke, 2015).

By objectively monitoring PA we were able to assess if differing intensities of PA were associated with cognition and mood. Across all domains, greater time spent in moderate-intensity PA was associated with improved cognition. During work hours, moderate-intensity PA was positively associated with accuracy on the two- and three-back n-back tasks, indicating improved working memory with more time spent in this intensity of PA. Furthermore, during weekdays and weekends increased moderate-intensity PA was associated with greater executive function and working memory performance. Collectively, our data suggests that moderate-intensity PA may be needed to enhance aspects of cognition. In support, existing research shows the intensity of the activity can influence cognitive outcomes, with higher intensities providing larger improvements in cognition than lower intensities (Mandolesi et al., 2018). This finding does not align with current workplace activity guidelines, which recommend light-intensity PA may improve workers' productivity (Buckley et al., 2015). Consequently, this may indicate that recommending low-intensity PA will not elicit improvements in workers' cognition and their subsequent productivity.

The finding that neither work hours, weekday or weekend sitting was associated with cognition contrasts previous work showing relationships between SB and cognition (Edwards and Loprinzi, 2017a, 2017b; Falck et al., 2017). However, such research has assessed older populations who experience an accelerated rate of age-related cognitive decline compared to younger adults (Salthouse,

2009), and in this study we have assessed young, working-age adults. Furthermore, in our cohort, age was only associated with two of our cognitive outcomes, the orienting and executive control networks of attention, and, after statistically controlling for age in our analyses of these variables, this had no influence on the relationships observed. Whilst sitting was not associated with cognition in this cohort, this study only assessed three domains of cognitive functioning and others may be more susceptible to the deleterious effects of sitting. Indeed, previous work has assessed cognitive domains such as processing speed and organisation and planning (Falck et al., 2017). Furthermore, The National Institutes of Health have identified executive function, episodic memory, language, processing speed, working memory, and attention as the cognition subdomains most important for health, success in school and work, and independence in daily functioning (Weintraub et al., 2013); which were not all assessed in our study, thus future research should consider these domains.

### 4.4.1. Limitations

This study is strengthened by the objective assessment of sitting, standing and stepping and different intensities of PA over an entire week which provided a complete picture of our participants' habitual SB and PA levels across various time domains. Nonetheless, we assessed a small number of cognitive domains and mood states that could influence workers' productivity, therefore others may be associated with sitting which should be explored. We did not control for factors such as sleep, stress and diet, which are important determinants of cognition and mood. The influence of the number or the length of breaks from sitting on cognition and mood were not considered, factors which are known to

have an important effect on cardiometabolic health markers (Healy et al., 2008). Furthermore, we did not measure or control for fidgeting during sitting, which attenuates the association between sitting and mortality risk (Hagger-Johnson et al., 2016). Nonetheless there is currently no evidence to suggest that fidgeting would influence the association between sitting, cognition, mood and work performance. It is possible that seasonality may have influenced participants' PA and SB levels, since levels can vary depending on the season when the assessment occurs (O'Connell et al., 2014). Some participants were employed in the same workplace which may increase the homogeneity of our data, owing to similar behaviour patterns during work hours. However, our sample appears representative of the typical English workers since weekday sitting (61.0%), standing (26.1%) and stepping (13.0%) time was similar to that previously reported by Smith et al. (2015) in English workers (weekday sitting 66.2%, standing 23.3% and stepping 10.5%). Finally, the cross-sectional study design means inferences about causality cannot be determined, results should therefore be interpreted with caution. A follow-up or interventional study design would provide more insight into the influence of the repeated exposure to sitting during work hours on cognition and mood, however this was beyond the scope of this study.

## 4.5. Conclusion

This study demonstrates that in healthy workers, sitting during work hours is negatively associated with the calm mood state. The time spent standing or stepping is however positively associated with aspects of mood and cognition, indicating that reducing sitting and taking regular PA breaks throughout the

workday may have beneficial effects on mood. Moderate-intensity PA was the only intensity of PA that was positively associated with cognitive outcomes across all domains. Further research is needed to determine whether increasing moderate-intensity PA during work hours positively influences cognition. Additionally, the influence of workplace sitting on other domains of cognition and mood and over a long-term follow up should be explored.

# 5. Acute effects of prolonged sitting on cerebrovascular function, cognition and mood

## 5.1. Introduction

It has recently been suggested that sitting-induced changes in cerebrovascular function may contribute to cognitive decline (Wheeler et al., 2017), however this hypothesis is yet to be tested. A recent systematic review concluded that increased SB was associated with lower cognitive function (Falck et al., 2017), yet the studies included in the review used subjective measures of SB and were not experimental designs, so were unable to elucidate the physiological mechanisms underlying this relationship. Increasing SB for up to two weeks also decreases mood (Edwards and Loprinzi, 2017b; Endrighi et al., 2016), however once again the mechanisms underlying this link are unknown, but may also relate to impaired cerebrovascular function. Consequently, whether sitting-induced impairment in cerebrovascular function results in decrements to cognition and mood is currently unclear.

This study therefore explored the acute effects of a prolonged, uninterrupted sitting period on cerebrovascular function, cognition, mood and work productivity. Secondly, this study assessed if any observed alterations in cerebrovascular function were associated with any changes in measures of cognition, mood and work productivity. We hypothesised that prolonged sitting would impair cerebrovascular function and that this decline would result in acute impairments in cognition, mood and work productivity.

## 5.2. Methods

### 5.2.1. Participants

Twenty-five healthy desk workers (18 male) volunteered and written informed consent was obtained prior to inclusion. Participants were screened prior to testing for exclusion criteria including: use of medication, smoker, BMI >35 or <18 $kg \cdot m^{-2}$, use of hormone-based contraception and diagnosis of cerebrovascular, cardiovascular or metabolic disease. Study procedures were approved by the Liverpool John Moores University Ethics Committee and adhered to the Declaration of Helsinki.

### 5.2.2. Study Design

Participants attended the laboratory on two occasions. Visit one was a familiarisation session, whereby participants were given the opportunity to practise a battery of computer-based cognitive performance and work productivity tests. The second experimental visit occurred on the following day. Participants arrived at the laboratory between 7.00-9.00 am and rested in the supine position for 20-min. This was followed by assessments of supine MCAv and CVR. Participants then moved to a seated position and underwent measures of seated MCAv and CA. Following this, the same battery of cognitive performance and work productivity tests as in the familiarisation visit were completed, in addition to two mood questionnaires. Following these tests (PRE) participants completed a continuous uninterrupted sitting period for 6-hr. The measurement of seated MCAv was repeated immediately after the 6-hr intervention. MCAv was assessed while seated to examine the posture of interest, sitting, and to prevent the effects of moving to a supine posture altering

hemodynamics. Participants then returned to a supine posture and all other measurements were repeated (POST). During the sitting period MAP and heart rate (HR) were assessed every 1-hr (Figure 5-1).

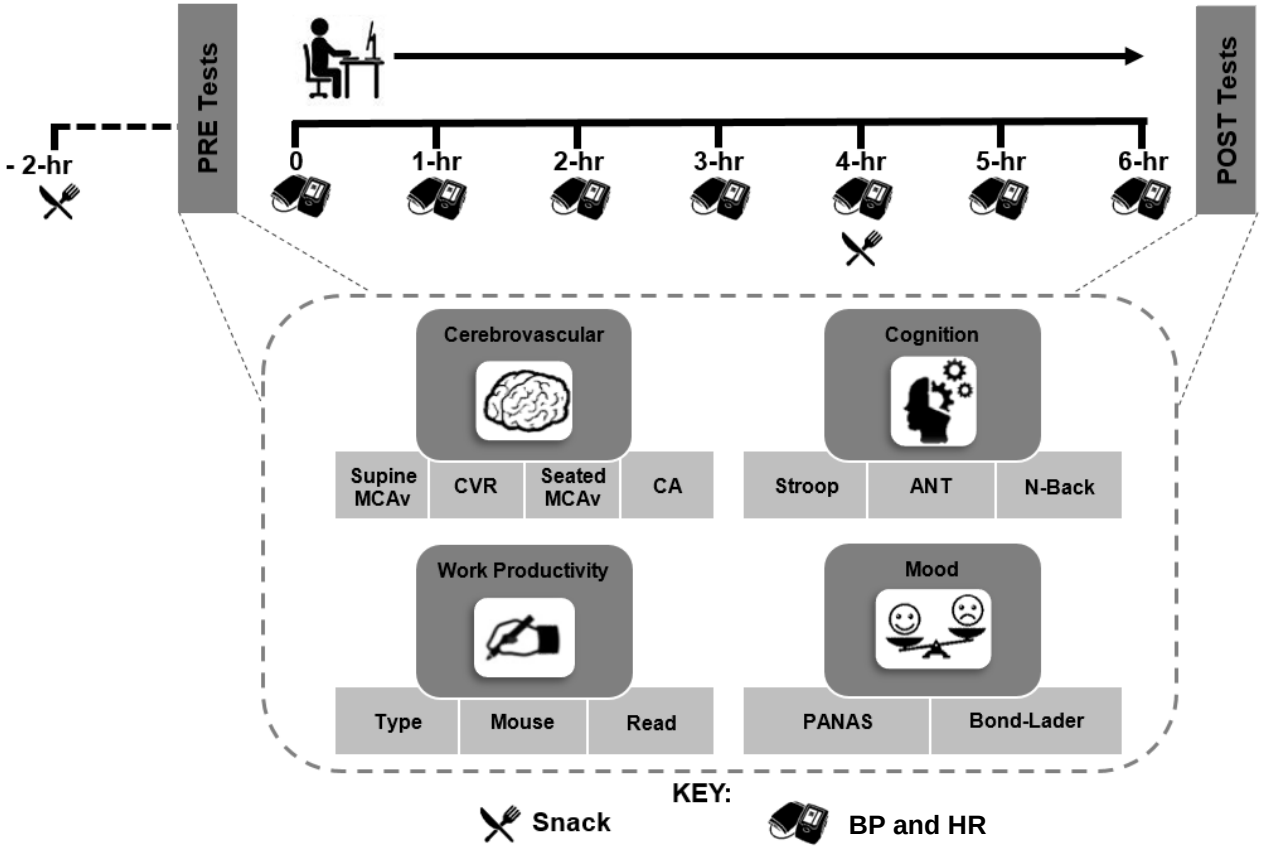


**Figure 5-1:** Study design for the experimental visit. MCAv- middle cerebral artery blood flow velocity; CVR- cerebrovascular carbon dioxide reactivity; CA- cerebral autoregulation; ANT- attention network test; PANAS- positive and negative affect schedule; BP- blood pressure; HR- heart rate.

### 5.2.3. Study Procedures

*5.2.3.1. Visit 1: Familiarisation Visit*

Participants completed the battery of cognitive performance and work productivity tests that would be used during the experimental visit to reduce any learning effects. During this visit participants were given the opportunity to ask questions to ensure full comprehension of each test. In preparation for the experimental visit on the following day, participants were given a standardised meal to take away with them. Participants were instructed to consume this meal in the morning, two hours prior to their scheduled arrival time (Figure 5-1). The meal consisted of 50g of porridge oats prepared with water (187kcal, 31.2g carbohydrate, 2.9g fat, 7.5g protein) and a banana (~100kcal, ~27.0g carbohydrate, ~0.3g fat, ~1.0g protein).

*5.2.3.2. Visit 2: Experimental Visit*

Prior to the experimental visit, participants were instructed to avoid strenuous exercise for 24-hr, and to abstain from caffeine and alcohol. Women were assessed in the follicular phase of the menstrual cycle (days 1-7). In the 2-hr between participants consuming the standardised meal and arriving at the laboratory, participants were asked to keep PA to a minimum. On arrival, participants completed the Workforce Sitting Questionnaire (WSQ) to assess sitting time on a working day and a non-working day (Chau et al., 2011). Participants were asked to verbally confirm they had consumed the standardised meal prior to arrival and the time at which this occurred. Participants were given this same low calorie, low fat, meal 2-hr prior to any POST measurements were taken, ensuring the time between food consumption and physiological measurements were matched between PRE and POST

assessments (Figure 5-1). Water was available to drink *ad libitum* throughout the testing session. During the uninterrupted sitting period, participants remained seated at a desk for 6-hr and were permitted to perform low cognitively demanding desk-based activities such as reading and watching television. The duration of sitting was selected based on previous research which observed reduced mood following 6-hr of uninterrupted sitting (Bergouignan et al., 2016). Participants were prevented from standing, walking or making vigorous movements during this period, limb movements were otherwise uncontrolled (i.e. fidgeting was permitted). Participants were wheeled to the bathroom if needed (on average participants visited the toilet once during the sitting period). Participants were continuously supervised to ensure these conditions were adhered to.

### 5.2.4. Measurements

The measurement of MCAv, CVR, CA, $PETCO_2$, BP, cognition, mood and work productivity are described in detail in Chapter 3. General Methods, hence here only specific features of this study are outlined. During data acquisition, supine and seated MCAv were acquired for a 2-min period. CVC was calculated by dividing MCAv by MAP. Participants were fitted with a photoplethysmographic cuff on the index or middle finger of the right hand (Finometer model 1, Finapres Medical Systems BV, Amsterdam, The Netherlands) and a 3-lead electrocardiogram to continuously assess MAP and HR throughout measurements. During the uninterrupted sitting period, MAP and HR were measured with an oscillometric cuff at the left brachial artery (Carescape V100, Dinamap, GE Healthcare, UK) every 1-hr. The battery of computer-based

cognitive performance and work productivity tests were completed in a randomised order between participants but not within an experimental visit. Participants were randomly assigned to the order in which they completed the tests using computer-generated random numbers.

### 5.2.5. Statistical Analyses

Data were analysed using statistical software (SPSS Version 23.0, IBM Corporation, Somers, NY, USA), with significance accepted as $p<0.05$. Results are presented as means±SD. Data were assessed for normal distribution using Shapiro-Wilk tests. Paired samples t-tests were used to compare the difference between PRE and POST for all outcome parameters, whilst Wilcoxon signed rank tests were used for any non-parametric data. Changes in HR and MAP during uninterrupted sitting were assessed using a one-way within-subjects ANOVA. Post-hoc analyses were performed using the least significant difference (LSD) method. Where significant changes were observed in our data following sitting, Pearson's bivariate correlation analysis (parametric data) and Spearman's correlation (non-parametric data) were used to assess the relationship between the change (POST-PRE) in these outcomes.

## 5.3. Results

All 25 participants completed the study and were included in analyses. Participants self-reported sitting for 12.1±3.3 hours during work days and 10.0±3.3 hours during non-work days. Full descriptive characteristics are shown in Table 5-1.

**Table 5-1:** Descriptive characteristics and self-reported sitting time of participants (n=25).

| | **Mean±SD** |
|---|---|
| Age (years) | 28.3±7.5 |
| Body Mass (kg) | 74.3±12.4 |
| Stature (cm) | 175.0±7.0 |
| Body Mass Index ($kg \cdot m^{-2}$) | 24.2±3.3 |
| Sitting Time Per Work Day (Hours) | 12.1±3.3 |
| Sitting Time Per Non-Work Day (Hours) | 10.0±3.3 |

### 5.3.1. Cardiorespiratory and Haemodynamic Measures

HR in the supine ($p=0.022$) and seated ($p=0.003$) postures were significantly reduced at POST compared to PRE (Table 5-2). There was also a significant reduction in seated MAP ($p=0.001$) between PRE and POST, but not for supine MAP ($p=0.966$; Table 5-2). There was no significant difference between PRE and POST supine ($p=0.365$) or seated ($p=0.306$) $PETCO_2$ (Table 5-2). During 6-hr of uninterrupted sitting HR was significantly decreased at all but one time point (5-hr, 62±9.2 bpm $p=0.059$), compared to baseline (Baseline: 66±10.1 bpm, 1-hr: 61±10.1 bpm, 2-hr: 56±13.9 bpm, 3-hr: 58±10.1 bpm, 4-hr: 58±9.7 bpm, 6-hr: 62±10.4 bpm, $p<0.05$). MAP was also significantly reduced at all time points compared to baseline (Baseline: 90±9.8 mmHg, 1-hr: 86±9.3 mmHg, 2-hr: 87±8.4 mmHg, 3-hr: 86±8.6 mmHg, 4-hr: 87±8.4 mmHg, 5-hr: 85±8.0 mmHg, 6-hr: 85±8.4 mmHg, $p<0.05$).

**Table 5-2:** Cardiorespiratory measures prior to (PRE) and following (POST) 6-hr of uninterrupted sitting (mean±SD).

| | PRE | POST |
|---|---|---|
| **Supine position** | | |
| MAP (mmHg) | 84±8.2 | 84±7.8 |
| HR (bpm) | 62±11.6 | 57±9.4* |
| $PETCO_2$ (mmHg) | 38.4±2.9 | 38.8±3.6 |
| **Seated position** | | |
| MAP (mmHg) | 90±9.8 | 85±8.4* |
| HR (bpm) | 66±210.1 | 61±10.7* |
| $PETCO_2$ (mmHg) | 36.6±3.0 | 36.8±3.9 |

MAP- mean arterial pressure; HR- heart rate; $PETCO_2$- pressure of end-tidal carbon dioxide.
* Significantly different to PRE ($p<0.05$).

### 5.3.2. Cerebral Blood Flow

Uninterrupted sitting for 6-hr significantly reduced seated MCAv (PRE: 58.2±7.3 $cm \cdot s^{-1}$, POST: 54.8±7.1 $cm \cdot s^{-1}$, p=0.001*)*, however there was no significant change in seated CVC (PRE: 0.65±0.12 $cm \cdot s^{-1} \cdot mmHg^{-1}$, POST: 0.65±0.11 $cm \cdot s^{-1} \cdot mmHg^{-1}$, p=0.950; Figure 5-2a). In the supine posture, significant reductions in MCAv (PRE: 63.5±8.1 $cm \cdot s^{-1}$, POST: 60.6±9.0 $cm \cdot s^{-1}$, p=0.012) and CVC (PRE: 0.77±0.15 $cm \cdot s^{-1} \cdot mmHg^{-1}$, POST: 0.74±0.15 $cm \cdot s^{-1} \cdot mmHg^{-1}$, p=0.018) were observed (Figure 5-2b).

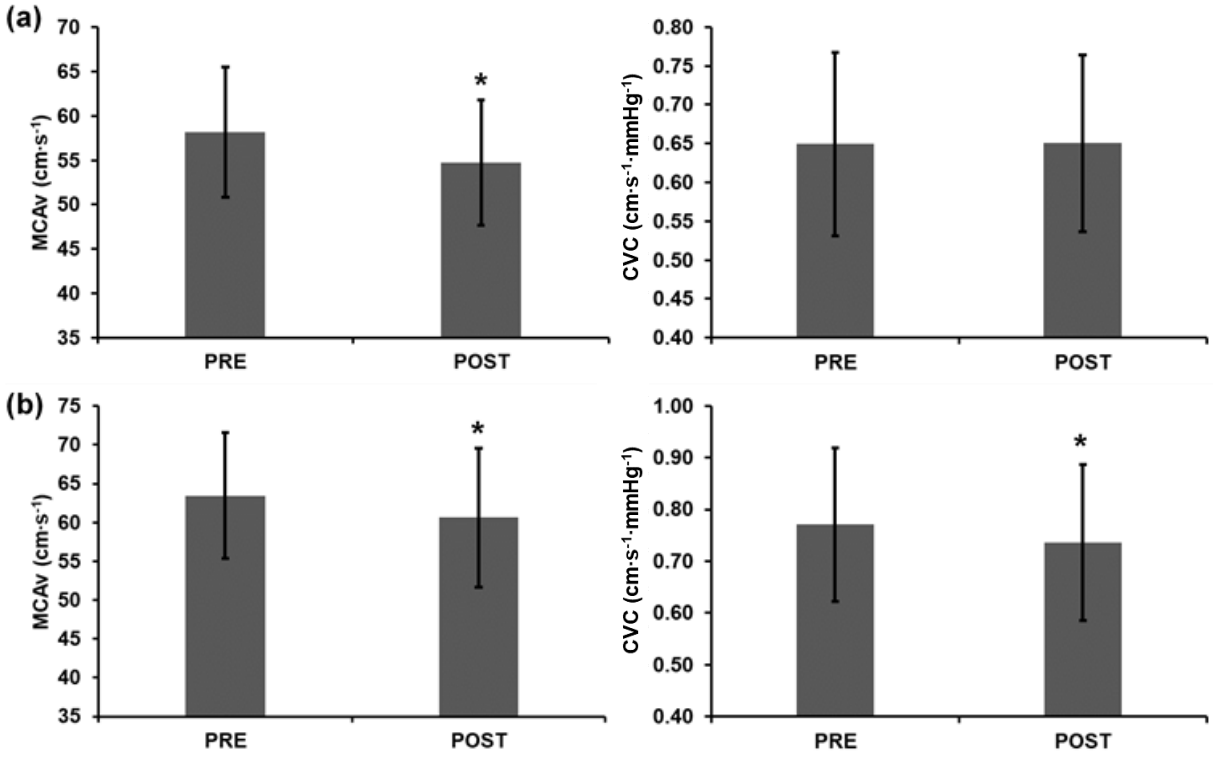


**Figure 5-2:** Middle cerebral artery blood flow velocity (MCAv) and cerebrovascular conductance (CVC) in (a) seated and (b) supine postures prior to (PRE) and following (POST) 6-hr of uninterrupted sitting. Error bars= ±SD. * Significantly different to PRE ($p<0.05$).

