UNIVERSITY OF
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EXERCISE AND CEREBRAL HAEMODYNAMICS FOR BRAIN HEALTH IN OLDER ADULTS

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ABSTRACT

The global population is ageing. With age, inevitably, comes declines in all aspects of health, including to brain health. Brain health is particularly important in later life, as this dictates cognitive function and thus our ability to live independently and maintain a good quality of life. Age-related changes in cerebral haemodynamics are hypothesised to contribute to age-related cognitive declines. Identifying strategies or individual characteristics that delay age-related changes to cerebral haemodynamics could help to enable a healthy ageing process. Cardiorespiratory fitness and exercise training are commonly assumed to increase cerebral blood flow; however, there are large disparities within the available literature. Therefore, the purpose of this thesis was to investigate the impact of cardiorespiratory fitness and exercise training on resting cerebral blood flow (CBF) and arterial transit time (ATT) in healthy older adults. Furthermore, the importance of these cerebral haemodynamic markers for cognitive function was assessed.

Chapter 1 describes some key concepts and theories in this field and provides an overview of the existing literature. **Chapter 2** includes cross-sectional analyses which revealed that older adults with a higher BMI had lower global CBF and a longer global ATT. Cardiorespiratory fitness was not associated with CBF, but fitter older adults unexpectedly had longer ATT in parietal and occipital regions. Regarding cognitive function, neither CBF or ATT were associated with processing speed, working memory, or attention performance. **Chapter 3** investigates the impact of exercise training on CBF and ATT. Older adults were randomised into a control or exercise group (lifestyle maintenance vs. six-month home-based high-intensity exercise programme). Following the intervention, there were no group-level differences in CBF or ATT, despite increased cardiorespiratory fitness in the exercise group. However, sub-group analysis of exercise participants revealed that those with the greatest cardiorespiratory fitness gains (>2 mL/kg/min)

experienced significant global CBF reductions. Moreover, across the whole exercise group, changes in cardiorespiratory fitness and global CBF were negatively associated. Regarding cognitive function, there were also no group-level differences, nor were changes in CBF or ATT associated with cognitive function changes. **Chapter 4** focusses on associations between accelerometer-derived physical activity behaviours and CBF or ATT, including both cross-sectional and longitudinal analyses. Neither set of analyses identified associations between cerebral haemodynamics and sedentary behaviour, light physical activity, or moderate-to-vigorous physical activity. **Chapter 5** explored whether adherence to the exercise intervention predicted subsequent changes to cardiorespiratory fitness. Exploratory analyses indicated that superior general adherence and adherence specifically to exercise volume or intensity were associated with greater fitness gains, but these associations were strongest for exercise volume. Finally, **Chapter 6** summarises conclusions relating to the preceding experimental chapters and discusses future work required to assess the impact of longer-term exercise training on cerebral haemodynamics in older adults, and whether cerebral haemodynamics predict cognitive function over the longer-term.

Overall, the findings from this thesis show that exercise training appears to reduce resting CBF in older adults, but only when cardiorespiratory fitness gains were evident. Further, changes in physical activity behaviours had no clear effect on cerebral haemodynamics within this population, and cerebral haemodynamics were not associated with cognitive function.

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Segaert, K., Tsvetanov, K., **Feron, J.**, Joyce, K., Gilani, A., Fernandes, E., Wetterlin, A., Wheeldon, L., Lucas, S., Rahman, F. (2023). *Neural and Cardiovascular Determinants of Tip-of-The-Tongue States in Healthy Ageing*. Oral presentation at the 15th Annual Meeting of the Society for the Neurobiology of Language, Marseille, France.

Yanina, P., Tsvetanov, K., Rahman, F., **Feron, J.**, Fernandes, E.G., Wetterlin, A., Lucas, S.J.E., Wheeldon, L., Segaert, K. (2024). *Does regular physical exercise mitigate age-related decline in word production?* Oral presentation at the 31st Annual Meeting of the Cognitive Neuroscience Society, Toronto, Canada.