### 5.3.3. Cerebrovascular Carbon Dioxide Reactivity

Absolute and relative MCA CVR $R^2$ values (values are the same for both) of linear regression were PRE: $R^2$=0.84±0.08 and POST: $R^2$=0.84±0.09. There was no significant difference between absolute or relative MCA CVR at PRE (Absolute 3.12±0.65 $cm \cdot s^{-1} \cdot mmHg^{-1}$, Relative 4.82±0.86 $cm \cdot s^{-1} \cdot mmHg^{-1}$) compared to POST (Absolute 3.12±0.79 $cm \cdot s^{-1} \cdot mmHg^{-1}$, Relative 4.13±1.23 $cm \cdot s^{-1} \cdot mmHg^{-1}$, p=0.992 and p=0.198 respectively). Similarly, no significant differences were observed between absolute CCA diameter CVR (PRE: 0.002±0.002 $cm \cdot s^{-1} \cdot mmHg^{-1}$, POST: 0.003±0.003 $cm \cdot s^{-1} \cdot mmHg^{-1}$, p=0.235) or relative CCA diameter CVR (PRE: 0.33±0.28 $cm \cdot s^{-1} \cdot mmHg^{-1}$, POST: 0.39±0.38 $cm \cdot s^{-1} \cdot mmHg^{-1}$, p=0.904). Finally, there were no significant differences between absolute CCA blood flow CVR (PRE: 0.32±0.31 $ml \cdot min^{-1} \cdot mmHg^{-1}$, POST: 0.41±0.25 $ml \cdot min^{-1} \cdot mmHg^{-1}$, p=0.164) or relative CCA blood flow CVR (PRE: 2.42±2.17 $ml \cdot min^{-1} \cdot mmHg^{-1}$, POST: 3.18±1.99 $ml \cdot min^{-1} \cdot mmHg^{-1}$, p=0.122).

### 5.3.4. Cerebral Autoregulation

Table 5-3 presents values for phase, gain, normalised gain and coherence for each frequency domain. In the VLF, there was a significant increase in normalised gain following 6-hr of uninterrupted sitting (p=0.016). There were no significant changes for any other parameters in any of the frequency domains (p>0.05).

**Table 5-3:** Values of phase, gain, normalised gain ($Gain_n$) and coherence before (PRE) and after (POST) 6-hr of uninterrupted sitting (mean±SD).

| | **VLF** | |
|---|---|---|
| | **PRE** | **POST** |
| Phase (degrees) | 41.0±16.7 | 42.1±13.9 |
| Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.50±0.13 | 0.65±0.17 |
| $Gain_n$ ($\% \cdot mmHg^{-1}$) | 0.86±0.20 | 1.09±0.29* |
| Coherence | 0.5±0.08 | 0.5±0.11 |
| | **LF** | |
| | **PRE** | **POST** |
| Phase (degrees) | 24.4±18.5 | 23.0±13.3 |
| Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.79±0.18 | 0.85±0.23 |
| $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.28±0.25 | 1.39±0.33 |
| Coherence | 0.6±0.11 | 0.6±0.11 |
| | **HF** | |
| | **PRE** | **POST** |
| Phase (degrees) | 10.6±34.8 | 12.4±21.2 |
| Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.83±0.34 | 0.80±0.27 |
| $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.34±0.51 | 1.32±0.39 |
| Coherence | 0.4±0.12 | 0.4±0.13 |

VLF- very low frequency; LF- low frequency; HF- high frequency
* Significantly different to PRE ($p<0.05$).

### 5.3.5. Cognition and Work Productivity

Following 6-hr uninterrupted sitting, there was no significant change in any measures of cognition ($p>0.05$; Table 5-4), however changes in work productivity parameters were observed. There were significant increases in both gross (PRE: 45.8±8.9 wpm, POST: 47.9±9.7 wpm, $p=0.035$) and net (PRE: 40.6±9.3 wpm, POST: 43.7±9.4 wpm, $p=0.005$) typing speed; however there was no significant change in typing accuracy (PRE: 88.6±9.8%, POST: 90.4±7.7%, $p=0.065$). Performance score for the mouse dexterity test significantly improved (PRE: 1031.7±53.6, POST: 1070.3±62.2, $p<0.001$), as did RT (PRE: 749.2±86.9 ms, POST: 678.7±88.5 ms, $p<0.001$). For the reading and correcting task, there was no significant difference in the number of characters participants read (PRE: 2791.8±929.7, POST: 3086.5±955.3, $p=0.117$), however the percentage of spelling errors missed significantly increased (PRE: 35.0±20.6%, POST: 41.6±20.4%, $p=0.048$).

**Table 5-4:** Cognition outcomes prior to (PRE) and after (POST) 6-hr of uninterrupted sitting (mean±SD).

| | **PRE** | **POST** | ***p-value*** |
|---|---|---|---|
| **Stroop Colour-Word Test** | | | |
| Interference Score (ms) | 184.5±120.1 | 170.6±132.6 | *0.425* |
| **Attention Network Test** | | | |
| Alerting Network (ms) | 13.0±21.2 | 15.1±17.3 | *0.638* |
| Orientating Network (ms) | 14.6±28.1 | 17.6±18.2 | *0.584* |
| Executive Control (ms) | 73.4±21.5 | 75.3±24.3 | *0.647* |
| **N-Back Task** | | | |
| Zero Back Accuracy (%) | 98.0±2.9 | 97.4±2.9 | *0.467* |
| Zero Back RT (ms) | 538.8±90.5 | 537.2±112.7 | *0.924* |
| One Back Accuracy (%) | 93.8±8.6 | 93.0±6.0 | *0.192* |
| One Back RT (ms) | 598.7±120.9 | 605.2±168.8 | *0.737* |
| Two Back Accuracy (%) | 92.8±10.3 | 85.6±21.3 | *0.153* |
| Two Back RT (ms) | 876.8±348.9 | 836.8±294.4 | *0.437* |
| Three Back Accuracy (%) | 80.8±17.2 | 75.8±20.7 | *0.112* |
| Three Back RT (ms) | 1325.9±810.9 | 1377.1±987.3 | *0.586* |

RT- reaction time.

### 5.3.6. Mood

There were significant decreases in positive affect (PRE: 27.1±7.2, POST: 22.5±7.9, p<0.001), and the alert (PRE: 53.6±15.0, POST: 43.0±18.5, p=0.002) and content (PRE: 67.7±13.7, POST: 60.3±15.5, p=0.006) mood states. Negative affect (PRE: 12.5±3.3, POST: 12.1±2.3, p=0.610) and the calm mood state (PRE: 48.2±10.6, POST: 45.9±8.9, p=0.392) did not significantly change.

### 5.3.7. Relationship Between Cerebrovascular Function, Mood and Work Productivity

There were no significant relationships between the change in seated or supine MCAv and the changes in mood or work productivity (p>0.05). Furthermore, the change in VLF normalised gain was not significantly associated with the observed changes in mood or work productivity (p>0.05).

## 5.4. Discussion

This study demonstrates for the first time that prolonged, uninterrupted sitting for six hours acutely decreases MCAv and impairs aspects of dynamic CA in healthy desk workers. In addition, prolonged sitting caused significant decreases in positive affect and alert and content mood states; although we demonstrate for the first time that the changes in mood were not related to changes in MCAv and dynamic CA. Finally, in line with previous findings (Bergouignan et al., 2016; Wennberg et al., 2016), we observed no changes in cognition in response to prolonged sitting. Our results indicate that prolonged, uninterrupted sitting acutely impairs cerebrovascular function and mood, but that acute impairments in cerebrovascular function do not appear to be associated with this lowered mood state. Nonetheless, mood and cerebrovascular function are important predictors of cerebrovascular health and mental health (Honda et al., 2014; Sabayan et al., 2012; Videbech, 2000; Wolters et al., 2017), therefore whether acute changes in these variables have implications for long-term mental and physical health of individuals who are repeatedly exposed to periods of uninterrupted sitting warrants further investigation.

Uninterrupted sitting for six hours reduced MCAv by 3.4 $cm \cdot s^{-1}$ while seated. Furthermore, there is an age-related decline in MCAv of 0.76 $cm \cdot s^{-1}$ per year (Ainslie et al., 2008), suggesting the reductions in MCAv observed following a one-off bout of uninterrupted sitting may equate to 2-4 years of age-related decline, albeit likely transient. Further research is needed to explore if the repeated reduction in MCAv of this magnitude would translate to long-term

impairment in CBF. Importantly, the decline in MCAv following sitting unlikely is the result of the daily circadian variation of CBF. Previous data indicate that CBF closely tracks the rhythm of core body temperature and is therefore lower during the morning than in the afternoon or evening (Conroy et al., 2005). Since our data shows a reduction in MCAv from baseline (am) to post test (pm), this decline most likely relates to prolonged sitting rather than a circadian rhythm. Seated CVC, was unchanged following six hours of sitting, indicating the observed reduction in MAP contributed to this decline in MCAv. In contrast, there was no change in supine MAP over the six-hour sitting period thus both supine MCAv and CVC were decreased, suggesting that mechanisms other than BP are involved in lowering supine CBF. Increased sympathetic activity causes cerebral vasoconstriction (Seifert and Secher, 2011) and progressive sympathoexcitation is suggested to contribute to age-associated decreases in CBF (Ainslie et al., 2008). As prolonged sitting elevates muscle sympathetic nerve activity (Ray et al., 1993) this heightened neural activity may therefore also induce vasoconstrictor effects on the cerebral vasculature.

In addition to CBF, aspects of dynamic CA were also impaired following uninterrupted sitting. Normalised gain, a measure of how changes in BP are transmitted into CBF, was significantly increased indicating a less efficient CA (i.e. greater changes in CBF for a given change in BP) (Claassen et al., 2009). Although the mechanisms of CA are not fully elucidated, it is suggested that sympathetic activity, endothelial NO production and myogenic factors all contribute (Tzeng and Ainslie, 2014). In peripheral vessels, sitting-induced impairments in vascular function are suggested to be partly due to reduced

blood flow and endothelial NO production (Carter et al., 2017). It is therefore possible that similar mechanisms contribute to the impaired CA observed in this study. Alternatively, sitting did not affect CVR. As $CO_2$ is considered the main regulator of CBF (Willie et al., 2011), it is plausible that the cerebrovasculature may exhibit an enhanced capability to preserve this function and resist any deleterious effects of sitting.

Collectively, our data showing prolonged sitting acutely impairs cerebrovascular function may have importance for long-term disease risk. Many cohorts of the population are highly sedentary, including UK office workers who spend 60-65% of their work time sitting (Clemes et al., 2014, 2016), and adults aged over 60 years, who spend on average 9.4 hours a day sedentary, equating to 65-80% of their waking day (Harvey et al., 2015). These populations are therefore at risk of a myriad of health issues related to sedentary behaviour (Young et al., 2016) and if evidence from this study can be replicated in the long-term, this may also include reduced cerebrovascular function. Chronic reduction in CBF is a risk factor for cognitive impairment (Wolters et al., 2017) and is associated with cerebrovascular diseases such as Alzheimer's disease and dementia (Sabayan et al., 2012; Wolters et al., 2017). Furthermore, impaired CA is observed in patients with Alzheimer's disease (den Abeelen et al., 2014). Consequently, long-term repeated exposure to sitting-induced decreases in CBF and CA could cause chronic impairment to cerebrovascular function and therefore have implications in the development of such diseases in highly sedentary cohorts.

Uninterrupted sitting also resulted in significant reductions in the alert and content mood states and positive affect. Improvements in mood have been observed previously when SB is reduced. Pleasantness increased after four days following a free-living 'sit less' strategy compared to four days of free-living SB (Duvivier et al., 2017), while breaking up six hours of uninterrupted sitting with hourly treadmill walking bouts improved measures of vigour and fatigue (Bergouignan et al., 2016). Our study also shows decreases in mood in response to an acute prolonged sitting period, and we are the first to highlight that these detriments do not appear to be correlated with reductions in MCAv or CA, indicating cerebrovascular function may not be a mechanism explaining mood alterations. This is unexpected since impaired cerebrovascular function is associated with mood disorders such as depression (Honda et al., 2014; Nobler et al., 2002; Videbech, 2000), but this is likely the result of chronic alterations, suggesting other mechanisms underlie our findings. Acutely elevating inflammatory markers in healthy participants increases negative mood (Wright et al., 2005), it has therefore been suggested that heightened inflammation may contribute to sitting-induced decreases in mood (Endrighi et al., 2016). Indeed, SB is associated with higher levels of C-reactive protein (Howard et al., 2015). Whilst this association is based on longer-term exposure to prolonged sitting, acutely reducing sitting time in adolescents improved apoB/apoA-1 ratio, a marker of inflammation (Penning et al., 2017), suggesting similar inflammatory responses may occur acutely in adults. Markers of inflammation are implicated in mood disorders (Rosenblat et al., 2014), consequently, sitting-induced inflammation may have contributed to the observed decreases in mood in this study, however further research is needed to test this hypothesis. Finally, the

reduction in mood following sitting may relate to the laboratory setting used for testing and participant boredom, which cannot be ruled out as potential contributing factors. In the long-term, repeated sitting-induced decreases in mood may be pertinent for mental health and well-being. A systematic review and meta-analyses demonstrated associations between SB and anxiety (Teychenne et al., 2015) and depression (Zhai et al., 2015). The mechanisms underlying these associations are understudied (Hallgren et al., 2016), but a chronically decreased mood state could contribute.

It has been hypothesised that hypoperfusion of the brain due to prolonged sitting contributes to cognitive decline (Wheeler et al., 2017), but despite sitting-induced decreases in MCAv observed in this study, this did not translate to changes in cognitive performance. This supports previous work showing no change in cognitive performance following up to six hours of uninterrupted sitting (Bergouignan et al., 2016; Wennberg et al., 2016). Chronically, decrements in CBF decrease oxygen and nutrient delivery, causing a breakdown of the blood brain barrier, neuronal damage (Wheeler et al., 2017) and slowing amyloid β clearance (Miners et al., 2018). Progressively, this results in amyloid β accumulation which may be a cause of neurodegeneration and cognitive impairment in dementia and Alzheimer's disease (Wheeler et al., 2017). A single, acute exposure to sitting would not have manifested such structural and function alterations, which may explain the lack of change in our cognitive outcomes. Whether chronic exposure to the transient reductions in CBF observed acutely in this study results in impaired cognition should be explored further.

This study also included tests of work productivity that represented an office worker's typical daily tasks, since impairments in these indices may impact overall work performance. After uninterrupted sitting for six hours, typing speed increased, with no change in typing accuracy, while mouse dexterity performance and RT both improved. In contrast, after sitting participants missed more spelling errors, despite reading the same number of characters. These changes in work productivity do not appear to be correlated with reductions in MCAv or CA, indicating cerebrovascular function may not be a mechanism explaining these results. However, caution must be taken with these findings since the tests of work productivity were not validated methods to assess these performance domains but were included as ecologically important measures representative of office workers' tasks. As such, these data provide preliminary evidence to support further research using validated productivity tests exploring whether prolonged sitting has a negative impact on markers of work productivity.

### 5.4.1. Limitations

By experimentally assessing prolonged sitting we inherently removed the habitual PA participants would perform during their work hours. However, recruited participants were desk workers and were minimally active during their work hours, therefore reductions in PA would have been minimal. Nonetheless the removal of activity may have contributed to the observed reduction in CBF. The lack of change in cognitive performance measures may indicate the tests were not sensitive enough to detect an effect of sitting, or that we assessed domains of cognition that are not influenced by sitting. However, we assessed

only the responses to an acute sitting period, therefore future work should examine the influence of chronic sitting. Additionally, it is also possible a learning effect may have occurred for the cognitive or work performance tests, however the inclusion of a familiarisation visit was used to reduce learning effects. The activities that participants completed during sitting were not controlled for, therefore it is possible that they may have differentially influenced cerebrovascular, cognitive and mood responses. However, the MCAs supply many of the brain regions involved in cognitive processing tasks (Li et al., 2014), therefore even if different tasks were performed during sitting, variation in cerebrovascular responses could be minimal. Furthermore, we did not measure or control for fidgeting during sitting, which attenuates the association between sitting and mortality risk (Hagger-Johnson et al., 2016), therefore may also influence the effect of sitting on cerebrovascular function and cognition. Due to the length of the experimental protocol, measurements could not be completed in a fasted state as is usual best practice. In an attempt to overcome the postprandial impact on outcome measures, the timing and content of the meals prior to each measurement time-point were matched so any influence on outcomes measures would be similar. Markers of inflammation were not assessed, which would have allowed for the exploration of the possibility that heighted inflammation contributed to the reduction in mood observed following sitting. The use of TCD to assess MCAv and cerebrovascular function is associated with known limitations, including the inability to measure actual blood flow (Willie et al., 2011), the assumptions that measures from the MCA are representative of other cerebral vessels and that MCA diameter is unaltered during varying levels of $CO_2$ (Skow et al., 2013). By recording the signal

parameters and photographically recording the TCD probe placement, it was ensured as closely as possible the probe was in the same location; small variations may have occurred, however our coefficient of variation was 7.8% indicating good reproducibility. Finally, the analysis of CA using TFA is a developing method and lacks references values, however we have collected and analysed data based on current guidelines (Claassen et al., 2016).

## 5.5. Conclusion

This study demonstrates that in healthy desk workers, prolonged, uninterrupted sitting for six hours acutely reduces CBF and impairs aspects of dynamic CA and mood, but does not result in decrements to cognition. The sitting-induced decline in cerebrovascular function was not related to changes in mood, suggesting other mechanisms underlie sitting-induced mood alterations. Further research is needed to understand how SB may impact cerebrovascular disease risk, mood and mental health in the long-term.

# 6. Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting

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# 7. Effects of a workplace intervention to break up sitting time on cerebrovascular function, cognition and mood: A pilot study

## 7.1. Introduction

The workplace is where most adults accumulate high amounts of SB (Parry and Straker, 2013; Ryan et al., 2011). Since SB is an established independent risk factor for cardiovascular morbidity and mortality (Young et al., 2016), workers are frequently exposing themselves to this potentially hazardous behaviour. Consequently, recent guidelines highlight the need to change workplace activity patterns by regularly breaking up seated work by replacing sitting with at least two hours of standing and light activity per work day. It is suggested that such a behaviour change would benefit workers' cardiometabolic health and also potentially enhance their productivity and overall performance (Buckley et al., 2015). However, there is little evidence from workplace intervention studies to support these recommendations; a fact that has been recently criticised (Stamatakis et al., 2018).

Cognition has been established as one of the best predictors of work performance across a range of professions (Fisher et al., 2017). Indeed, cognitive ability is negatively associated with counterproductive work behaviours (Dilchert et al., 2007) and employees with greater cognitive capabilities perform more work-related tasks (Morgeson et al., 2005). Workers' mood also influences task performance. During periods of pleasant mood, workers are more efficient and effective in their job role (Miner and Glomb, 2010; Rothbard and Wilk, 2011). Furthermore, positive affect is positively related to task performance and negatively related to counterproductive work behaviours, whilst opposite associations are observed for negative affect (Kaplan et al., 2009; Shockley et al., 2012).

Using PA breaks to interrupt sitting has however shown mixed effects on cognition and mood. Interrupting an acute sitting period with walking breaks had no effect on cognition (Bergouignan et al., 2016; Wennberg et al., 2016) but did enhance mood state (Bergouignan et al., 2016). Furthermore, no differences in cognition were observed following four days of a free-living 'sit' strategy compared to a 'sit less' strategy, but pleasantness increased (Duvivier et al., 2017). However, these studies were only acute assessments, consequently there is a need to investigate the long-term effect of reducing SB on cognition and mood, especially in a high SB environment, such as the workplace.

A range of workplace interventions to reduce SB have been examined, with meta-analyses and reviews concluding interventions using active workstations are an effective strategy to reduce workplace sitting time (Commissaris et al., 2015; Neuhaus et al., 2014; Torbeyns et al., 2014). However, despite workplace interventions reducing sitting time and increasing PA, no changes in work productivity have been observed (Brakenridge et al., 2016; Carr et al., 2015). Nonetheless, no workplace intervention study has directly assessed cognition or mood. Importantly, as shown in Chapter 6, breaking up SB with frequent walking bouts can prevent an impairment in cerebrovascular function that is otherwise observed. Since cerebrovascular function contributes to the maintenance of cognitive functioning (Bertsch et al., 2009; Wolters et al., 2017) and mood (Evans et al., 2017), this indicates that a workplace intervention to maintain or improve cerebrovascular function could in turn enhance cognition, mood and work performance.

More recently, the need to examine workplace interventions that are low-cost to consider workplaces with limited financial resources has been highlighted (Shrestha et al., 2018). One such alternative low-cost method to reduce workplace SB is using prompting devices to encourage workers to take a break from sitting. Exertime is a computer-based prompting software that has been previously used as a workplace intervention to break up sitting, increasing standing time during working hours by up to 7.99 minutes per day by performing activity breaks from sitting 4.95 to 6.28 times per day (Mainsbridge et al., 2014, 2016; Pedersen et al., 2014). In turn this has resulted in increases in self-reported health and wellbeing (Mainsbridge et al., 2016), energy expenditure (Pedersen et al., 2014) and reductions in MAP (Mainsbridge et al., 2014). Whether such an intervention can improve other aspects of health and wellbeing, such as cerebrovascular function, cognition and mood is unknown.

This study therefore aimed to assess changes in SB and PA levels, cerebrovascular function, cognition, mood and work productivity following an 8-week intervention designed to break up prolonged sitting at work. A secondary aim was to assess changes in peripheral artery endothelial function, since previously an eight-week intervention using sit-stand workstations significantly reduced sitting time and led to likely beneficial improvements in brachial artery endothelial function (Graves et al., 2015). Hence this study aimed to assess whether breaking up workplace sitting could produce systemic improvements to vascular health. Finally, owing to the influence of sleep on cognition and mood (Walker, 2009), this study also aimed to assess changes in sleep following the 8-week intervention. It was hypothesised that, firstly, following the 8-week

intervention to break up sitting at work, sitting time at work would be reduced and the time spent walking would increase. Secondly, it was hypothesised that following the intervention cerebrovascular and peripheral vascular function, cognition and mood would be improved.

## 7.2. Methods

### 7.2.1. Participants

Office-based workers from one University (Liverpool John Moores University, Liverpool, UK) were recruited. Departmental managers were contacted to gain consent for employee recruitment and participation in the study protocol, of which 17 approved (Figure 7-1). Staff within these departments were contacted via email with a study overview and those who expressed an interest received a participant information sheet and were screened for exclusion criteria including: aged >65 years, use of medication, smoker, BMI >35 or <18 $kg \cdot m^{-2}$, use of hormone-based contraception and diagnosis of cerebrovascular, cardiovascular or metabolic disease, or a mental health condition. Following this, ten healthy desk workers (4 male) were enrolled into the study and written informed consent was obtained prior to inclusion.

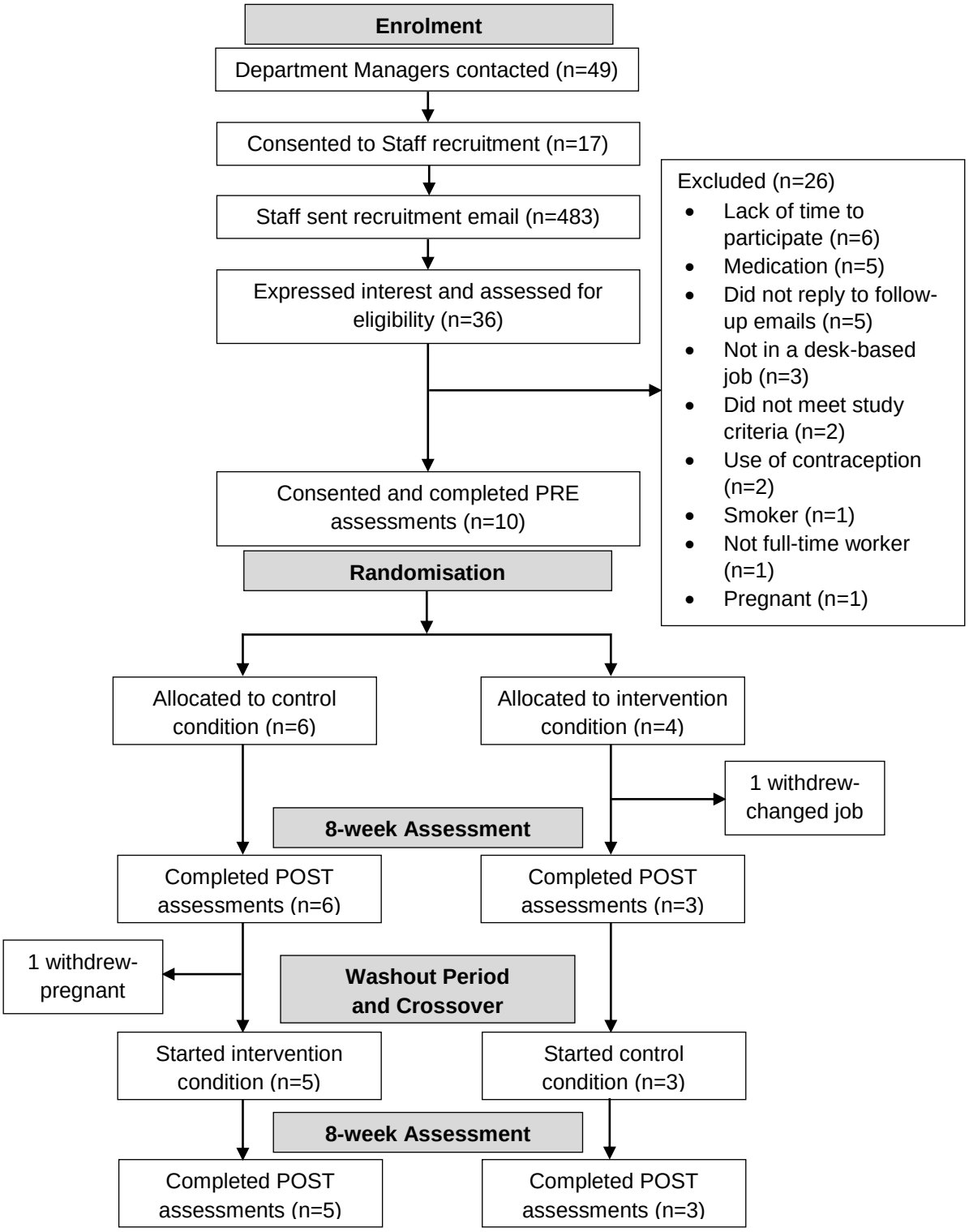



**Figure 7-1:** Consort flow diagram of enrolment, allocation and follow-up.

### 7.2.2. Study Design and Procedures

Study procedures were approved by the Liverpool John Moores University Ethics Committee and adhered to the Declaration of Helsinki. The study was conducted between September 2017 to May 2018. The study was a randomised crossover trial design, consisting of two conditions: intervention and control. Each condition lasted 8-weeks and conditions were separated by a 6-week wash-out period. Participants were randomly assigned to the order in which they completed conditions using computer-generated random numbers. During the intervention, participants used a computer-based prompting software designed to break up their workplace sitting, whilst in the control period participants did not have access to the software and were asked to maintain their normal workplace activity patterns. Participants and researchers were not blinded to group allocation.

Participants attended the laboratory on four separate occasions; before (PRE) and after (POST) each condition. Prior to each laboratory visit, participants were instructed to avoid strenuous exercise for 24-hr, and to abstain from caffeine and alcohol. Women were assessed in the follicular phase of the menstrual cycle (days 1-7). Anthropometric measures of stature and body mass were acquired at the start of each visit. After a 20-min supine rest, baseline measures of HR, BP, cardiovascular (brachial and femoral artery FMD) and cerebrovascular (CBF, NVC, CVR, CA) function were obtained. Participants were then given a 15-min break and a standardised snack to consume (a banana ~100kcal, ~27.0g carbohydrate, ~0.3g fat, ~1.0g protein and a cereal bar 192kcal, 27.1g carbohydrate, 7.2g fat, 3.4g protein). Following this, a

battery of computer-based cognitive performance (Stroop Colour-Word Test, Attention Network Test, N-Back Task) and productivity tests (Typing Performance, Reading and Correcting, Mouse Dexterity) were conducted and participants completed a mood questionnaire (PANAS) and a work performance questionnaire (Health and Work Questionnaire). Participants were then fitted with three monitors to measure habitual SB, PA and sleep which were worn for the next seven consecutive days. Following this, the 8-week trial began. At the start of the final week of the trial (week 7), SB, PA and sleep were assessed. All other measures were then repeated after the 8-week period (Figure 7-2).

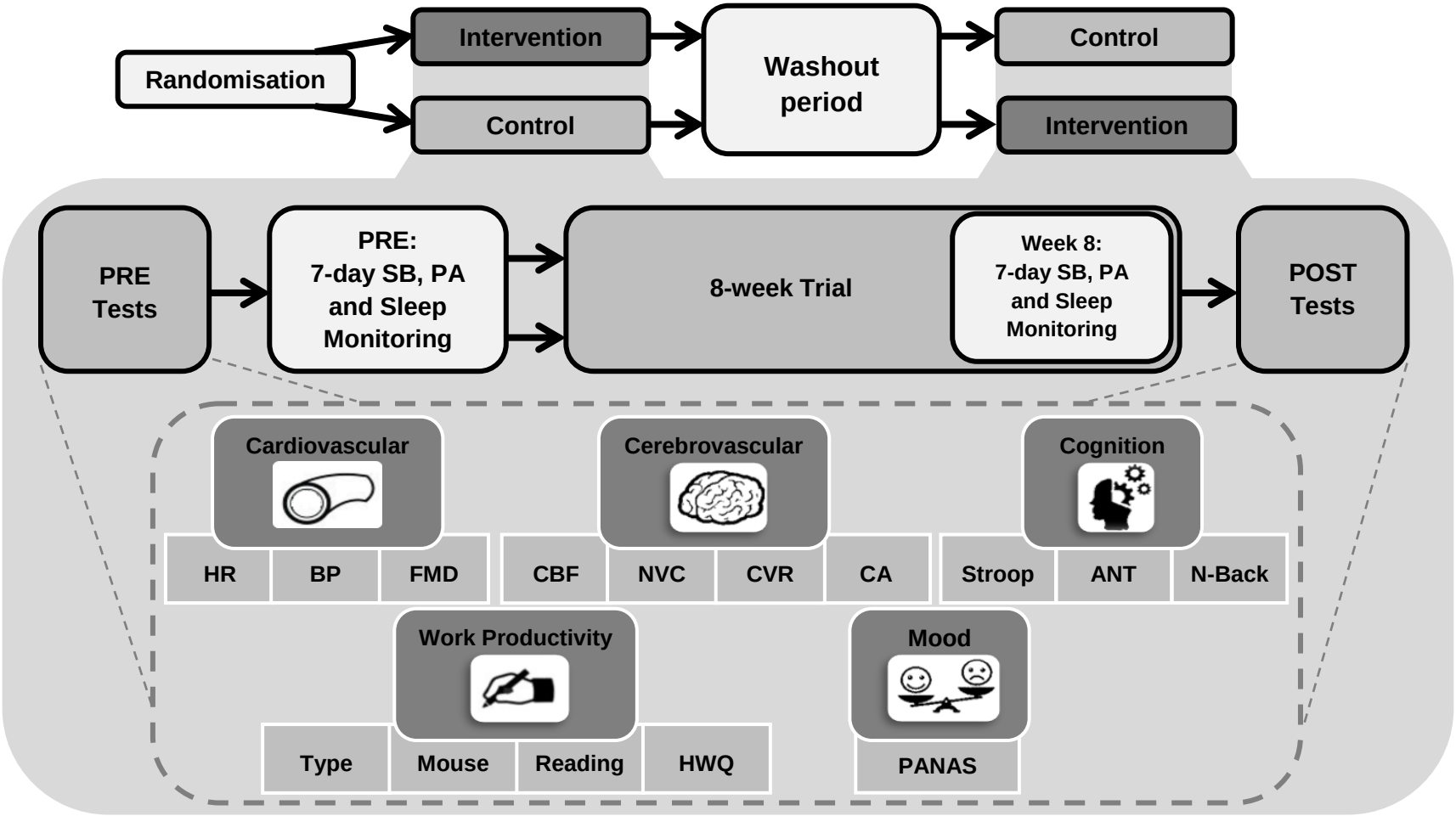



**Figure 7-2:** Study design. SB- sedentary behavior; PA- physical activity; HR- heart rate; BP- blood pressure; FMD- flow-mediated dilation; CBF- cerebral blood flow; NVC- neurovascular coupling; CVR- cerebrovascular carbon dioxide reactivity; CA- cerebral autoregulation; ANT- attention network test; HWQ- health and work questionnaire; PANAS- positive and negative affect schedule.

### 7.2.3. Intervention: Exertime

The intervention utilised a computer-based software programme called 'Exertime' which was installed onto the participant's work computer. Participants were instructed to install the software the morning after the seven-day baseline habitual SB, PA and sleep monitoring period was complete. This was confirmed via emailing the participant and monitoring the Exertime administrator online portal which tracks participants' use of the software. Exertime is designed to prompt employees to interrupt long bouts of sitting by standing up to engage in a brief bout of non-purposeful movement periodically during work hours (Mainsbridge et al., 2016; Pedersen et al., 2014). Based on data from Chapter 6, participants' activity selection was limited to only a walk during their breaks from sitting. The Exertime software was initiated every 45 minutes as a prompt bubble appearing on the bottom right hand side of the participant's computer screen (Figure 7-3). The prompting time was selected based on a combination of existing research using Exertime software and the results from Chapter 6. In previous studies using Exertime, the prompting time has been based on Australian national guidelines for office employees which specifies all computer-based employees should remove themselves from a sedentary position for a short period every hour (Worksafe Australia, 1996). Data from Chapter 6 demonstrated taking a break from sitting every 30 minutes attenuated sitting-induced reductions in MCAv. Collectively this indicates that a break frequency of between 30 minutes to one hour is required, hence, a 45 minutes prompt frequency was chosen as a trade-off between the two.

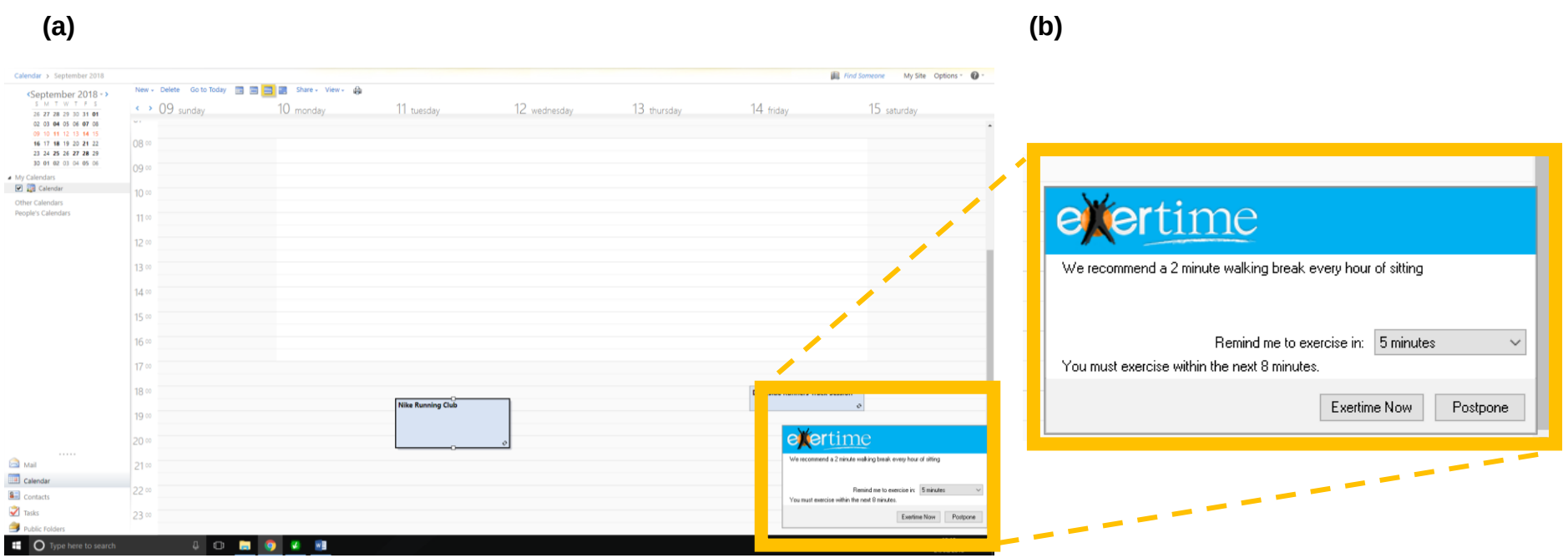
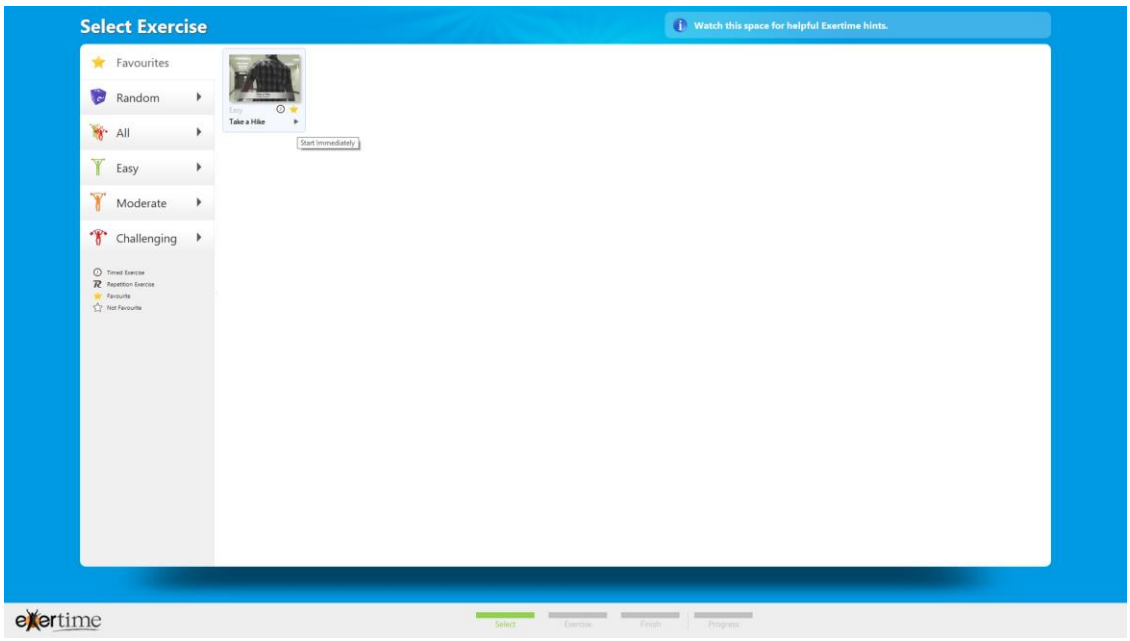



**Figure 7-3:** (a) Screenshot of the Exertime prompt at the bottom of the computer screen. (b) Close-up of the Exertime prompt.

The Exertime prompt indicated it was time for participants to take a break from sitting. Participants could choose to either engage with or postpone the prompt. If engage was selected, the Exertime software was activated and displayed across the whole computer screen. The software could not be shut down, forcing the participants to click onto the Exertime software before being able to regain control of their computer screen. If postpone was selected, it enabled participants to temporarily delay the prompt for a selected time period (5, 10 or 15 minutes) before it then reappeared. This function could only be activated for a maximum time of 15 minutes, after which the Exertime was automatically activated. The inclusion of the postpone function accounted for occasions where participants may be in a situation, such as a phone conversation or meeting, whereby they needed important access to their computer. When Exertime was activated, participants were required to select the ‘Take a Hike’ option to signify the beginning of their walking break and to then stand up and commence their walk. This started a clock which timed the duration the participant was away from their computer (Figure 7-4). The break duration was self-selected, however participants were advised to take a 2-min walking break based on the data from Chapter 6. Upon returning, the participant clicked to stop the timer, logging the total duration of their non-sedentary time. Once participant’s data was recorded the Exertime sequence terminated and the participant regained control over their computer screen.