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ABBREVIATIONS

ASL	Arterial spin labelling
ATT	Arterial transit time
ATT_{GM}	Arterial transit time in grey matter
BMI	Body mass index
CASL	Continuous arterial spin labelling
CBF	Cerebral blood flow
CBF_{GM}	Cerebral blood flow in grey matter
HIIT	High-intensity interval training
HR_{peak}	Peak heart rate
LPA	Light physical activity
MCA_V	Middle cerebral artery blood velocity
MICT	Moderate-intensity continuous training
MPA	Moderate physical activity
MRI	Magnetic resonance imaging
MVPA	Moderate-to-vigorous physical activity
NIRS	Near-infrared spectroscopy
PASL	Pulsed arterial spin labelling
PCASL	Pseudo-continuous arterial spin labelling
PC-MRI	Phase-contrast magnetic resonance imaging
PET	Positron emission tomography
PLD	Post-labelling delay
sCoV	spatial coefficient of variation in ASL signal
TCD	Transcranial Doppler ultrasound
$\dot{V}O_{2max/peak}$	Maximum/Peak rate of oxygen consumption
VPA	Vigorous physical activity

THESIS OVERVIEW

The global population is rapidly ageing (United Nations Department of Economic and Social Affairs, Population Division, 2022). As we age, the risk of developing cardiovascular and neurodegenerative diseases increases. Not only does this hinder quality of life in later life, but it poses a substantial financial and logistical burden on health services globally. Maintaining brain health is of particular importance to a healthy ageing process because this dictates cognitive function. Maintaining a level of cognitive function that enables the completion of both basic and instrumental activities of daily living allows us to live independently in later life (Salthouse, 2012). However, most aspects of cognitive function progressively, and unavoidably, decline with age (Gorbach et al., 2017; Park et al., 2002; Rönnlund et al., 2005; Salthouse, 2010; Schaie, 1994), although large individual variability exists (Gorbach et al., 2017; Habib et al., 2007; Nyberg et al., 2020). The ageing brain experiences a plethora of adverse structural and functional changes, including to cerebral haemodynamics (Alwatban et al., 2021; Damestani et al., 2023), carotid artery stiffness (Pewowaruk et al., 2022), cerebrovascular reactivity (Bakker et al., 2004; Miller et al., 2019), cerebral artery stenosis (Johnsen et al., 2023), functional connectivity (Baghernezhad and Daliri, 2024), grey matter volume (Frangou et al., 2022; Raz et al., 2005), and white matter hyperintensities (Scharf et al., 2019). This cocktail of unfavourable changes is likely to cause declines in cognitive function and contribute to the rise in neurodegenerative and cerebrovascular diseases of the global ageing population. However, robust links between changes to specific aspects of brain health and cognitive function are lacking, and thus the brain health metrics of most importance to a healthy ageing process are poorly understood. As such, it is important to identify modifiable characteristics of individuals that best preserve their cognitive function in later life so that strategies can be developed that target these characteristics, which can then be implemented within the general population.

In recent years, cerebral haemodynamics has attracted more attention as a potentially important meditator of cognitive ageing. The brain lacks intracellular energy stores (Öz et al., 2007) and thus blood flow to the brain ultimately dictates the delivery of oxygen and nutrients to the cerebral tissue and thus energy availability (Zimmerman et al., 2021). Any tissue, including the cerebral tissue, will cease to function in an optimal manner without sufficient energy availability, potentially resulting in cell death in severe instances. It is therefore hypothesised that age-related changes in cerebral haemodynamics may be an underpinning mechanism to the plethora of age-related structural and functional declines in brain health that ultimately influence behavioural outcomes (de la Torre, 2013).

The overall purpose of this PhD was to investigate relationships between modifiable lifestyle factors and resting cerebral haemodynamics in healthy older adults. Pseudo-continuous arterial spin labelling with multiple post-labelling delays was used to accurately estimate cerebral blood flow (CBF) and arterial transit time (ATT) in grey matter (i.e., CBF_{GM} ATT_{GM}). The impact of cardiorespiratory fitness and exercise training on these variables was of particular interest, because, despite their known beneficial effects on other structural, functional, and behavioural brain health outcomes, how they affect cerebral haemodynamics in older adults is poorly understood. Furthermore, because the exact mechanisms underpinning both age- and fitness-related changes to cognitive function are also poorly understood, this thesis aimed to assess the links between cerebral haemodynamics and behavioural outcomes (i.e., cognitive function).