(a)




(b)

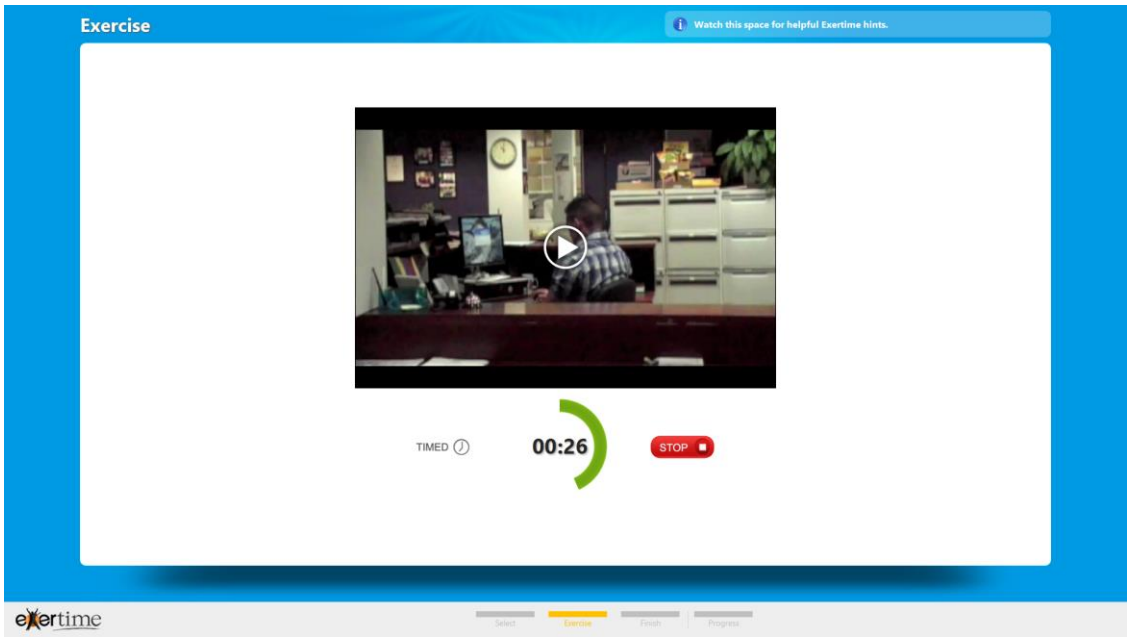



**Figure 7-4:** Screen shot of the activation of Exertime software. (a) The screen where participants select the 'Take a Hike' option. (b) The timer recording the duration of the activity break.

Prior to the start of the intervention, participants were given an educational booklet which included videos and information about the importance of reducing SB and how to install the software onto their work computer (Appendix 1: Participant Educational Booklet). Previous studies utilising Exertime software have included this prior to beginning the intervention period (Mainsbridge et al., 2014, 2016; Pedersen et al., 2014) and importantly, a pre-educational session combined with Exertime was more effective at decreasing prolonged sedentary periods and increasing workday movement compared to using the Exertime software alone (Smith et al., 2013). Participants were contacted via email to check they had watched the video and read the booklet. During the 8-week intervention, participants' use of the software was monitored by the research team using the online portal for Exertime administrators. If a participant was not logging activities, they were contacted via email by the principal researcher to check the software was working correctly and were prompted to use the software. To increase participants' motivation to engage with Exertime, participants also received a weekly email from the principal researcher at the start of each new week using the software which detailed the number of breaks and activity minutes they had logged in the previous week. Additionally, each week this email contained a suggestion as to how they could break up their sitting with walking breaks (Appendix 2: Email Suggestions for Participants to Break Up Their Sitting). The use of weekly emails was based on previous studies using Exertime that have phoned participants during the intervention to remind them to accurately report their activities and engage with the software (Cooley and Pedersen, 2013; Mainsbridge et al., 2014).

*7.2.3.1. Exertime Analysis*

Exertime usage data was recorded and accessible using the Exertime administrator online portal. For each participant, the date, time and duration of each break logged over the 8-week intervention period was extracted. This was subsequently broken down into weekly averages for the number of breaks taken and the duration of each break. Exertime data are based on the participants' self-report activity and is unable to determine if participants actually take a break from sitting. Consequently, to objectively validate the Exertime software, activPAL data for all weekdays from the final week (week 8) of the intervention trial were compared to data from the Exertime software log for this corresponding time period. Participants were not aware their monitor data was being compared with their Exertime data. This was achieved using the Exertime data output for the time when each break ended and the duration of this break. For each break, the time when the break ended was matched to the time in the activPAL data file. Based on the duration of the break logged in the Exertime file, the corresponding time duration in the activPAL data file was examined prior to the time the break ended. It was then determined firstly, if the participant actually took a break from sitting and, secondly, whether the duration of this break accurately matched that in the Exertime output. This was initially achieved using the 'sedentary to upright' and 'upright to sedentary' data output from activPAL, which provides a marker when a participant has transitioned from a sitting to standing or standing to sitting position. If it was confirmed that a participant had taken a break it was identified as a valid break and the epochs during the time period between these transitions were summed to calculate the time that was spent standing or stepping. If the participant had not taken a break at this time point, it was identified as a missed break. Using this

approach, it allowed the total logged break time to be compared between Exertime and activPAL to calculate any difference between the methods. This comparison was completed for total breaks (i.e. all breaks logged in Exertime (missed and validated breaks)), and validated breaks (i.e. only Exertime breaks with corresponding validated activPAL data). The compliance of taking a break when prompted was also determined by comparing the number of breaks reported in the Exertime output to the number of breaks actually achieved based on the activPAL output.

### 7.2.4. Measurements

The measurement of CBF, NVC, CVR, CA, $PETCO_2$, brachial and femoral artery FMD, SB, BP, cognition, mood, and work productivity are described in detail in Chapter 3. General Methods, hence here only specific features of this study are outlined. The battery of computer-based cognitive performance and work productivity tests were completed in a randomised order between participants but not within experimental visits. Participants were randomly assigned to the order in which they completed the tests using computer-generated random numbers. During data acquisition, supine MCAv and PCAv were acquired as a 2-min average. CVC was calculated by dividing MCAv by MAP. Participants were fitted with a photoplethysmographic cuff on the index or middle finger of the right hand (Finometer model 1) and a 3-lead electrocardiogram to continuously assess MAP and HR throughout measurements.

*7.2.4.1. Health and Work Questionnaire*

Participants completed the Health and Work Questionnaire (HWQ; Shikiar et al., 2004) which assesses workplace productivity in relation to worker health. The questionnaire is formed of 24 questions which then create subscales for: work productivity, concentration/focus, work satisfaction, non-work satisfaction, supervisor relations, impatience/irritability and stress (Shikiar et al., 2004). The HWQ is also designed to reduce social desirability bias by including questions that require an individual to rate their work quality, quantity, and efficiency from their supervisor's and their co-worker's perspective, as well as their own. Participants were required to rate each item in the questionnaire on a ten-point scale, with the end points of the scale tailored to each specific question. Subscale scores were then derived by averaging items within a subscale. The internal consistency of these scores, assessed using Cronbach's alpha, has been shown to be high (α 0.84-.096) (Shikiar et al., 2004). The HWQ has also been shown to significantly correlate to call agents' objective work performance (Shikiar et al., 2004).

*7.2.4.2. Physical Activity*

PA was objectively measured using the Actigraph GT3X monitor (Actigraph, Pensacola, Florida), a tri-axial accelerometer that assesses movement over three axes. The Actigraph reliably measures adult PA levels under free-living conditions (Aadland and Ylvisåker, 2015) and is widely used in research to assess PA levels (Aguilar-Farías et al., 2014). For seven consecutive days, participants wore the Actigraph GT3X accelerometer on an elastic waist belt positioned on the right hip. They were instructed to wear the monitor during all waking hours, removing only for water-based activities such as showering. Each

participant was allocated their own Actigraph monitor, using the monitor's unique serial number. This ensured the same monitor was used for each separate 7-day monitoring period. Participants were instructed to complete a log sheet detailing the times the monitor was removed and replaced each day. Data were downloaded at 60 second epochs and analysed using ActiLife software (Version 6.2, Actigraph). Raw accelerometery data were presented in counts per minute (counts·min$^{-1}$). Non-wear time was defined as 90 consecutive minutes of zero counts·min$^{-1}$ (Choi et al., 2011) and this was excluded from all analyses. PA intensity was determined using the cut points: light-intensity (≤2689 counts·min$^{-1}$), moderate-intensity (≤6166 counts·min$^{-1}$), and vigorous-intensity (>6167 counts·min$^{-1}$); which are validated cut points for healthy, normal or overweight adults (Sasaki et al., 2011), thus suited to the population assessed in this study. Participant's data were only included for analyses if the following criteria were met: ≥10 hours of wear time per day, for a minimum of four days, including one weekend day (Trost et al., 2005). For each day the time spent in each category of activity was determined and then mean values were calculated for each variable for a working day and a weekend day. Participant log-book recording of their working hours were used to determine time spent at work. By applying filters in the ActiLife software to correspond to these reported work hours, activity intensities were then calculated for working hours for each work day.

*7.2.4.3. Sedentary Behaviour*

SB was assessed using the activPAL, as described in detail in section 3.11.3.1, hence here only specific features of this study are outlined. For each separate 7-day monitoring period it was ensured the same activPAL monitor was worn be

each participant. This was achieved by allocating each participant a specific monitor using the monitor's unique serial number. Data were considered a valid day if the monitor was worn ≥10 hours per day and if wear time corresponded with the participant's self-report wear time diary. The latter was achieved by visually inspecting the activPAL graphical data and event file outputs following analyses to assess if self-report wake up and bed time corresponded with activPAL data. When assessing working hours, it was required that the monitor was worn for 100% of work time. Furthermore, data were only included if a valid activPAL wear day had a corresponding valid Actigraph wear day, thus both SB and PA data were valid for the same day (i.e. if the participant only wore one of the monitors this day was excluded). Based on these criteria, activPAL data was only included if there were a minimum of four valid wear days, including one weekend day.

*7.2.4.4. Sleep*

Sleep was assessed using the Actiwatch 4 (Cambridge Neurotechnology Ltd, Cambridge, UK), a small, light-weight wrist accelerometer which has been shown to have acceptable performance compared to the gold standard measures of sleep (polysomnography) for all sleep measures, except for sleep onset latency (Tonetti et al., 2008). The Actiwatch was worn on participants' non-dominant wrist continuously for seven days, as recommended to reliably measure sleep (Sadeh and Acebo, 2002), with an epoch length set to one minute. Participants pressed a marker button on the Actiwatch for two seconds upon lights out and repeated this process the following morning upon lights on to indicate bedtime and wake up time. In combination with the Actiwatch, participants completed the Consensus Sleep Diary (Carney et al., 2012), as

recommended in conjunction with actigraphy to prevent periods of non-wear time being incorrectly classed as sleep (Sadeh and Acebo, 2002). The Consensus Sleep Diary is the recommended sleep diary as it was generated in order to standardise sleep diaries within sleep research (Carney et al., 2012). Participants were instructed to fill in the consensus sleep diary immediately after getting out of bed, which asked questions relating to bedtime, wake up time, number of awakenings and sleep quality. From the Actiwatch and the consensus sleep diary, the principal investigator was able to manually determine bedtime, time of sleep onset (sleep start), wake up time (sleep end) and get up time so that sleep behaviour could be automatically calculated using Actiwatch software (Sleep Analysis 5.24, Cambridge Neurotechnology Ltd). Data were analysed using the default medium sensitivity, where an integrated activity count equal to or greater than 40 within a one minute epoch is designated as being awake. Each night of sleep was analysed using the Actiwatch software for the following sleep parameters: sleep duration, sleep latency, sleep efficiency and fragmentation index. Fragmentation index provides a measure of restlessness during sleep, using the percentage of epochs where activity is >0, while sleep efficiency describes the percent of time sleeping whilst in bed defined by the bedtime and get-up time by the participant.

### 7.2.5. Statistical Analyses

Data were analysed using statistical software (SPSS Version 22.0, IBM Corporation, Somers, NY, USA), with significance accepted as $p<0.05$. Results are presented as means±SD. To assess for condition, time and any interaction effect, parameters were analysed using linear mixed models. Femoral and

brachial FMD were also analysed using an allometric approach that controls for changes in baseline diameter (Atkinson and Batterham, 2013). Paired samples t-tests were used to compare the difference between Exertime and activPAL break data. Post-hoc analyses were performed using the least significant difference (LSD) method.

## 7.3. Results

From the originally recruited sample size of 10, eight participants completed the study and were included in analyses. As shown in Figure 7-1, one participant withdrew after the PRE assessment due to a change of job and another participant withdrew during the washout period due to pregnancy. Full descriptive characteristics are shown in Table 7-1.

**Table 7-1:** Participant descriptive characteristics (n=8, 3 male).

| | **Mean±SD or n of group** |
|---|---|
| Age (years) | 43.4±11.6 |
| Body Mass (kg) | 70.5±11.9 |
| Stature (cm) | 168.8±7.8 |
| Body Mass Index ($kg \cdot m^{-2}$) | 24.6±3.0 |
| White British | 8 |
| Married | 6 |
| Job Category | |
| Manager/Director | 0 |
| Clerical/Services/Other | 8 |
| Time at Current Workplace | |
| < 1 year | 1 |
| 1-3 years | 4 |
| >3 years | 3 |
| Work Hours (per week) | 38.1±9.8 |
| Work Hours (per day) | 8.0±1.7 |
| Number of People in Office | |
| 0 | 0 |
| 1-3 People | 3 |
| >3 People | 5 |
| Occupational Transport | |
| Car | 3 |
| Train | 4 |
| Bus | 1 |

### 7.3.1. Exertime

Week-by-week Exertime data for the number and duration of activity breaks recorded by participants from the automated software log are shown in Figure 7-5. There were no significant differences between weeks for the number or duration of activity breaks completed ($p>0.05$). Over the 8-week intervention, the automated Exertime software logged that participants recorded a daily average of 7.6±3.5 minutes taking breaks from sitting, as assessed by time standing or stepping, which was achieved by logging an average of 6.1±1.4 breaks per day. This equated to 175.4±66.8 minutes taking breaks from sitting per week, achieved by taking 24.7±7.9 breaks. The corresponding activPAL data indicated that participants actually took a break from sitting for 73.6% of the breaks that they logged in Exertime. For total weekly breaks (validated and missed breaks) there was no significant difference between Exertime (159.4±94.2 minutes/week) or objective break data (143.3±56.6 minutes/week, $p=0.497$). Hence, there was a 16.1 minute/week overestimation by the Exertime software. For only validated breaks (Exertime breaks with corresponding objective activPAL data) there was no significant difference between Exertime (149.9±85.6 minutes/week) or objective break data ($p=0.722$), hence there was a 6.6 minute/week overestimation by the Exertime software.

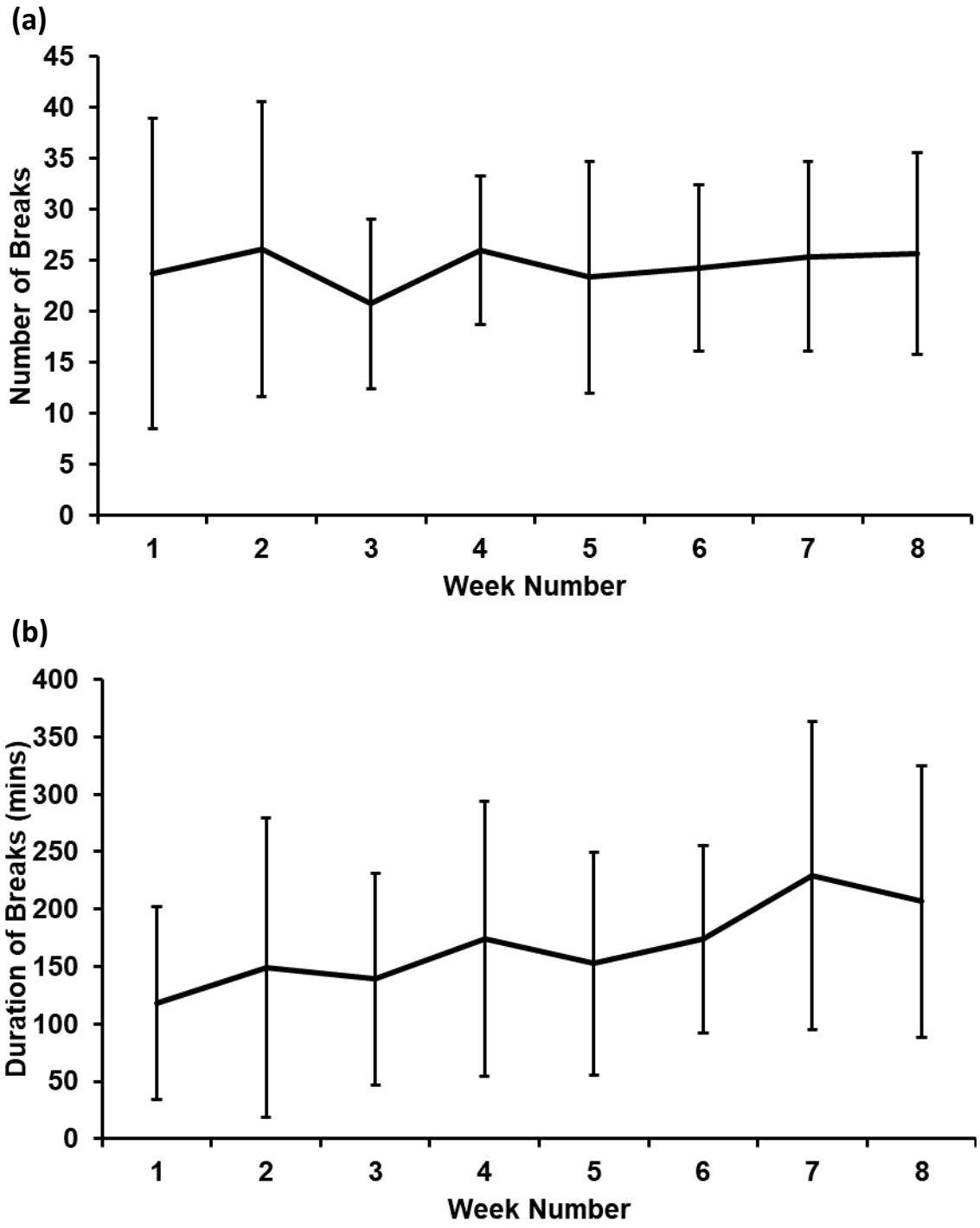



**Figure 7-5:** Weekly Exertime self-report data for (a) the number of activity breaks recorded, and (b) the duration of the activity breaks (Mean±SD).

### 7.3.2. Sedentary Behaviour and Physical Activity

SB and PA data for work hours are presented in Table 7-2, for weekdays in Table 7-3 and for the weekend in Table 7-4. There were no significant main effects for any domain of PA or SB for work hours, weekdays or the weekend ($p>0.05$).

**Table 7-2:** Time spent sitting, standing and stepping and in light-, moderate- and vigorous-intensity physical activity (PA) during work hours at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | **Control** | | **Exertime** | | ***p-value*** | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Work Hours** | | | | | | | |
| Sitting time (min) | 325.9±93.9 | 375.1±75.5 | 357.9±43.6 | 315.7±64.5 | *0.659* | *0.883* | *0.331* |
| Standing time (min) | 116.9±79.0 | 106.1±73.5 | 78.6±36.4 | 92.3±42.6 | *0.179* | *0.911* | *0.480* |
| Stepping time (min) | 50.1±16.1 | 63.4±35.9 | 54.1±19.0 | 51.9±26.5 | *0.551* | *0.545* | *0.165* |
| Sitting time (% of work hours) | 66.2±17.0 | 70.1±9.6 | 73.2±8.2 | 69.0±13.1 | *0.517* | *0.962* | *0.271* |
| Standing time (% of work hours) | 23.7±16.0 | 18.6±7.3 | 15.9±6.7 | 19.9±8.5 | *0.224* | *0.889* | *0.109* |
| Stepping time (% of work hours) | 10.1±3.2 | 11.3±4.0 | 10.9±3.5 | 11.1±5.3 | *0.738* | *0.502* | *0.428* |
| Light-intensity PA (min) | 117.1±44.7 | 115.2±30.4 | 110.4±45.8 | 105.7±34.2 | *0.350* | *0.743* | *0.825* |
| Moderate-intensity PA (min) | 23.5±13.0 | 29.4±15.6 | 26.8±12.3 | 26.6±14.7 | *0.909* | *0.414* | *0.217* |
| Vigorous-intensity PA (min) | 4.5±5.8 | 5.0±6.0 | 7.0±8.0 | 4.6±6.8 | *1.00* | *1.00* | *1.00* |
| Light-intensity PA (% of work hours) | 24.3±8.9 | 22.9±6.3 | 22.9±9.1 | 22.9±7.8 | *0.629* | *0.634* | *0.511* |
| Moderate-intensity PA (% of work hours) | 4.8±2.6 | 5.9±3.0 | 5.5±2.4 | 5.8±3.2 | *0.612* | *0.356* | *0.463* |
| Vigorous-intensity PA (% of work hours) | 0.9±1.2 | 1.0±1.3 | 1.4±1.6 | 0.9±1.3 | *0.495* | *0.283* | *0.057* |

**Table 7-3:** Time spent sitting, standing and stepping and in light-, moderate- and vigorous-intensity physical activity (PA) during weekdays at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | **Control** | | **Exertime** | | ***p-value*** | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Weekday** | | | | | | | |
| Sitting time (min) | 590.6±103.0 | 586.3±137.2 | 620.1±62.6 | 621.8±166.0 | *0.366* | *0.969* | *0.944* |
| Standing time (min) | 255.2±86.6 | 201.6±64.6 | 209.4±36.2 | 226.7±45.5 | *0.456* | *0.443* | *0.183* |
| Stepping time (min) | 123.5±35.5 | 111.8±35.0 | 121.3±35.2 | 126.7±30.1 | *0.399* | *0.541* | *0.290* |
| Sitting time (% of waking hours) | 60.8±10.0 | 65.6±4.8 | 65.2±5.7 | 63.2±8.4 | *0.546* | *0.389* | *0.260* |
| Standing time (% of waking hours) | 26.5±9.6 | 21.9±3.8 | 22.1±4.1 | 23.5±5.7 | *0.487* | *0.372* | *0.193* |
| Stepping time (% of waking hours) | 12.7±3.2 | 12.4±2.2 | 12.7±3.6 | 13.3±3.6 | *0.359* | *0.809* | *0.410* |
| Light-intensity PA (min) | 270.9±44.7 | 267.5±33.6 | 256.0±27.1 | 267.3±52.1 | *0.536* | *0.655* | *0.509* |
| Moderate-intensity PA (min) | 56.8±30.9 | 56.8±22.7 | 56.7±32.8 | 63.0±20.7 | *0.382* | *0.555* | *0.307* |
| Vigorous-intensity PA (min) | 9.0±8.3 | 10.4±11.2 | 11.0±8.8 | 8.7±8.8 | *0.950* | *0.743* | *0.064* |
| Light-intensity PA (% of waking hours) | 30.3±6.3 | 28.2±3.4 | 28.6±4.9 | 29.4±6.4 | *0.782* | *0.389* | *0.378* |
| Moderate-intensity PA (% of waking hours) | 6.2±3.1 | 6.0±2.3 | 6.2±3.4 | 6.9±2.2 | *0.089* | *0.721* | *0.298* |
| Vigorous-intensity PA (% of waking hours) | 1.0±0.9 | 1.1±1.1 | 1.2±1.0 | 1.0±1.0 | *0.754* | *0.597* | *0.200* |

**Table 7-4:** Time spent sitting, standing and stepping and in light-, moderate- and vigorous-intensity physical activity (PA) during the weekend at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | **Control** | | **Exertime** | | ***p-value*** | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Weekend** | | | | | | | |
| Sitting time (min) | 451.3±86.9 | 452.5±76.6 | 429.6±99.6 | 462.9±111.4 | *0.828* | *0.562* | *0.638* |
| Standing time (min) | 309.8±55.0 | 302.6±67.4 | 332.2±94.9 | 304.3±95.5 | *0.534* | *0.427* | *0.672* |
| Stepping time (min) | 121.3±31.3 | 130.3±32.3 | 131.5±56.9 | 129.9±34.4 | *0.580* | *0.785* | *0.548* |
| Sitting time (% of waking hours) | 51.0±8.3 | 51.1±8.0 | 48.6±12.6 | 51.6±12.3 | *0.715* | *0.626* | *0.665* |
| Standing time (% of waking hours) | 35.2±6.2 | 34.2±7.5 | 36.8±9.2 | 34.0±10.6 | *0.734* | *0.430* | *0.737* |
| Stepping time (% of waking hours) | 13.8±3.8 | 14.7±3.4 | 14.6±5.7 | 14.4±3.6 | *0.809* | *0.804* | *0.586* |
| Light-intensity PA (min) | 326.4±84.8 | 361.1±70.7 | 322.0±108.7 | 335.1±102.2 | *0.540* | *0.261* | *0.381* |
| Moderate-intensity PA (min) | 46.8±27.6 | 47.3±34.4 | 46.1±48.6 | 53.6±25.2 | *0.849* | *0.824* | *0.794* |
| Vigorous-intensity PA (min) | 5.4±10.8 | 4.9±10.9 | 3.3±8.8 | 4.9±11.4 | *0.292* | *0.822* | *0.546* |
| Light-intensity PA (% of waking hours) | 41.3±10.2 | 42.2±9.0 | 40.9±7.7 | 43.0±9.1 | *0.822* | *0.455* | *0.733* |
| Moderate-intensity PA (% of waking hours) | 6.0±3.6 | 5.5±4.4 | 5.7±5.7 | 7.2±3.4 | *0.636* | *0.795* | *0.474* |
| Vigorous-intensity PA (% of waking hours) | 0.7±1.4 | 0.5±1.1 | 0.4±1.1 | 0.8±1.9 | *0.752* | *0.838* | *0.343* |

### 7.3.3. Cardiorespiratory and Haemodynamic Measures

No significant main effects were observed for resting HR (Control PRE: 60.7±10.1 bpm, Control POST: 59.6±9.0 bpm, Exertime PRE: 56.3±10.2 bpm, Exertime POST: 59.6±9.2 bpm, $p>0.05$). Similarly, there were no significant main effects for resting MAP (Control PRE: 83.5±4.8 mmHg, Control POST: 85.5±8.6 mmHg, Exertime PRE: 83.6±10.5 mmHg, Exertime POST: 83.0±7.5 mmHg, $p>0.05$). No significant main effects were also observed for $PETCO_2$ (Control PRE: 37.9±4.2 mmHg, Control POST: 39.6±3.1 mmHg, Exertime PRE: 38.1±4.1 mmHg, Exertime POST: 38.3±3.2 mmHg, $p>0.05$).

### 7.3.4. Cerebrovascular Function

CA data are presented in Table 7-5 and MCAv, PCAv, NVC, CVR data are presented in Table 7-6. For CA, in the 10-sec squat protocol a significant interaction effect was observed for phase in the VLF domain ($p=0.042$), with post hoc analyses revealing at POST the Exertime condition had a higher phase compared to the control condition ($p=0.010$). There was also a significant main effect for time in the LF domain for gain ($p=0.033$), with gain higher at POST (0.84±0.18 $cm \cdot s^{-1} \cdot mmHg^{-1}$) compared to PRE (0.76±0.15 $cm \cdot s^{-1} \cdot mmHg^{-1}$). No other significant main effects were observed for other CA parameters in the 10-sec squat protocol ($p>0.05$). In the 5-sec squat protocol there were no significant main effects for any parameter in each of the frequency domains ($p>0.05$). For NVC, there was a significant main effect for time for the absolute change in PCAv ($p=0.047$), with PCAv higher at POST (11.97±2.10 $cm \cdot s^{-1}$) compared to PRE (7.20±3.01 $cm \cdot s^{-1}$). No other significant main effects were observed for NVC outcomes ($p>0.05$). Absolute and relative MCA CVR $R^2$ values (values are the same for both) of linear regression are presented in

Table 7-6. No significant main effects were observed for absolute or relative MCA CVR ($p>0.05$). Similarly, no significant main effects were observed for absolute or relative CCA CVR for both CCA diameter or CCA blood flow ($p>0.05$). Finally, no significant main effects were observed for MCAv or PCAv ($p>0.05$).

**Table 7-5:** Measures of cerebral autoregulation (CA) at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | Control | | Exertime | | *p-value* | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **CA: 5-sec Squats** | | | | | | | |
| VLF Phase (degrees) | 47.36±17.90 | 45.93±14.95 | 45.07±14.34 | 50.96±14.20 | *0.980* | *0.834* | *0.262* |
| VLF Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.66±0.16 | 0.68±0.20 | 0.72±0.27 | 0.65±0.13 | *0.979* | *0.912* | *0.889* |
| VLF $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.13±0.25 | 1.10±0.24 | 1.15±0.24 | 1.06±0.23 | *0.928* | *0.996* | *0.578* |
| VLF Coherence | 0.46±0.10 | 0.50±0.06 | 0.51±0.10 | 0.45±0.11 | *0.660* | *0.823* | *0.090* |
| LF Phase (degrees) | 25.78±6.17 | 18.90±9.43 | 27.99±19.96 | 20.89±7.96 | *0.907* | *0.263* | *0.587* |
| LF Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.86±0.22 | 0.87±0.10 | 0.81±0.28 | 0.97±0.28 | *0.465* | *0.156* | *0.585* |
| LF $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.44±0.25 | 1.42±0.18 | 1.30±0.30 | 1.57±0.41 | *0.988* | *0.054* | *0.156* |
| LF Coherence | 0.63±0.10 | 0.64±0.08 | 0.68±0.11 | 0.60±0.08 | *0.821* | *0.221* | *0.212* |
| HF Phase (degrees) | 2.99±11.83 | 3.68±14.93 | -2.16±15.13 | 3.09±11.17 | *0.433* | *0.448* | *0.107* |
| HF Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.65±0.20 | 0.65±0.13 | 0.61±0.19 | 0.84±0.28 | *0.372* | *0.071* | *0.185* |
| HF $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.11±0.30 | 1.12±0.35 | 1.00±0.24 | 1.37±0.37 | *0.655* | *0.242* | *0.212* |
| HF Coherence | 0.43±0.08 | 0.44±0.03 | 0.46±0.10 | 0.40±0.05 | *0.638* | *0.311* | *0.111* |
| **CA: 10-sec Squats** | | | | | | | |
| VLF Phase (degrees) | 40.82±15.08 | 37.12±7.40 | 45.21±21.81 | 48.38±17.88* | *0.241* | *0.611* | *0.042* |
| VLF Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.66±0.25 | 0.79±0.22 | 0.70±0.27 | 0.76±0.17 | *0.800* | *0.159* | *0.457* |
| VLF $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.14±0.38 | 1.26±0.31 | 1.16±0.28 | 1.27±0.28 | *0.736* | *0.138* | *0.877* |
| VLF Coherence | 0.82±0.18 | 0.88±0.08 | 0.87±0.12 | 0.88±0.08 | *0.896* | *0.154* | *0.913* |
| LF Phase (degrees) | 19.10±15.77 | 16.19±7.47 | 23.63±28.83 | 20.88±13.57 | *0.376* | *0.515* | *0.991* |
| LF Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.74±0.23 | 0.81±0.22# | 0.76±0.18 | 0.87±0.20# | *0.422* | *0.033* | *0.898* |
| LF $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.33±0.43 | 1.31±0.33 | 1.26±0.15 | 1.47±0.34 | *0.628* | *0.198* | *0.327* |
| LF Coherence | 0.63±0.15 | 0.64±0.16 | 0.63±0.10 | 0.64±0.11 | *0.776* | *0.979* | *0.651* |
| HF Phase (degrees) | 0.11±4.68 | 3.11±10.67 | 17.06±32.25 | 1.63±7.13 | *0.373* | *0.498* | *0.327* |
| HF Gain ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.69±0.22 | 0.76±0.27 | 0.64±0.11 | 0.67±0.09 | *0.726* | *0.393* | *0.919* |
| HF $Gain_n$ ($\% \cdot mmHg^{-1}$) | 1.22±0.41 | 1.121±0.40 | 1.09±0.12 | 1.14±0.17 | *0.605* | *0.633* | *0.633* |
| HF Coherence | 0.55±0.10 | 0.49±0.05 | 0.50±0.14 | 0.48±0.08 | *0.835* | *0.447* | *0.509* |

CA- cerebral autoregulation; VLF- very low frequency; LF- low frequency; HF- high frequency; $Gain_n$- normalised gain.

* Significant interaction effect (p=0.042), significantly different to Control at POST (p=0.010).

# Significant main effect for time (p=0.033), with POST higher than PRE.

**Table 7-6:** Measures of cerebrovascular function at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | **Control** | | **Exertime** | | ***p-value*** | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Resting CBF** | | | | | | | |
| MCAv ($cm \cdot s^{-1}$) | 65.2±15.2 | 66.9±8.6 | 68.0±16.9 | 66.7±9.9 | *0.663* | *0.924* | *0.616* |
| PCAv ($cm \cdot s^{-1}$) | 54.0±17.1 | 53.0±11.2 | 50.4±11.8 | 51.7±12.0 | *0.587* | *0.802* | *0.734* |
| **NVC** | | | | | | | |
| Absolute Peak PCAv ($\Delta cm \cdot s^{-1}$) | 10.0±4.2 | 18.7±2.7# | 4.4±3.7 | 7.3±6.2# | *0.380* | *0.047* | *0.376* |
| Relative Peak PCAv (%) | 20.7±10.7 | 38.7±10.6 | 9.3±6.7 | 16.6±16.4 | *0.057* | *0.084* | *0.543* |
| Absolute Peak MCAv ($\Delta cm \cdot s^{-1}$) | 8.6±3.8 | 2.9±0.6 | 3.1±2.5 | 6.0±1.8 | *0.756* | *0.654* | *0.252* |
| Relative Peak MCAv (%) | 10.3±7.6 | 5.0±0.6 | 5.0±4.2 | 9.7±4.4 | *0.652* | *0.486* | *0.723* |
| **CVR** | | | | | | | |
| MCA CVR ($R^2$) | 0.90±0.01 | 0.87±0.03 | 0.86±0.01 | 0.90±0.02 | *0.970* | *0.554* | *0.152* |
| Absolute MCA CVR ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 3.02±1.07 | 3.21±1.17 | 3.47±1.24 | 3.31±0.75 | *0.363* | *0.921* | *0.613* |
| Relative MCA CVR ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 4.63±1.06 | 4.73±1.49 | 5.29±1.82 | 5.23±1.14 | *0.371* | *0.854* | *0.937* |
| Absolute CCA Diameter CVR ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.001±0.001 | 0.001±0.001 | 0.001±0.001 | 0.001±0.001 | *0.675* | *0.492* | *0.502* |
| Relative CCA Diameter CVR ($cm \cdot s^{-1} \cdot mmHg^{-1}$) | 0.12±0.15 | 0.11±0.09 | 0.19±0.18 | 0.12±0.18 | *0.491* | *0.567* | *0.594* |
| Absolute CCA Blood Flow CVR ($ml \cdot min^{-1} \cdot mmHg^{-1}$) | 0.24±0.20 | 0.32±0.29 | 0.25±0.27 | 0.21±0.29 | *0.718* | *0.797* | *0.411* |
| Relative CCA Blood Flow CVR ($ml \cdot min^{-1} \cdot mmHg^{-1}$) | 1.81±1.53 | 2.84±2.99 | 2.02±2.08 | 1.98±2.62 | *0.955* | *0.756* | *0.767* |

CBF- cerebral blood flow; MCAv- middle cerebral artery blood flow velocity: PCAv- posterior middle cerebral artery blood flow velocity; NVC- neurovascular coupling; CVR- cerebrovascular carbon dioxide reactivity; CCA- common carotid artery.
# Significant main effect for time (p=0.047), with POST higher than PRE.

### 7.3.5. Cognition and Work Productivity

There were no significant main effects observed for any measures of cognition (Table 7-7) or work productivity ($p>0.05$; Table 7-8). However, a significant main effect for time was observed for the impatience subscale of the HWQ ($p=0.027$), with the impatience score higher at PRE (4.2±2.2) compared to POST (2.8±1.6). No other significant main effects were observed for the other subscales of the HWQ ($p>0.05$).

### 7.3.6. Mood

No significant main effects were observed for positive affect (Control PRE: 38.0±6.7, Control POST: 36.3±6.5, Exertime PRE: 35.9±7.0, Exertime POST: 37.4±5.6, $p>0.05$) or negative affect (Control PRE: 16.1±6.4, Control POST: 14.3±2.5, Exertime PRE: 16.4±8.5, Exertime POST: 14.9±4.5, $p>0.05$).

**Table 7-7:** Measures of cognition at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | Control | | Exertime | | *p-value* | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Stroop Colour-Word Test** | | | | | | | |
| Interference Score (ms) | 84.0±89.7 | 116.5±59.0 | 97.5±74.8 | 129.5±69.3 | *0.525* | *0.197* | *0.989* |
| **Attention Network Test** | | | | | | | |
| Alerting Network (ms) | 8.7±22.0 | 9.5±26.6 | 16.7±24.2 | 29.0±20.2 | *0.122* | *0.238* | *0.419* |
| Orientating Network (ms) | 7.0±24.1 | 23.8±27.5 | 16.4±29.3 | 9.8±13.7 | *0.705* | *0.345* | *0.092* |
| Executive Control (ms) | 74.2±33.4 | 71.2±23.2 | 57.7±32.3 | 67.0±30.6 | *0.187* | *0.601* | *0.201* |
| **N-Back Task** | | | | | | | |
| Zero Back Accuracy (%) | 96.9±2.6 | 96.9±4.6 | 98.1±2.6 | 98.9±2.3 | *0.106* | *0.718* | *0.763* |
| Zero Back RT (ms) | 590.5±82.3 | 581.0±102.7 | 589.2±124.5 | 581.6±108.0 | *0.982* | *0.294* | *0.972* |
| One Back Accuracy (%) | 93.1±5.3 | 93.8±5.8 | 89.4±9.4 | 90.0±16.6 | *0.496* | *0.722* | *1.00* |
| One Back RT (ms) | 664.0±100.2 | 654.7±117.2 | 676.0±109.1 | 685.1±132.9 | *0.416* | *0.997* | *0.671* |
| Two Back Accuracy (%) | 90.6±7.8 | 78.8±24.2 | 83.8±18.7 | 78.1±16.0 | *0.530* | *0.076* | *0.571* |
| Two Back RT (ms) | 1072.2±368.6 | 1116.5±189.8 | 988.8±370.7 | 1086.4±424.0 | *0.469* | *0.476* | *0.779* |
| Three Back Accuracy (%) | 75.6±25.7 | 67.5±24.1 | 64.4±30.9 | 69.4±24.6 | *0.268* | *0.703* | *0.133* |
| Three Back RT (ms) | 1515.0±939.9 | 1156.7±383.0 | 1225.9±517.3 | 1183.5±677.2 | *0.275* | *0.112* | *0.415* |

RT- reaction time.



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**Table 7-8:** Measures of work productivity at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | Control | | Exertime | | *p-value* | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Typing Performance** | | | | | | | |
| Gross Speed (wpm) | 41.8±8.3 | 42.0±9.8 | 42.4±10.6 | 44.0±12.1 | *0.547* | *0.561* | *0.596* |
| Net Speed (wpm) | 38.9±7.6 | 39.3±9.1 | 38.9±9.3 | 40.6±10.1 | *0.659* | *0.449* | *0.492* |
| Accuracy (%) | 92.8±3.0 | 93.4±5.4 | 91.6±4.4 | 92.1±3.0 | *0.296* | *0.590* | *0.957* |
| **Mouse Dexterity** | | | | | | | |
| Performance Score | 1099.4±111.9 | 1115.1±89.2 | 1108.3±102.7 | 1124.1±116.0 | *0.530* | *0.088* | *0.996* |
| Reaction Time (ms) | 678.9±130.0 | 626.6±105.9 | 646.3±118.1 | 637.9±116.1 | *0.512* | *0.056* | *0.197* |
| **Reading and Correcting** | | | | | | | |
| Number of Characters Read | 2359.6±778.8 | 2825.5±644.3 | 2703.0±1031.7 | 2787.9±881.3 | *0.583* | *0.051* | *0.091* |
| Percent of Errors Missed (%) | 36.9±24.9 | 31.9±30.4 | 25.4±18.1 | 33.1±30.6 | *0.253* | *0.811* | *0.249* |
| **HWQ** | | | | | | | |
| Productivity | 7.5±1.9 | 7.3±1.5 | 7.3±1.6 | 7.5±1.8 | *0.953* | *0.985* | *0.412* |
| Concentration/Focus | 4.3±2.6 | 3.8±2.6 | 4.4±2.7 | 4.1±2.5 | *0.496* | *0.137* | *0.831* |
| Impatience/Irritability | 4.0±2.5[#] | 3.2±2.2 | 4.5±3.1[#] | 2.5±1.4 | *0.833* | *0.027* | *0.366* |
| Work Satisfaction | 7.5±1.7 | 6.7±2.0 | 7.0±2.0 | 7.7±1.6 | *0.500* | *0.863* | *0.184* |
| Stress | 5.6±2.8 | 4.6±2.4 | 5.6±2.9 | 4.4±2.2 | *0.825* | *0.104* | *0.866* |
| Supervisor Relations | 6.9±3.0 | 7.2±2.7 | 6.6±3.2 | 7.0±2.8 | *0.675* | *0.589* | *0.944* |
| Non-work Satisfaction | 8.5±1.4 | 8.5±1.1 | 8.3±1.4 | 8.0±1.9 | *0.205* | *0.462* | *0.590* |

HWQ- health and work questionnaire.