Chapter 1 describes the key concepts and theories within this field and provides an overview of relevant existing literature. This includes the impact of age, cardiorespiratory fitness, exercise training, and physical activity behaviours on resting cerebral haemodynamics (i.e., CBF, ATT, volumetric CBF, and cerebral artery blood velocity) in healthy older adults. An overview of the different techniques available to measure these haemodynamics measures is also provided.

Furthermore, existing knowledge regarding the importance of cerebral haemodynamics to cognitive function in older adults will also be discussed. This chapter concludes with a summary of the research questions this thesis aimed to address.

Chapter 2 investigates cross-sectional associations between resting cerebral haemodynamics (CBF_{GM} and ATT_{GM}) and modifiable lifestyle factors, including blood pressure, body mass index (BMI), and cardiorespiratory fitness (i.e., $\dot{V}O_{2peak}$). Associations between cerebral haemodynamics and cognitive function were also assessed (i.e., working memory, processing speed, and attention).

Chapter 3 includes a randomised controlled trial that investigated the impact of a six-month, home-based, high-intensity exercise intervention on cerebral haemodynamics and cognitive function in 65 healthy older adults. This study builds upon earlier cross-sectional research that does not allow for causal inferences to be made. A particular focus was given to individual variability in exercise-induced cardiorespiratory fitness gains, and whether these explained any observed changes in cerebral haemodynamics or cognitive function.

Chapter 4 includes both cross-sectional and longitudinal analyses investigating associations between cerebral haemodynamics and accelerometer-derived physical activity behaviours or physical function in older adults. Compared with cardiorespiratory fitness, which has a large genetic component, physical activity behaviours provide a more holistic and possibly more accurate depiction an individual's health and/or lifestyle. Therefore, these alternative measures may have stronger associations with cerebral haemodynamics.

Chapter 5 includes an exploratory analysis of whether objectively measured adherence to the six-month exercise intervention predicts changes in cardiorespiratory fitness in older adults.

Chapter 6 provides a general discussion to the previous four empirical data chapters. Findings are summarised and potentially valuable avenues of future research are highlighted.

CHAPTER ONE

BACKGROUND AND LITERATURE REVIEW

1.1 Chapter overview

This chapter will primarily focus on how age, cardiorespiratory fitness, physical activity behaviours, and exercise training affect cerebral haemodynamics in healthy older adults. Furthermore, the importance of cerebral haemodynamics to cognitive function in older adults will also be discussed. This chapter will also review some of the most extensively studied haemodynamic measures, including large artery cerebral blood velocity, CBF (volumetric, whole-brain, and grey matter), and grey matter arterial transit time (ATT_{GM}). Both CBF_{GM} and ATT_{GM} are primary outcome measures in the following three data chapters. As part of these discussions, common methodological approaches to measure these haemodynamic markers will also be reviewed, highlighting some of the inconsistent findings reported in the literature due to the different approaches used as well as providing the rationale for the imaging methodology used in the subsequent data chapters.

1.2 Age-related changes to cerebral haemodynamics

Similar to most aspects of brain health, brain vascular health and cerebral haemodynamics undergo adverse age-related changes. These include reductions in resting cerebral artery blood velocity (Alwatban et al., 2021; Bakker et al., 2004; Tegeler et al., 2013), volumetric CBF (Amin-Hanjani et al., 2015; Roberts et al., 2023), whole-brain CBF (Lu et al., 2011; Vernooij et al., 2008), and CBF_{GM} (Damestani et al., 2023; Leidhin et al., 2021; Yetim et al., 2023) as well as increases in ATT_{GM} (Damestani et al., 2023; Fujiwara et al., 2017; Mutsaerts et al., 2015) and cerebral blood flow pulsatility (Alwatban et al., 2021; Bakker et al., 2004; Roberts et al., 2023; Tegeler et al., 2013). Figures 1.1 and 1.2 show examples from existing literature highlighting commonly reported age-

related changes in a selection of these haemodynamics measures. These figures also demonstrate that although declines are seen across the board, there is considerable individual variability. Understanding why this is the case is important for prolonging functional brain health during ageing. It should also be considered that the majority of studies within the literature are cross-sectional (including those presented in Figure 1.1 and 1.2) and thus may not accurately reflect the true longitudinal age-related changes in resting cerebral haemodynamics. Interestingly, regarding CBF, a small longitudinal study in healthy older adults (n=14) found that, over seven-years, regional CBF declines were evident, but these were also accompanied by CBF increases in other regions (Figure 1.3) (Beason-Held et al., 2007).

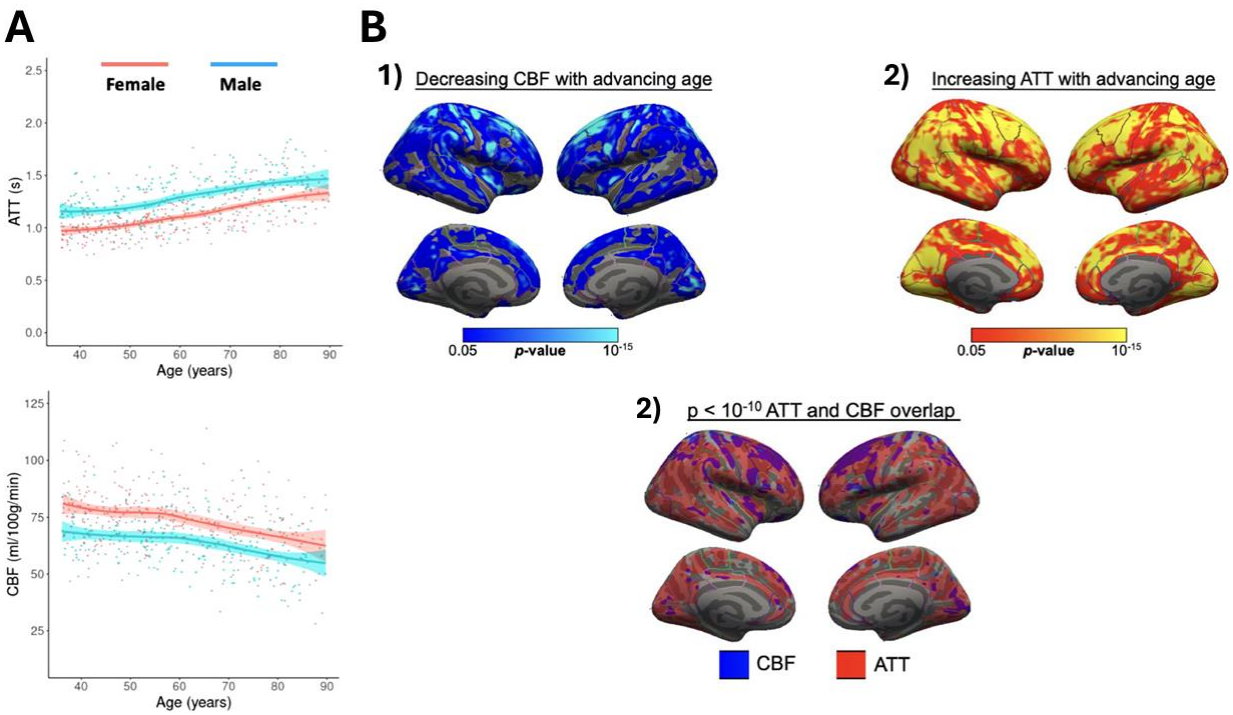

Figure 1.1: Cross-sectional changes in cortical grey matter cerebral blood flow (CBF) and arterial transit time (ATT) over the lifespan (n=562). From Damestani et al. (2023).

A: CBF lowers, and ATT lengthens with age, but there is large individual variation. Females tend to have higher CBF and shorter ATT across the lifespan compared with males.

B: Spatial maps of regions where age affects CBF (1), ATT (2), or overlap between CBF and ATT (3). Age-effects on ATT are more widespread than CBF, with overlap in frontal and superior parietal cortices.