# Significant main effect for time (p=0.027), with PRE higher than POST.



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### 7.3.7. Brachial and Femoral Flow-Mediated Dilation

When analysed using general linear mixed models, a significant main effect for time was observed for femoral artery relative FMD (p=0.020), with FMD higher at POST (8.57±3.43%) compared to PRE (5.84±2.63%). However, after allometric modelling this main effect for time was no longer significant (p=0.062). There were no significant main effects observed for femoral artery baseline diameter, absolute FMD, or SR area under the curve (AUC) (p>0.05; Table 7-9). When analysed using both general linear mixed models and allometric modelling, there were no significant main effects observed for brachial artery baseline diameter, absolute or relative FMD, or SR AUC (p>0.05; Table 7-9).

### 7.3.8. Sleep

No significant main effects were observed for any parameters of sleep (p>0.05; Table 7-10).

**Table 7-9:** Brachial and femoral artery flow-mediated dilation (FMD) at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | Control | | Exertime | | *p-value* | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Brachial Artery** | | | | | | | |
| Baseline Diameter (cm) | 0.32±0.08 | 0.33±0.08 | 0.33±0.06 | 0.32±0.06 | *0.753* | *0.717* | *0.358* |
| FMD (%) | 6.5±3.2 | 8.0±4.2 | 8.4±3.7 | 7.5±4.6 | *0.361* | *0.817* | *0.329* |
| FMD (%) allometric modelling | 6.1±3.5 | 7.6±3.5 | 8.0±3.5 | 7.1±3.5 | *0.495* | *0.883* | *0.448* |
| Absolute FMD (cm) | 0.02±0.01 | 0.02±0.01 | 0.03±0.01 | 0.02±0.01 | *0.204* | *0.932* | *0.218* |
| SR AUC ($s^{-1}$ x $10^3$) | 22.53±13.92 | 26.50±10.58 | 28.00±19.10 | 27.47±14.33 | *0.279* | *0.730* | *0.575* |
| **Femoral Artery** | | | | | | | |
| Baseline Diameter (cm) | 0.59±0.16 | 0.60±0.13 | 0.61±0.13 | 0.59±0.13 | *0.680* | *0.260* | *0.886* |
| FMD (%) | 7.0±2.6 | 7.4±4.9# | 5.1±3.0 | 9.7±6.7# | *0.812* | *0.020* | *0.363* |
| FMD (%) allometric modelling | 6.5±4.3 | 7.6±4.0 | 4.7±4.0 | 9.6±4.0 | *0.943* | *0.062* | *0.210* |
| Absolute FMD (cm) | 0.04±0.02 | 0.04±0.02 | 0.03±0.01 | 0.05±0.03 | *0.908* | *0.069* | *0.121* |
| SR AUC ($s^{-1}$ x $10^3$) | 19.05±11.53 | 15.90±6.54 | 17.00±12.98 | 18.02±13.43 | *0.951* | *0.817* | *0.495* |

SR- shear rate; AUC- area under the curve.
# Significant main effect for time (p=0.020), with POST higher than PRE.

**Table 7-10:** Measures of sleep at the start (PRE) and following (POST) the 8-week Control and Exertime trials (Mean±SD).

| | **Control** | | **Exertime** | | ***p-value*** | | |
|---|---|---|---|---|---|---|---|
| | **PRE** | **POST** | **PRE** | **POST** | ***Condition*** | ***Time*** | ***Interaction*** |
| **Weekdays** | | | | | | | |
| Sleep Duration (hrs:mins) | 06:41±00:45 | 06:46±00:47 | 06:45±00:35 | 06:46±00:48 | *0.707* | *0.839* | *0.444* |
| Sleep Latency (hrs:mins) | 00:11±00:07 | 00:08±00:05 | 00:11±00:05 | 00:11±00:04 | *0.229* | *0.365* | *0.248* |
| Sleep Efficiency (%) | 85.7±6.2 | 86.6±5.3 | 84.9±7.0 | 85.2±5.8 | *0.193* | *0.361* | *0.866* |
| Fragmentation Index | 24.4±11.1 | 28.3±9.5 | 27.4±10.8 | 26.0±9.1 | *0.814* | *0.714* | *0.166* |
| **Weekend** | | | | | | | |
| Sleep Duration (hrs:mins) | 07:16±01:13 | 07:07±01:02 | 07:38±01:17 | 06:42±01:08 | *0.913* | *0.151* | *0.251* |
| Sleep Latency (hrs:mins) | 00:26±00:29 | 00:08±00:07 | 00:09±00:04 | 00:13±00:06 | *0.238* | *0.284* | *0.077* |
| Sleep Efficiency (%) | 81.6±9.2 | 83.6±8.1 | 85.2±6.7 | 83.2±7.2 | *0.278* | *0.978* | *0.300* |
| Fragmentation Index | 23.5±10.3 | 28.8±16.1 | 24.5±11.5 | 27.8±9.4 | *0.995* | *0.341* | *0.783* |

## 7.4. Discussion

This study assessed, for the first time, whether using Exertime, a computer-based prompting software to break up workplace sitting, could alter cerebrovascular function, cognition, mood and work productivity in healthy, university-based office workers. We have shown that workplace sitting was reduced after using Exertime, albeit it not a statistically significant reduction, and was replaced predominantly by increased time spent standing. We observed improvements in aspects of dynamic CA, but no changes in other measures of cerebrovascular function, cognition, mood or work productivity. These data provide preliminary support for further examination of the use of Exertime to reduce workplace sitting and improve some markers of cerebrovascular health. Whether longer-term use of Exertime has an impact on workers' physical and mental health and wellbeing should be further explored in more diverse populations of office workers.

This study was the first to use objective measures of SB and PA when using Exertime to assess the influence of the software on workplace activity patterns. Following the Exertime trial there was an average 42.2 minute reduction in sitting time during work hours, which equated to a 4.2% reduction in sitting when expressed as a percent of total work hours. Despite not reaching statistical significance, a reduction in sitting time of this magnitude may be clinically meaningful at a population health level. Additionally, by using objective PA and SB monitoring, we have been able to characterise the type of break participants used to break up their sitting. The observed reduction in sitting appears to have been replaced with increased standing rather than walking,

since there was a 4% increase in standing time following the trial, but only a 0.2% increase in stepping time. This apparent increase in standing time is slightly surprisingly considering participants were instructed to take a walk when the Exertime software initiated. This may indicate that participants' work environments were not suited to take walking breaks and they instead opted to stand. Furthermore, standing potentially allowed participants to stay in the vicinity of their desk, which may be less disruptive to work tasks such as using the phone.

Participants completed 73.6% of the breaks logged in Exertime. When using Exertime, participants reported an average 7.6 additional minutes per day taking breaks from sitting, which was achieved by an average of 6.1 activity breaks per work day. This is comparable to previous studies using Exertime with office workers which report an additional 7.51-7.99 minutes of additional activity per work day achieved by taking breaks 4.95 to 6.28 times per day (Mainsbridge et al., 2014, 2016; Pedersen et al., 2014). Importantly, the comparison between Exertime data and objective postural monitor data showed there were no significant differences between the duration of recorded breaks time. Collectively, these data suggest Exertime software may be a valid and effective method to reduce workplace sitting, which larger scale, longer-term studies should explore.

The reductions in time spent sitting at work using Exertime appear to have enhanced aspects of cerebrovascular function. The Exertime condition had a higher phase compared to the control condition following the trial period,

indicating enhanced buffering capacity of CA (Panerai, 2009). This is in line with data from Chapter 6, showing frequent walking breaks from sitting leads to enhanced phase compared to uninterrupted sitting. The improvement in phase in this study is likely due to increased standing time rather than walking, highlighting that simply a postural change from sitting when taking a break, rather than the mode of activity during the break, may be the important aspect to improve markers of cerebrovascular function, however further research is needed to confirm this hypothesis. The mechanisms underlying the increase in phase are unclear. Changes in CBF precede changes in BP, termed phase lead, as a protective mechanism to minimise the acute effects of a change in posture (van Beek et al., 2008). Hence, as participants were altering their posture more frequently by standing up to break up their sitting, the cerebrovasculature may have enhanced its ability to respond to the corresponding change in BP. It is also proposed that autonomic nervous activity contributes to CA, since phase lead decreases following ganglion blockade (Zhang et al., 2002). Heightened sympathetic nervous system activity during standing or walking breaks may have therefore enhanced phase.

In Chapter 6, increases in CBF were observed when four hours of sitting was regularly interrupted with walking breaks. The lack of change in CBF in this current study suggests this does not translate into a longer-term effect over a period of months. Nonetheless, the 8-week time-frame used in this study is not representative of a full-time workers annual exposure to prolonged sitting, hence a long-term intervention may still elicit chronic changes in CBF. Alternatively, since participants appear to have predominantly increased their

standing time to break up their sitting this may suggest ambulation is needed to positively affect CBF. The lack of chronic change in CBF despite acute improvements observed in Chapter 6, could also provide indication of the cerebrovascular regulatory mechanisms adapting to the activity break stimuli. Indeed, in the peripheral vasculature, despite initial improvements in artery function across the first weeks of exercise training, this improvement in function returns towards baseline, most likely due to subsequent arterial remodelling and chronic adaptation to the exercise stimuli (Tinken et al., 2008, 2010). A similar response could be present in the cerebrovasculature, with initial improvements in CBF returning to baseline following an adaptation to the PA breaks. Indeed, CBF did not increase following a 6-month aerobic exercise training in chronic stroke patients (Ivey et al., 2011), whilst in healthy young and old adults, CBF remained unchanged following a 12-week aerobic exercise training programme, with a reported increase only occurring after correcting data for post-training hypocapnia (Murrell et al., 2013).

The lack of effect on CVR is in line with results from Chapter 5 and Chapter 6 demonstrating no change in CVR following acute sitting. However, in Chapter 6 breaking up this time with walking breaks did acutely improve MCA and CCA CVR compared to sitting and such a beneficial effect of PA was not observed in this study. In the longer term, a 12-week aerobic exercise training programme also increased CVR (Murrell et al., 2013). This difference in our results may relate to the intensity of the activity, since when using Exertime participants appear to have broken up their sitting with standing, which is of a much lower intensity than the exercise used in these previous studies. Exercise and

training-induced improvements in CVR are suggested to be in part due to increased NO production, since exercise elevates its bioavailability (Green et al., 2004) and cerebrovascular vasodilatation in response to increased $CO_2$ is partially dependent upon NO (Murrell et al., 2013). Increased blood flow stimulates NO production (Green et al., 2004), hence breaking up sitting with low-intensity standing, as in this study, may not have caused a large enough blood flow response to augment sufficient amounts of NO to enhance CVR.

Despite the Exertime trial appearing to enhance aspects of cerebrovascular function, cognition and mood were unchanged. Previous studies have also observed no effect on cognition when using PA breaks to interrupt sitting (Bergouignan et al., 2016; Duvivier et al., 2017; Wennberg et al., 2016), however these have been acute in design, lasting a maximum of four days. In contrast, our study shows that eight weeks of reducing workplace sitting also has no influence on cognition. This suggests the magnitude of the improvement in cerebrovascular function may not have been a large enough stimulus, or that eight weeks is still a too acute period to alter cognition. Additionally, it may indicate that other factors linked to cognition, such as diet (Spencer et al., 2017), have a stronger influence on cognitive performance. In contrast, previous acute studies have observed increased mood state using PA to break up sitting periods (Bergouignan et al., 2016; Duvivier et al., 2017). However, in these studies walking has predominantly been used as the PA intervention, either by using treadmill breaks or by increasing total walking time. As participants in our study mainly substituted sitting with standing, it may indicate the intensity was not a large enough to stimulus to enhance mood.

Exertime also had no influence on markers of cardiovascular health, with FMD, HR and MAP all unchanged compared to the control trial. Previously, following a thirteen week intervention using Exertime, MAP was significantly reduced compared to a control group (Mainsbridge et al., 2014). Our cohort of workers however had a baseline MAP nearly 20 mmHg lower than in Mainsbridge et al. (2014), therefore the health status of our population may explain this disparity in our findings. The lack of a statistically significant change in femoral FMD following Exertime compared to the control trial contrasts previous work showing breaking up 3-hrs of sitting with walking bouts acutely enhances femoral endothelial function (Thosar et al., 2015), however the acute study design rather than chronic intervention as in our study may explain this difference. Furthermore, since participants appear to have stood up rather than walked to break up their sitting, it may contribute to this disparity in findings. Nonetheless, the 4.6% improvement in femoral FMD observed following Exertime, although statistically not significant, may have important health implications since a 1% decrease in FMD is associated with a 13% higher risk of a future cardiovascular event (Inaba et al., 2010). Whilst likely beneficial improvements in brachial FMD were observed after office workers used a sit-stand workstation for 8-weeks (Graves et al., 2015), in this study, a slight reduction in brachial FMD was observed following the Exertime intervention, albeit not statistically significant. Sit-stand workstations permit an individual to continue working whilst standing, such as using a computer, whilst in our study when participants stood, this would not have been possible. Upper limb movements during sitting are suggested to prevent sitting-induced impairment

in endothelial function (Thosar et al., 2014), hence the absence of such movements when standing may explain the lack of improvement in our study. Collectively, this indicates that standing alone may be sufficient to elicit improvements in artery function, but the activities performed during the standing may contribute to this response.

Recently the dearth of research assessing breaking up sitting and its influence on sleep has been highlighted (Vincent et al., 2017). Short sleep is associated with negative changes to many of the cardiometabolic markers that are positively influenced by breaking up sitting, emphasising sleep should be controlled for so not to confound findings (Vincent et al., 2017). Importantly in this study, sleep was assessed prior to and at the end of each trial period and no significant differences were observed in sleep duration. A small body of work has begun to examine the influence of breaking up sitting on sleep quality, however only in sleep restricted individuals (Vincent et al., 2018). Improvements in sleep quality have been observed when sitting is broken up with walking breaks, however this had no influence on cognition (Vincent et al., 2018). Although sleep quality was unchanged in our population of healthy sleepers, using Exertime with a larger sample size, over longer intervention period, or with a population of sleep-restricted workers such as shift workers, may alter sleep parameters, and this should be explored.

### 7.4.1. Limitations

The small sample size in this study is a limitation, however the work was pilot in nature since it was the first use of Exertime software in a UK workforce.

Furthermore, the data presented in this thesis is a sub-sample of the final study population, since participants were still completing the Exertime intervention at the time of thesis submission. The population assessed were all employees from a University, meaning the use of the Exertime software and compliance may differ in different worksites and professions. For the purpose of this thesis, the activity that participants could select to complete when using the Exertime software was limited to taking a walk, whereas full use of Exertime includes a range of activity modalities. Despite, instructing participants to take a walk, the increased standing time observed following the Exertime intervention suggests this was not achievable or adhered to by all participants, however we do not have the qualitative data to elucidate the reasons behind this. The lack of activity break choice may have reduced participant engagement with the software. It is also possible that seasonality may have influenced participants' PA and SB levels, since levels can vary depending on the season when the assessment occurs (O'Connell et al., 2014). The use of TCD to assess MCAv and cerebrovascular function is associated with known limitations, including the inability to measure actual blood flow (Willie et al., 2011), the assumptions that measures from the MCA are representative of other cerebral vessels and that MCA diameter is unaltered during varying levels of $CO_2$ (Skow et al., 2013). By recording the signal parameters and photographically recording the TCD probe placement, it was ensured as closely as possible the probe was in the same location; small variations may have occurred, however our coefficient of variation was 7.8% indicating good reproducibility. Finally, the analysis of CA using TFA is a developing method and lacks references values, however we

have collected and analysed data based on current guidelines (Claassen et al., 2016).

## 7.5. Conclusion

This pilot study demonstrates using a computer-based prompting software for eight-weeks may reduce workplace sitting in a cohort of university desk workers. Furthermore, aspects of dynamic CA were improved after using the software, but there was no influence on cognition, mood or work productivity. Workplace sitting was replaced with increased standing time rather than walking, suggesting the intensity of the breaks from sitting may not have been large enough to enhance these parameters. Further research with a larger sample size and longer intervention period is needed to examine if longer-term use of Exertime has an impact on workers' sitting time, cerebrovascular function, cognition, mood and work productivity.

# 8. Synthesis of Findings

## 8.1. Aims and Objectives

The main aims of this thesis were to explore the acute effects of prolonged, uninterrupted sitting on cerebrovascular function, cognition and mood, and whether breaking up prolonged sitting with short bouts of light-intensity PA could alter these parameters in healthy but sedentary adults. Additionally, this thesis aimed to assess the relationship between workplace SB, cognition and mood, and to investigate whether an 8-week intervention designed to break up prolonged sitting in an office-based workplace would affect cerebrovascular function, cognition and mood.

## 8.2. Major Findings

### 8.2.1. Workplace Sedentary Behaviour, Cognition and Mood

Given that office workers spend 65–75% of their work hours sitting, recent guidelines suggest reducing workplace SB could improve employee health and wellbeing, as well as their productivity (Buckley et al., 2015). However, there is currently no data supporting these guidelines to suggest that workplace SB influences or is correlated with factors contributing to productivity, such as cognition and mood. Consequently, Chapter 4 assessed if there was a relationship between cognition, mood and the time spent sitting, stepping or standing whilst at work as well as during a weekday and a weekend. Results showed that sitting whilst at work was not associated with cognition (attention, working memory and executive function), and this result also extended to weekdays and the weekend. Workplace sitting was however negatively associated with the calm mood state. Individuals that are less calm may exhibit a heightened stress response (Klaperski et al., 2013). Chronic work stress is

related to increased risk of CVD morbidity and mortality (Chandola et al., 2006; Kivimäki et al., 2002; Kivimäki and Kawachi, 2015) and mental health conditions (Harvey et al., 2017). In the long term, sitting at work may therefore have negative implications for employee health and well-being. In contrast, increased time spent standing and stepping at work were associated with improved mood and cognitive performance. Importantly, workers who are in a positive mood have enhanced work performance (Miner and Glomb, 2010; Rothbard and Wilk, 2011), indicating PA during work hours could be beneficial for work productivity. Further analyses revealed that during work hours only moderate-intensity PA was positively associated with working memory and attention, suggesting this intensity of PA is needed to positively influence cognitive performance. This observation is important since current workplace guidelines recommend replacing SB with light activity to improve workers' health and productivity (Buckley et al., 2015). This may indicate that low-intensity PA during work hours may not have a beneficial effect on cognition and that recommending moderate-intensity PA may be the most effective way to elicit improvements in workers' cognitive performance and their subsequent productivity. However, whether it would be feasible for sedentary workers to complete this intensity of PA within the constraints of their work environment is unclear. Further research is therefore required investigating differing intensities of PA breaks on cognition and mood, especially in a work environment, which can then provide evidence to support or modify guidelines for a healthy workplace.

### 8.2.2. Prolonged Sitting, Cerebrovascular Function, Cognition and Mood

*8.2.2.1. Prolonged Sitting and Cerebrovascular Function*

Previous research indicates that acute periods of prolonged sitting causes peripheral artery endothelial dysfunction (Restaino et al., 2015; Thosar et al., 2015), however whether other vascular beds are also influenced by prolonged sitting is currently unknown. Since the delivery of CBF is vital for normal brain function and survival (Willie et al., 2011), effective cerebrovascular function is essential for maintaining constant cerebral perfusion (Willie et al., 2014), preventing cognition decline (Wolters et al., 2017) and impaired mood (Evans et al., 2017). Owing to this critical role in preserving brain health and function, understanding the potential impact of SB on cerebrovascular function is needed. Hence in Chapter 5 and Chapter 6, the aim was to examine whether acute, prolonged sitting impairs cerebrovascular function.

For the first time, this thesis presents data showing that CBF is acutely reduced following four and six hours of uninterrupted sitting (Chapter 6 and Chapter 5). Interestingly, a dose-response effect of sitting appears to occur, with the reduction in CBF increasing in magnitude as sitting time increases. Following four hours of uninterrupted sitting, MCAv was decreased by 1.4 $cm \cdot s^{-1}$, whilst uninterrupted sitting for six hours reduced MCAv by 3.4 $cm \cdot s^{-1}$. If these data are compared to the age-related decline in MCAv of 0.76 $cm \cdot s^{-1}$ per year (Ainslie et al., 2008), it indicates the transient reduction observed following a single uninterrupted sitting period may equate to 2-4 years of age-related decline. Importantly, transient reductions in CBF impairs cognitive performance (Marshall et al., 2001), demonstrating the findings in this thesis may have acute

implications for individuals who sit for prolonged periods. However, further research is required to assess whether this transient reduction in CBF may contribute to a chronic reduction, as this would have meaningful implications for the development of cerebrovascular diseases.