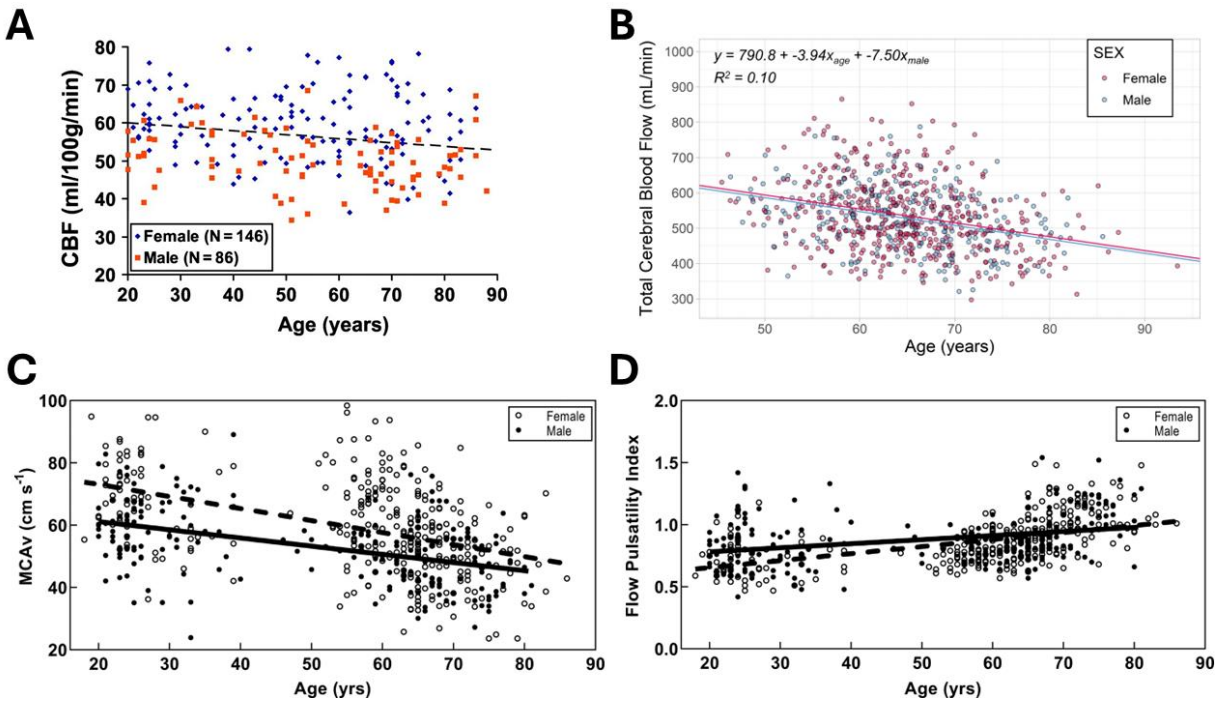

Figure 1.2: Cross-sectional changes in cerebral blood flow (CBF), cerebral blood velocity, and cerebral blood velocity pulsatility measures over the lifespan.

A: Global CBF declines with age (n=232). Volumetric blood flow through the internal carotid and vertebral arteries was determined using phase-contrast MRI, summed, and then normalised to total brain volume to provide global CBF (mL/100 g/min). From Lu et al. (2011).

B: Total volumetric CBF declines with age (n=759). Volumetric blood flow through the cervical internal carotid arteries and basilar artery was determined using four-dimensional (4D) flow MRI and summed to provide total CBF (mL/min). From Roberts et al. (2023).

C: Cerebral blood velocity within the middle cerebral artery (MCA_V) declines with age (n=525). Measured using transcranial Doppler. From Alwatban et al. (2021).

D: Cerebral blood velocity pulsatility within the middle cerebral artery declines with age (n=525). Measured using transcranial Doppler. From Alwatban et al. (2021).