Exploring the mechanisms underlying the sitting-induced reduction in MCAv were beyond the scope of this thesis but may relate to the sympathetic nervous system. Prolonged sitting elevates muscle sympathetic nerve activity (Ray et al., 1993) and heightened sympathetic activity causes cerebral vasoconstriction (Seifert and Secher, 2011), therefore sitting may induce transient global vasoconstrictor effects on the vasculature. Alternatively, changes in cerebral glycaemic regulation may also contribute to sitting-induced reductions in MCAv, as the brain is highly sensitive to perturbations in circulating glucose levels. Indeed, prolonged sitting increases postprandial glycemia (Dunstan et al., 2012; Peddie et al., 2013), which can cause microvascular damage, impair endothelial function and reduce CBF (Wheeler et al., 2017). Furthermore, the decline in MCAv may relate directly to the function of cerebrovascular endothelial cells, which contribute to the regulation of CBF (Toda, 2012), since elevated levels of tissue plasminogen activator and Von Willebrand factor, markers of endothelial dysfunction, are associated with reduced CBF in older adults (Sabayan et al., 2014).

A common observation across both Chapter 5 and Chapter 6 was that CVR was unaffected following acute prolonged sitting periods. Since $CO_2$ is considered the main regulator of CBF (Willie et al., 2011), it is plausible that the

cerebrovasculature may exhibit an enhanced capability to preserve this function and resist any deleterious effects of sitting. Impairments in CVR are observed in Alzheimer's disease patients, however the mechanisms underlying this decrease likely relate to factors such as β-amyloid accumulation in the brain, atherosclerosis and anatomical changes to the microvasculature (Glodzik et al., 2013). These factors develop chronically, thus would not be evident following an acute period of prolonged sitting in healthy adults. Therefore, if sitting does impair CVR, it may only be evident after chronic exposure to prolonged sitting.

Nonetheless, this thesis presents data showing for the first time that aspects of dynamic CA are impaired following uninterrupted sitting. In Chapter 6, following four hours of uninterrupted sitting, an acute reduction in phase, the synchronicity between BP and CBF responses, in the VLF range was observed indicating a less efficient CA. In Chapter 5, after six hours of uninterrupted sitting an increase in normalised gain, the damping effect of CA on the magnitude of BP oscillations, in the VLF range was observed, indicating impaired CA. These data present two interesting observations; firstly, different parameters of CA were impaired depending on the period of uninterrupted sitting, and secondly, both impairments were only observed in the VLF range of CA.

The differential impairment in CA parameters between the duration of the uninterrupted sitting period may relate to a potential progressive impairment of CA. It is suggested that impairment of autoregulation first affects phase, or the latency of response, before affecting gain, or the efficiency of the response (Tiecks et al., 1995; Wright et al., 2018). In support, following a concussion

injury, phase is impaired up to two weeks following the injury, with no change in gain during this time period (Wright et al., 2018). Whilst in this thesis the time difference between the sitting periods in Chapter 5 and Chapter 6 is only two hours, the reduction in phase after four hours of sitting and the reduction in normalised gain after six hours may further support that the impairment of CA is based on the duration of the exposure to the negative stimuli.

The lack of changes observed in the low and high frequency ranges of CA is in line with previous data examining the effects of high altitude exposure on dynamic CA which observed reduced phase and increased gain but only in the VLF range (Iwasaki et al., 2011). It is suggested that dynamic CA in the VLF is partially modulated by the autonomic nervous system (Zhang et al., 2002), since gain increased and phase decreased in the VLF but not at the higher frequency ranges following ganglion blockade. Furthermore, although several other mechanisms are suggested to contribute to CA, such as myogenic and cholinergic factors, it is has been shown that neurogenic influences are mainly responsible for the homeostatic maintenance of the relationship between BP and CBF (Hamner and Tan, 2014). It must however be noted that when modelling the relative contributions of the mechanisms underlying CA, 38% remained unexplained, suggesting a potential role for other physiological mechanisms such as NO production contributing to CA (Hamner and Tan, 2014). In peripheral vessels, sitting-induced impairments in vascular function are suggested to be partly due to reduced blood flow and endothelial NO production (Carter et al., 2017). Hence, a similar mechanism may have been present in the cerebrovasculature with the reductions in CBF observed in

Chapter 5 and Chapter 6, reducing SR and the release of local vasodilators such as NO, which in turn contributed to the subsequent impairment in CA.

*8.2.2.2. Prolonged Sitting and Cognition*

Based on previous suggestions that sitting is a risk factor for cognitive decline due to impairments in CBF (Wheeler et al., 2017), Chapter 5 aimed to assess if an acute, prolonged sitting period impaired cognition and, if changes in cognition were observed, whether these were related to changes in cerebrovascular function. However, following six hours of uninterrupted sitting, cognition (attention, working memory and executive function) was not acutely impaired, despite concurrent reductions in CBF and dynamic CA. This supports the cross-sectional observations in Chapter 4, where SB was not associated with cognition in any domain. Collectively these data indicate that in young healthy adults, SB does not impact cognition, however the cross-sectional and acute study designs used in this thesis do not provide sufficient data to conclude this. Chronically, decrements in CBF lead to a breakdown of the blood brain barrier, neuronal damage and amyloid β accumulation which may cause neurodegeneration and cognitive impairment (Wheeler et al., 2017). The suggestion that sitting is a risk factor for cognitive decline (Wheeler et al., 2017) may therefore be the result of the repeated exposure to acute reductions in CBF, which over time lead to structural changes in the brain, subsequently impairing cognition. Hence, whether chronic exposure to the transient reductions in CBF observed acutely in Chapter 5 and Chapter 6, result in impaired cognition should be explored further.

*8.2.2.3. Prolonged Sitting and Mood*

Recent guidelines suggest reducing workplace SB may improve employee productivity (Buckley et al., 2015) and worker mood is related to work performance and work efficiency (Kaplan et al., 2009; Shockley et al., 2012). Importantly, experimentally increasing SB decreases mood (Edwards and Loprinzi, 2017b; Endrighi et al., 2016), however the mechanisms underlying this are unknown, but may relate to cerebrovascular function owing to its own relationship with mood (Evans et al., 2017; Honda et al., 2014; Nobler et al., 2002; Videbech, 2000). Consequently, Chapter 5 aimed to investigate if an acute, prolonged sitting period impaired mood and, if changes in mood were observed, whether this was related to any observed changes in cerebrovascular function.

Following uninterrupted sitting for six hours, positive affect and the alert and content mood states were decreased. This is an important finding since a six-hour time period could replicate an adult's working day and may therefore have implications for worker productivity, since when workers' mood is lowered their work-specific task performance reduces (Miner and Glomb, 2010). Furthermore, in the long-term, repeated sitting-induced decreases in mood may have repercussions for workers mental health and well-being, a suggestion supported by previous research indicating that SB is associated with anxiety (Teychenne et al., 2015) and depression (Zhai et al., 2015). However, these data contrast with results from the cross-sectional observations in Chapter 4 showing workplace sitting was not associated with these mood outcomes. This disparity in findings may be explained by the single day assessment period in a laboratory setting in Chapter 5 whilst in Chapter 4 participants SB was

assessed over five days of work, thus could be more susceptible to other external factors influencing mood state.

Interestingly the observed reduction in mood in Chapter 5 was not related to the concurrent impairments in cerebrovascular function, indicating that other mechanisms underlie acute sitting-induced mood impairments. It has been suggested that heightened inflammation may contribute to sitting-induced decreases in mood (Endrighi et al., 2016). Indeed, SB is associated with higher levels of C-reactive protein (Howard et al., 2015) and other markers of low-grade inflammation (Yates et al., 2012). Importantly, markers of inflammation are implicated in mood disorders (Rosenblat et al., 2014) and negative mood state (Wright et al., 2005). Indeed, it is suggested that that inflammatory markers, such as interleukin-6 and tumour necrosis factor, act on multiple levels to induce mood symptoms, including deceasing serotonin levels and activating microglia cells leading to synaptic pruning and neuronal death (Rosenblat et al., 2014). Consequently, further research is needed examining prolonged sitting, mood and inflammation. Alternatively, other reasons for the reduction in mood following sitting observed in this thesis may relate to the laboratory setting used for testing and participant boredom. Future research in an environment more representative of an office would explore this theory.

### 8.2.3. Breaking Up Sitting, Cerebrovascular Function, Cognition and Mood

#### *8.2.3.1. Breaking Up Sitting and Cerebrovascular Function*

Based on the findings in Chapter 5, showing sitting acutely impairs cerebrovascular function, Chapter 6 investigated if walking breaks to interrupt

prolonged sitting periods could attenuate this reduction in function. Furthermore, two different walking break strategies were assessed: frequent, short duration walks (two-minute walking break every thirty minutes) and less frequent, longer duration walks (eight-minute walking break every two hours). For the first time, this thesis presents data showing that short duration, regular walking breaks, rather than less frequent, longer duration walking breaks prevented the acute reduction in MCAv and the impairment of the VLF phase metric of dynamic CA that otherwise occurred during uninterrupted sitting for four hours. This implies the frequency of the breaks may be more important than the duration of the PA to maintain aspects of cerebrovascular function. In contrast, both walking break strategies caused a larger increase in CVR compared to prolonged sitting. This indicates that, for this aspect of cerebrovascular function, any duration or frequency of PA may have acute benefits.

Following on from these acute observations, Chapter 7 investigated whether an eight-week intervention designed to break up sitting over a longer time period, thus a more ecological representative of adults' typical exposure to sitting, could enhance cerebrovascular function. Furthermore, the intervention took place in office workplaces, where SB is typically accrued. Using a computer-based prompting software called Exertime, participants were prompted to take a break from sitting every 45-minutes during their work hours. Based on the results from Chapter 6, participants were encouraged to complete two minutes of walking during these breaks.

Interestingly, analysis of participants' usage of the Exertime software showed that there was a non-significant, but potentially clinically meaningful, reduction in workplace sitting which appeared to be replaced by a non-significant increase in the time spent standing rather than walking. In support of the results from Chapter 6, the VLF phase metric of CA improved following the use of Exertime software compared to the control condition. Intriguingly, although the intensity of the standing activity was less than that of the walking breaks used in Chapter 6, VLF phase still improved. This suggests it is the frequency at which sitting it broken up rather than the type or intensity of PA used that is important for maintaining this aspect of cerebrovascular function. This observation is in line with previous research showing the pattern in which SB is accumulated impacts a range of cardiometabolic risk factors. Individuals who accumulate their SB in long, uninterrupted bouts have a worse cardiometabolic risk factor profile than individuals with the same total SB, but who regularly interrupt this sedentary time with PA bouts (Healy et al., 2008; 2011). In contrast to the acute observations in Chapter 6, CVR was not improved following the use of Exertime software. In this study, participants appeared to replace their sitting time with standing, whereas in Chapter 6, participants completed walking breaks to interrupt sitting. This may indicate higher intensity activities are needed to improve this aspect of cerebrovascular function.

Breaking up sitting influenced only the phase metric of dynamic CA in Chapter 6 and Chapter 7 and not gain. Improved phase indicates enhanced buffering capacity of CA to changes in BP (Panerai, 2009). Hence, as participants were altering their posture more frequently by either standing up or walking to break

up their sitting, the cerebrovasculature may have enhanced its ability to respond to the corresponding change in BP. Since it is suggested that impairments in phase occur before gain (Tiecks et al., 1995; Wright et al., 2018), a similar pattern of response may occur for improvements in these parameters of CA. Therefore, the duration of the intervention periods in Chapter 6 (four hours) and Chapter 7 (eight weeks) may not have been long enough for improvements in gain to occur. There is a dearth of research examining interventions to improve CA that can support this hypothesis, therefore future research is needed examining this possibility.

In contrast, the acute improvement in MCAv observed in Chapter 6 using regular walking breaks to interrupt sitting was not replicated in Chapter 7, as there was no change in MCAv following the Exertime intervention compared to the control trial. This indicates that ambulatory breaks or a longer intervention period may be required to result in a chronic improvement in CBF. It could also be possible that the cerebrovasculature may have adapted to the PA break stimuli. In the peripheral vasculature, despite initial improvements in artery function across the first weeks of exercise training, this improvement then returns towards baseline, possibly due to subsequent arterial remodelling and chronic adaptation to the exercise stimuli (Tinken et al., 2008, 2010). A similar response could be present in the cerebrovasculature, with initial improvements in CBF returning to baseline following an adaptation to the PA breaks. Indeed, following 12-week and 6-month aerobic exercise training programmes, CBF was not increased in stroke patients (Ivey et al., 2011) and healthy young and

old adults (Murrell et al., 2013). Further research is therefore needed to explore this hypothesis.

#### *8.2.3.2. Breaking Up Sitting and Cognition*

In Chapter 6 acutely breaking up sitting increased aspects of cerebrovascular function. Since cerebrovascular function influences cognitive performance (Bertsch et al., 2009; Marshall et al., 2001), Chapter 7 investigated whether breaking up sitting over a period of 8-weeks could improve cognition. However, following the Exertime software intervention no improvements in cognition (attention, working memory and executive function) were observed. This indicates that relatively small increases in standing may be insufficient to impact cognition. Previous studies have also observed no effect on cognition when using PA breaks to interrupt sitting (Bergouignan et al., 2016; Duvivier et al., 2017; Wennberg et al., 2016), however these have been acute in design, lasting a maximum of four days. Since an 8-week intervention also had no influence on cognitive function it suggests changes to cognition may occur over longer time periods. In support, improvements in CBF and cognition have been observed following short-term exercise training (Chapman et al., 2013), however this was 12-weeks in duration and used walking or cycling, thus a greater activity stimulus than standing.

#### *8.2.3.3. Breaking Up Sitting and Mood*

Improvements in mood have been previously observed when SB is acutely reduced (Bergouignan et al., 2016; Duvivier et al., 2017). Cerebrovascular function contributes to the maintenance of mood (Evans et al., 2017) and, as observed in Chapter 6, breaking up sitting can improve cerebrovascular

function. Consequently, interventions to reduce sitting may also increase mood via enhancing cerebrovascular function. Hence, Chapter 7 investigated whether breaking up sitting over a period of 8-weeks could improve mood. However, no changes in mood were observed following the Exertime intervention, implying replacing sitting time with standing is insufficient to effect mood. This finding is supported by the cross-sectional observations in Chapter 4 assessing relationships between sitting, standing, stepping and mood. Work hours stepping was positively associated with positive affect and the calm and contented mood states, whilst standing was not related to any aspects of mood. Collectively, data indicates that sitting may represent a risk factor for decreased mood and that engagement in PA is needed when breaking up sitting to potentially ameliorate this risk. However, whether it would be feasible for sedentary workers to complete PA within the constraints of their work environment is unclear.

## 8.3. Implications of Findings

### 8.3.1. Workplace Sedentary Behaviour and Work Productivity

A less sedentary workplace has been suggested to increase worker productivity (Buckley et al., 2015), yet there is no data to support this recommendation. In Chapter 4 the time spent stepping at work was associated with increased mood, whilst in Chapter 5 an acute prolonged sitting period, replicating office workers' typical daily exposure, impaired mood. Since workers' mood influences their performance (Kaplan et al., 2009; Miner and Glomb, 2010; Rothbard and Wilk, 2011; Shockley et al., 2012), these data provide some initial evidence to support further exploration of the hypothesis that the promotion of PA during work hours and the reduction of SB may increase worker mood and potentially productivity. If proven correct, this indicates that employers could look to encourage their workforce to reduce their SB by taking PA breaks and consider developing a work environment and work culture that allows this to occur.

Current workplace SB guidelines recommend replacing sitting with standing and light-intensity PA to improve worker's health and productivity (Buckley et al., 2015). Data from this thesis however suggest this may not be the optimal intensity to improve cognition. Chapter 4 showed that moderate-intensity PA was positively associated with cognition and not light-intensity PA. Furthermore, standing during work hours was not associated with any aspects of mood. In support of this, in Chapter 7, standing to break up workplace sitting did not result in any changes in mood. However, whether is it feasible for workers in highly sedentary professions to perform moderate-intensity PA at work needs to be considered due to restrictions in work environments, such as contact centre

employees who are not permitted to leave their desk, or perceptions about being active in front of colleagues, for example in shared office spaces. In contrast, results from Chapter 6 and Chapter 7 suggest it is the frequency at which sitting is broken up, rather than the type or intensity of PA used, that is important for maintaining cerebrovascular function, since using both standing and walking to interrupt sitting were effective break modalities. Hence, in work environments where performing moderate-intensity PA is not suitable, this indicates it could be encouraged to break up sitting with any type of PA as this may still confer health benefits to the cerebrovasculature. Collectively, less generic workplace SB guidelines may be required to factor in the differential intensities and frequencies of PA needed to promote worker health and mental wellbeing.

To gain the potential health benefits of breaking up sitting demonstrated throughout this thesis, an effective intervention strategy is needed. In Chapter 7 Exertime software was used as the workplace intervention, designed to prompt workers to take a break from sitting at their desk every 45-minutes. Prior to this thesis, Exertime software had previously been used successfully in Tasmania to reduce workplace SB (Cooley and Pedersen, 2013; Mainsbridge et al., 2014, 2016; Pedersen et al., 2014). This thesis therefore demonstrates Exertime software may also contribute to reducing sitting in UK workplaces within a University setting. The number and duration of breaks recorded using Exertime were comparable to previous studies using the software in Tasmanian workplaces (Mainsbridge et al., 2014, 2016; Pedersen et al., 2014). By, for the first time, objectively assessing participants' SB prior to and when using the

Exertime software, a 42.2 minute or 4.2% reduction in workplace sitting was observed. Whilst this was not statistically significant, this provides promising data to indicate Exertime may contribute to reducing sitting time at work. Importantly, a systematic review and meta-analyses of workplace intervention strategies observed a pooled intervention effect of 40-minute reductions in sitting time per day, which is in line with our Exertime data (Chu et al., 2016). Furthermore, multi-component interventions have been shown to be particularly effective at reducing workplace sitting (Chu et al., 2016), likely due to the ecologic model of SB which outlines there are multiple levels of influence on SB including individual, social and environmental (Owen et al., 2011). Exertime was a multi-component intervention, combining the computer-based prompting software with a pre-intervention educational booklet and videos, and weekly motivational emails, thus targeting different levels of influence. However, as previously outlined, since participants appeared to stand to break up their sitting rather than the walking as was suggested, it could indicate Exertime does not consider environmental factors within a workplace that can determine workers' SB and their ability to reduce this behaviour. Altogether, this thesis provides preliminary evidence for Exertime to be employed in other workplaces in the UK and further afield, and future larger-scale studies should investigate the effectiveness of Exertime as a workplace intervention.

### 8.3.2. Sedentary Behaviour as a Cerebrovascular Health Risk Factor

Prior to this thesis, research had only focused on the influence of SB on the peripheral vasculature (Restaino et al., 2015; Thosar et al., 2015). This thesis shows, for the first time, that prolonged sitting also has a deleterious effect on

cerebrovascular function (Chapter 5 and Chapter 6), which may have clinical importance for both cognition and disease risk. Owing to the relationship between cerebrovascular function and neurodegenerative diseases (Gommer et al., 2012; Roher et al., 2012; Wolters et al., 2017), this indicates SB may be a risk factor for cerebrovascular health and therefore may have implications for the prevention of diseases such as dementia and Alzheimer's disease. This is of critical importance since the prevalence of dementia is increasing worldwide. Deaths due to dementia more than doubled between 2000 and 2016, making it the 5$^{th}$ leading cause of global deaths in 2016, compared to 14$^{th}$ in 2000 (World Health Organization, 2018). Hence, identifying interventions to decrease the risk of dementia is imperative, and reducing sitting time could represent a potential, low cost option to contribute towards this, and further prospective research is needed to explore this.

### 8.3.3. Sedentary Behaviour Guidelines

Recently, it has been stated that the current SB evidence base is underdeveloped to provide quantitative guidelines regarding sitting less and breaking up prolonged sitting periods, and that further research is required before research-informed guidelines are produced (Stamatakis et al., 2018). Data from Chapter 6, provides a small contribution to this by showing that more regular, shorter duration PA breaks compared to less regular, longer duration PA breaks are needed to prevent sitting-induced impairments in cerebrovascular function; a hypothesis supported by previous findings examining the influence of break frequency on cardiometabolic health risk factors (Healy et al., 2008; 2011). Further data would be needed showing a regular break strategy improves long-term cerebrovascular health and function

before inclusion in guidelines, but this initial work provides a basis for further research.

## 8.4. Future Research

### 8.4.1. Mechanistic Research

Whilst this thesis presents data showing impairments in cerebrovascular function with sitting and improvements when this sedentary time is broken up with PA breaks, the underlying mechanisms for these observed results are unclear. The decrease in CBF following sitting observed in Chapter 5 and Chapter 6 could possibly relate to increase sympathetic nerve activity, hence studies including measures of sympathetic activation, such as microneurography, circulating levels of plasma noradrenaline, or heart rate variability could explore this further. Alternatively, in Chapter 6 it is suggested that endothelial dysfunction of cerebral vessels or impaired glycaemic regulation may also contribute to reductions in CBF. Experimental work combining measures of CBF alongside blood glucose concentrations and makers of endothelial dysfunction, such as markers of inflammation, tissue plasminogen activator and Von Willebrand factor, would investigate these theories.

The improvements in CBF seen after breaking up sitting in Chapter 6 may relate to sustained cholinergic activity due to the frequency of the walking breaks. Acetylcholine blockade abolishes exercise-induced increases in CBF (Seifert et al., 2010), therefore a similar study could be conducted investigating the impact of acetylcholine blockade during regular walking breaks to interrupt sitting. The mechanisms underlying the improvement in the phase metric of dynamic CA after breaking up sitting time in Chapter 6 and Chapter 7, and the increase in normalised gain in Chapter 5 should also be explored. It is suggested that sympathetic activity, endothelial NO production and myogenic factors all

contribute to CA (Tzeng and Ainslie, 2014; Xiong et al., 2017), consequently studies either measuring or manipulating these variables could assess these theories.

In Chapter 5, a single, period of prolonged sitting decreased aspects of mood and this reduction was not related to the observed impairments in CBF and dynamic CA, suggesting other mechanisms were involved, possibly relating to inflammatory markers. Hence future research should assess changes in mood following prolonged sitting and include markers of inflammation. Previously SB has been associated with higher levels of C-reactive protein (Howard et al., 2015) and adipokines linked to low-grade inflammation (Allison et al., 2012; Yates et al., 2012), suggesting these markers should be assessed.

### 8.4.2. Physical Activity Break Modality

Chapter 6 demonstrated using walking PA breaks to interrupt prolonged sitting could enhance aspects of cerebrovascular function. Walking breaks were chosen based on previous research showing this break modality was effective at improving other cardiometabolic health parameters, such as enhancing glucose and insulin concentrations (Bailey and Locke, 2015; Dunstan et al., 2012). However, whether this can be translated from a controlled, laboratory setting to real-world workplaces is unclear. Indeed, as evidenced in Chapter 7, even though participants were instructed to take a walking break when using the Exertime prompting software, it appears they instead replaced their sitting with standing. This indicates either their workplace was not suitable to take walking breaks, or they wanted to remain near their desk. However, in this

Chapter, this apparent increase in time spent standing did enhance aspects of dynamic CA indicating ambulation may not be needed to improve cerebrovascular function. Consequently, other break modalities that require less movement away from a workers' desk environment should be explored, such as simply standing up, the use of active workstations, or the full version of the Exertime software, which includes many exercises that can be performed in the vicinity of the desk. Research should investigate, firstly, if these interventions are effective at reducing SB at work, secondly, if different PA modalities can still elicit improvements in cerebrovascular function, and finally, whether they can be practically applied into a workplace environment.

### 8.4.3. Long-Term Research

A limitation to the studies in this thesis, and that of most of the existing SB literature, is that only acute time periods have been assessed, either in the form of a day (Chapter 5 and Chapter 6) or a few months (Chapter 7), which is not representative of the typical SB exposure of most working adults. Consequently, more ecologically valid research is needed assessing individuals over longer time periods, such as six months to one year. Throughout this thesis there were no changes in cognition either following prolonged sitting (Chapter 5) or breaking up sitting time (Chapter 7), furthermore during cross-sectional analyses sitting was not associated with cognition (Chapter 4). Whether chronic exposure, such as six-months or a year, to the transient reductions or elevations in CBF observed acutely in this thesis (Chapter 5 and Chapter 6) could result in changes to cognitive functioning should be explored further. Furthermore, research should consider populations who are at higher risk for

cognitive decline, such as older adults, who spend 65-80% of their waking day sedentary (Harvey et al., 2015). Finally, as discussed in Chapter 7, the lack of translation of the acute improvement in CBF observed following regular walking breaks (Chapter 6) to a longer-term improvement after using the Exertime software could be be due to the adaptation of the cerebrovasculature to the PA break stimuli. As has been conducted when assessing the peripheral vasculature's response to exercise training (Tinken et al., 2008, 2010), the CBF responses to activity breaks from sitting should be examined regularly over a period of weeks to profile the time course of any changes in CBF.

In Chapter 5 and Chapter 6 it is suggested that sitting-induced impairments in cerebrovascular function may contribute to the development of cerebrovascular diseases such as vascular dementia and Alzheimer's disease. Furthermore, in Chapter 6 is it implied that the improvement observed in cerebrovascular function using frequent walking breaks could have implications in the prevention of such diseases. Consequently, longitudinal research is needed assessing SB, cerebrovascular function and cognition, alongside utilising clinical tests for the development of dementia, such as the mini mental state examination and the Addenbrooke's cognitive assessment (Cooper and Greene, 2005).

## 8.5. Summary

The primary aims of this thesis were to investigate the effects of sitting on cerebrovascular function, cognition and mood. The main findings from this thesis with reference to the known and potential interactions between SB, cerebrovascular function, cognition and mood are summarised in Figure 8-1. Cross-sectional analyses specifically focusing on the workplace showed sitting during work hours was negatively associated with the calm mood state, but not cognition. However, increased time spent standing and stepping at work was positively associated with aspects of mood and cognition. Using controlled laboratory experiments, this thesis presents data showing for the first time that periods of prolonged sitting acutely impair aspects of cerebrovascular function, namely CBF and dynamic CA. However, interrupting this sitting time with frequent, short duration walking breaks, rather than longer duration, less frequent walking breaks can attenuate this impairment. Prolonged sitting also acutely impairs mood, which was unrelated to the observed decrements in cerebrovascular function; whilst cognition was not acutely affected by prolonged sitting. This thesis also assessed the effect of a longer-term intervention designed to reduce workplace sitting on cerebrovascular function, cognition and mood. Using Exertime, a computer prompting software designed to break up workplace sitting, for 8-weeks resulted in statistically non-significant but a potentially clinically meaningful reduction in the time workers spent sitting, which appeared to be replaced with increased time spent standing. Following this intervention, dynamic CA but not CBF was improved, and no changes in mood or cognition were observed. Taken together this thesis provides the first evidence that SB negatively effects cerebrovascular function and demonstrates

that breaking up sitting can prevent this deleterious outcome. Future research is needed examining the chronic influence of sitting to assess whether these acute impairments translate into cerebrovascular disease risk.

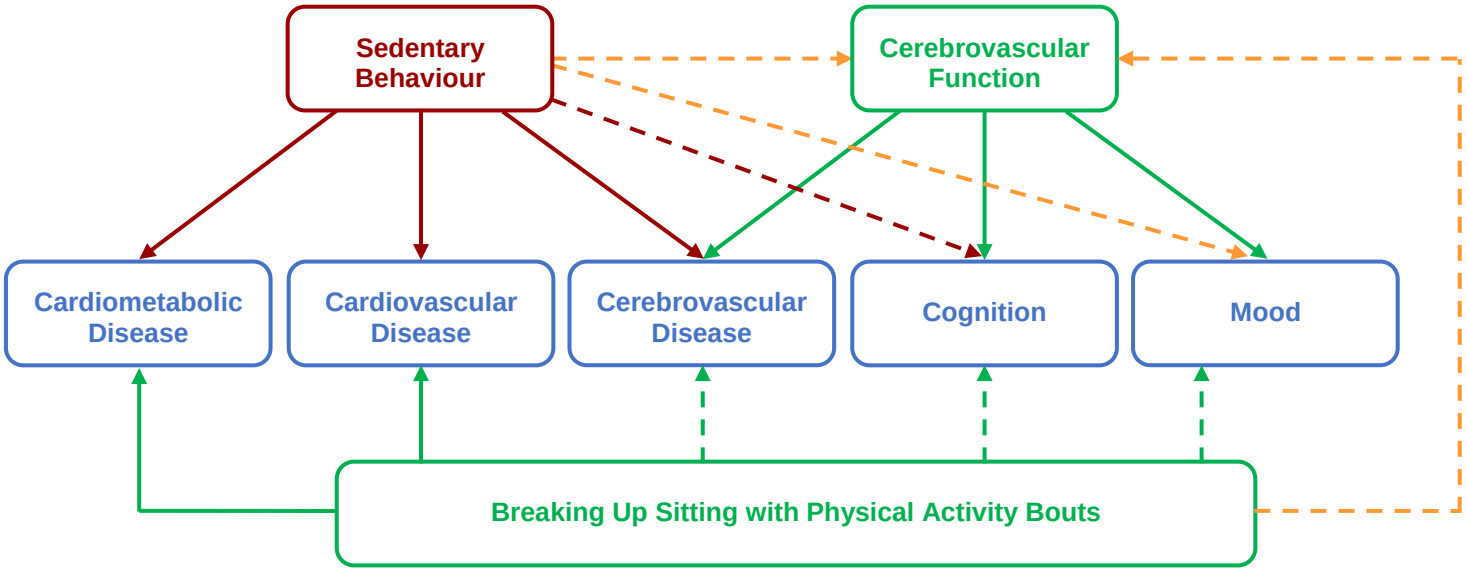


**Figure 8-1:** A summary of the major findings in this thesis with reference to the known and potential interactions between sedentary behaviour, cardiometabolic, cardiovascular and cerebrovascular disease; cerebrovascular function; cognition and mood. Orange lines indicate data from this thesis suggest a potential influence; dashed lines indicate a potential influence; red lines indicate negative influence; green lines indicate positive influence.

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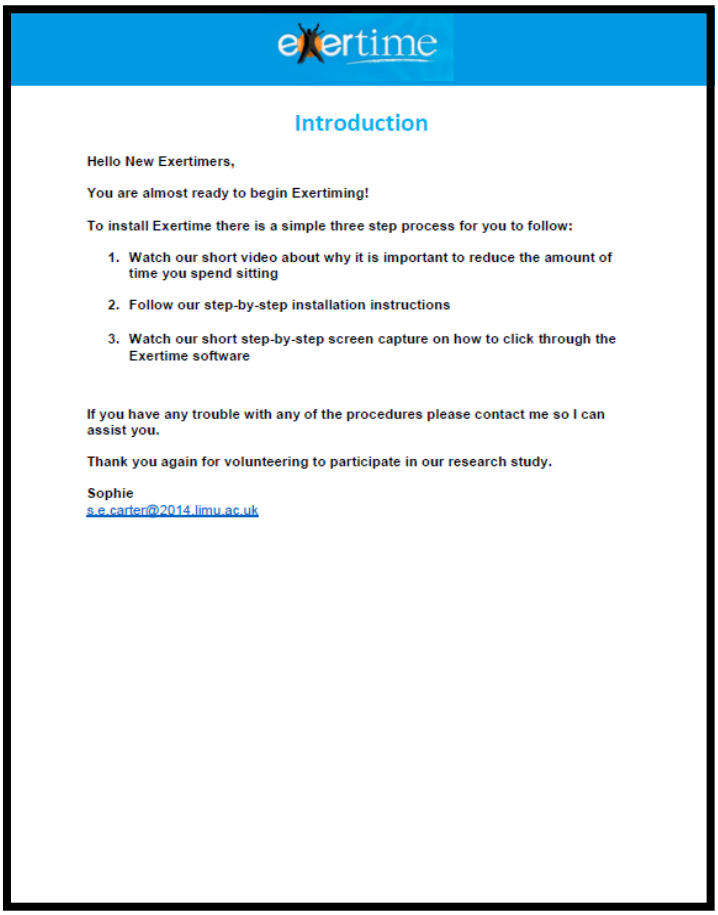
# 10. Appendices

## 10.1. Appendix 1: Participant Educational Booklet

exertime

### Introduction

Hello New Exertimers,

You are almost ready to begin Exertiming!

To install Exertime there is a simple three step process for you to follow:

1. Watch our short video about why it is important to reduce the amount of time you spend sitting
2. Follow our step-by-step installation instructions
3. Watch our short step-by-step screen capture on how to click through the Exertime software

If you have any trouble with any of the procedures please contact me so I can assist you.

Thank you again for volunteering to participate in our research study.

Sophie
s.e.carter@2014.limu.ac.uk

## Stage 1. Why should we break up our sitting time?

**As you may have seen in the media, it is now advised that we reduce the amount of time we spend sitting for long periods each day**


**We have created a short video on why prolonged sitting is bad for your health and why it is important to reduce the amount of time we spend sitting.**

**You can access the video using this link:**

https://goanimate.com/videos/0n8nYM3M6Pms?utm_source=linkshare&utm_medium=linkshare&utm_campaign=usercontent

**You can also watch a brief TED Talk on the negative effects of sitting here:**

https://ed.ted.com/lessons/why-sitting-is-bad-for-you-murat-dalkilinc

## Stage 2. Self-Install Process for Exertime

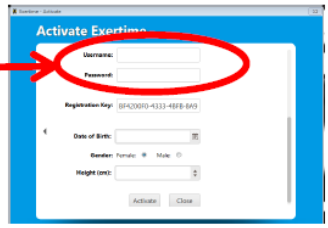
Below is a step-by-step procedure with screen shots to allow you to download and register your user details to begin the Exertime program.

It is recommended that you have a look through the brief instructions before beginning as there may be times during this process that you do not have access to this file.

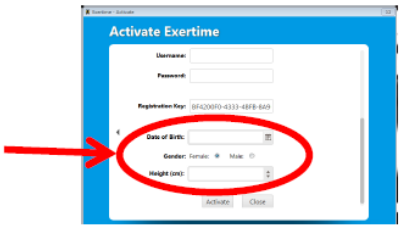
**Step 1.** Open the **LJMU AppPlayer** from the icon on the desktop.

**Step 2.** Click the **Search** icon to open a search box.

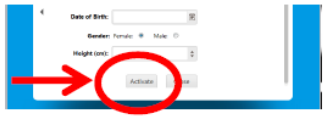
**Step 9.** Scroll down to create a Username and Password for Exertime. Do not alter the registration key details as this is assigned to your workplace.



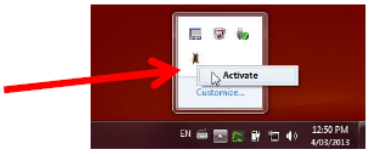

**Step 10.** Enter your Date of Birth, Gender, and Height. These data are used to generate individual-specific feedback within the Exertime program.



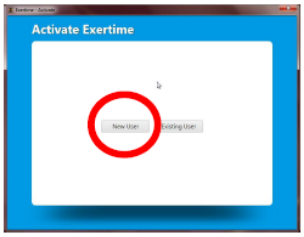

**Step 11.** Click Activate to complete the process.



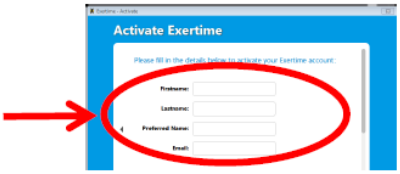
**Step 6.** Right Click and Choose 'Activate'.



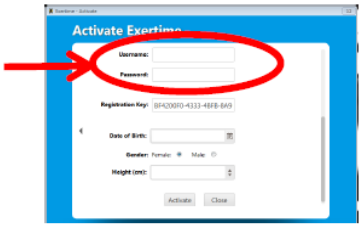

**Step 7.** Click on 'New User'



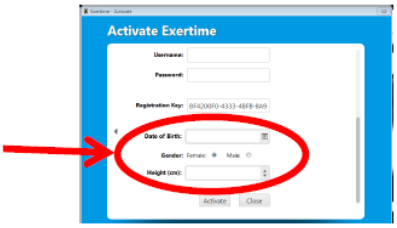

**Step 8.** Enter your personal details including your Firstname, Lastname, any preferred name and an email address



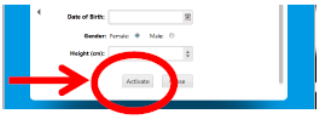
**Step 9.** Scroll down to create a Username and Password for Exertime. Do not alter the registration key details as this is assigned to your workplace.



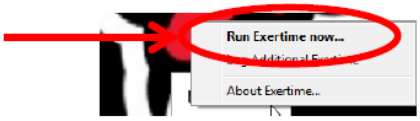

**Step 10.** Enter your Date of Birth, Gender, and Height. These data are used to generate individual-specific feedback within the Exertime program.



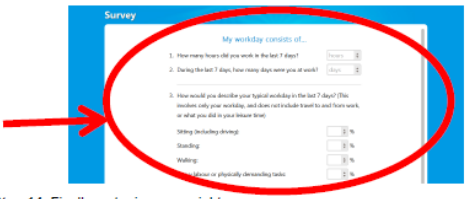

**Step 11.** Click Activate to complete the process.



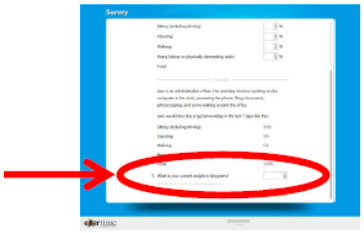

**Step 12.** On your desktop click on the Exertime icon in taskbar and then click '**Run Exertime now…**'



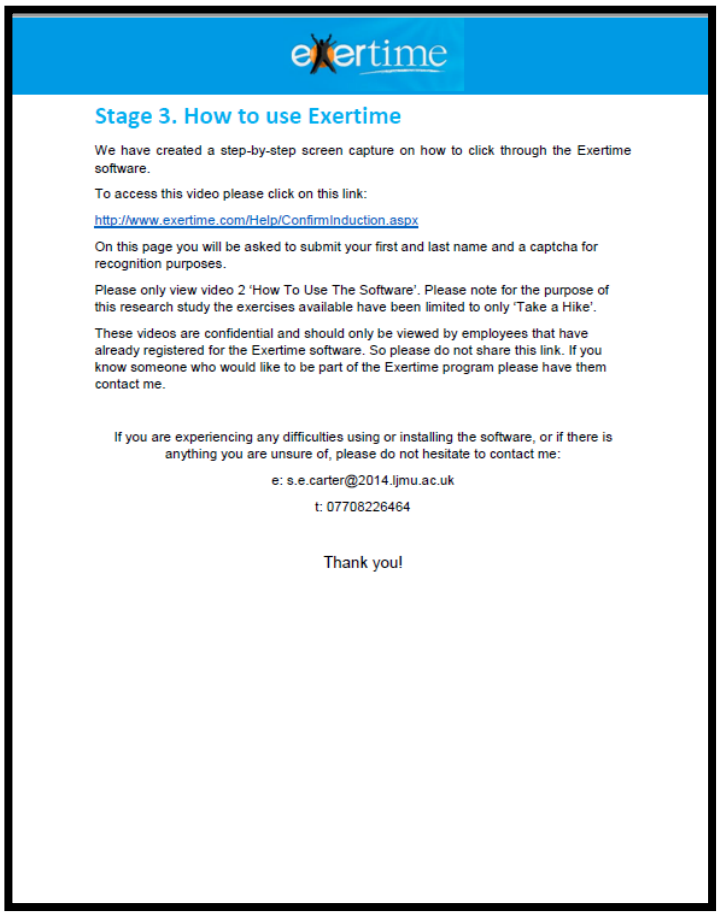

**Step 13.** Before running Exertime for the first time, you are required to complete a short survey about your workplace behaviour. The questions ask about the time you spend at work, and to consider how much time spent sitting, standing, walking, and heavy labour you do during a typical workday in terms of percentages.




**Step 14.** Finally, enter in your weight.




**Step 15.** Click **Save**.



## Stage 3. How to use Exertime

We have created a step-by-step screen capture on how to click through the Exertime software.

To access this video please click on this link:

http://www.exertime.com/Help/ConfirmInduction.aspx

On this page you will be asked to submit your first and last name and a captcha for recognition purposes.

Please only view video 2 'How To Use The Software'. Please note for the purpose of this research study the exercises available have been limited to only 'Take a Hike'.

These videos are confidential and should only be viewed by employees that have already registered for the Exertime software. So please do not share this link. If you know someone who would like to be part of the Exertime program please have them contact me.

If you are experiencing any difficulties using or installing the software, or if there is anything you are unsure of, please do not hesitate to contact me:

e: s.e.carter@2014.ljmu.ac.uk

t: 07708226464

Thank you!

## 10.2. Appendix 2: Email Suggestions for Participants to Break Up Their Sitting

| Week of Intervention | Suggestion |
|---|---|
| 1 | A tip for taking a two-minute walking break is to use the toilets a floor higher or lower than the one you work on |
| 2 | A tip to frequently break up your sitting is to use a smaller drinks bottle, so you have to get up and fill it up more regularly |
| 3 | A simple way to take a break from sitting is to go speak to a colleague rather than send an email |
| 4 | To walk more at work why not move the bin to the other side of your office so you have to get up and walk to use it |
| 5 | A simple way to take a two-minute walking break is to get up and a do a lap of your corridor, try even incorporating going up and down some stairs! |
| 6 | Going for a coffee break? Rather than sit with a colleague have a walking coffee break |
| 7 | If you can, use a printer in a different room or floor to the one you work on so you have to walk to collect your printing |
| 8 | Get some fresh air! If you've been inside all day, take a walk outside your building to get some fresh air! |