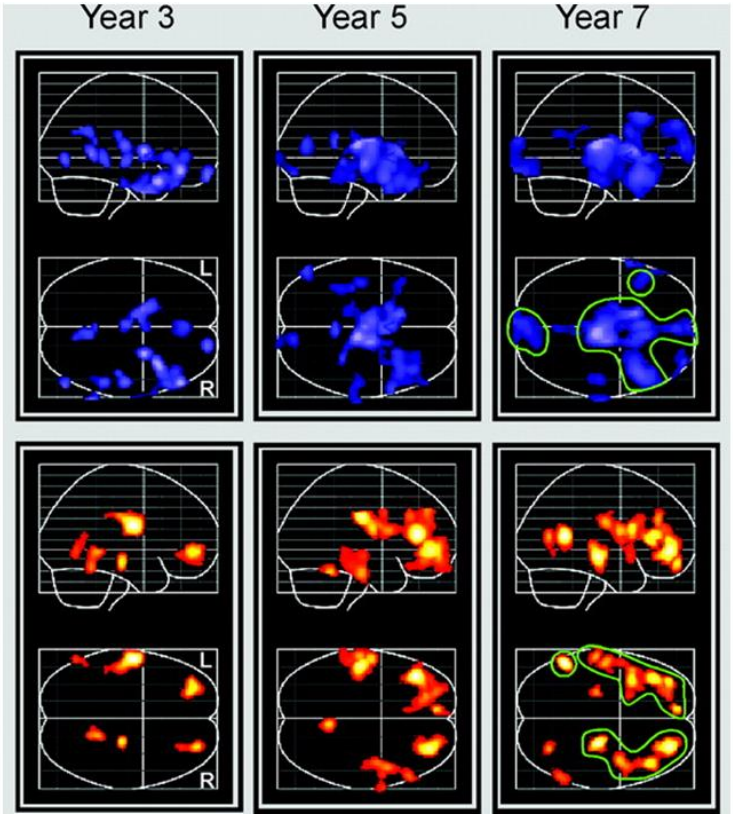

Figure 1.3: Longitudinal changes, relative to baseline, in resting cerebral blood flow (CBF) after three, five, and seven years in healthy older adults (n=14). Blue; decreased CBF, red; increased CBF. CBF measured with positron emission tomography. Adapted from Beason-Held et al. (2007).

Of the aforementioned variables, ATT is the least studied, defined as the time taken for blood to travel from large cerebral arteries in the neck to the cerebral tissue, and is primarily dictated by cerebral artery blood velocity and the vascular path length. The vascular path length is the physical distance the blood must travel through the cerebrovascular tree to reach the cerebral tissue. Evidence to date indicates that cortical CBF_{GM} declines by 3.1±1.1 mL/100 g/min per decade whereas cortical ATT_{GM} lengthens by 77±13 ms per decade (Figure 1.1A) (Damestani et al., 2023). Interestingly, compared with CBF_{GM} where the strongest age-related changes are primarily limited to superior frontal regions, age-related changes to ATT_{GM} are far more widespread across the whole cortex (Figure 1.1B) (Damestani et al., 2023). Cerebral artery blood velocity, measured with transcranial Doppler ultrasound, is commonly used as a proxy for volumetric CBF, under the

assumption that vessel diameter remains constant during the measurement process (Willie et al., 2011). However, research indicates that cerebral artery blood velocity is more closely related to ATT_{GM} than CBF_{GM} (Figure 1.8) (Burley et al., 2021). This is likely because ATT_{GM} indicates how fast blood travels through the cerebrovascular tree and thus is more strongly dictated by cerebral blood velocity, shown to reduce by -0.5 [-0.6, -0.4] cm/s per year (Bakker et al., 2004). A more detailed comparison of the differences and similarities between methodologies to measure cerebral haemodynamics is provided later in this chapter (see Section 1.4).

Age-related cerebral atrophy and/or reductions in cerebral metabolic rate have been suggested to explain CBF declines (Mokhber et al., 2021). However, regional reductions in CBF can be independent of atrophy (Chen et al., 2011) and the relationship between CBF and brain volume appears to be bidirectional (Zonneveld et al., 2015). Furthermore, although overall cerebral metabolic rate declines with ageing, proposed to be caused by cerebral atrophy, the metabolic rate of remaining tissue is in fact greater in older adults (Lu et al., 2011). Moreover, it is likely that systemic age-related cardiovascular and cerebrovascular changes also contribute to CBF reductions (Bliss et al., 2023; Mokhber et al., 2021). For example, hypertension, arterial stiffness, and endothelial dysfunction typically increase with age (Thijssen et al., 2016; Toth et al., 2017), and have all been associated with lower resting CBF (Jefferson et al., 2018; Leidhin et al., 2021; Lu et al., 2011; Sabayan et al., 2014). Regarding ATT_{GM}, age-related lengthening may be also be explained by structural changes in the cerebrovasculature, including increases in cerebral vessel tortuosity (Bullitt et al., 2010; Z. Sun et al., 2022) or partial intracranial artery stenosis (Johnsen et al., 2023), increasing both vascular path length and blood flow resistance. In addition, the aforementioned reductions in cerebral blood velocity are likely to also contribute to ATT_{GM} prolongation, potentially resulting from similar age-related deteriorations in vessel health (i.e., endothelial dysfunction and arterial stiffness).

1.3 The importance of cerebral haemodynamics to brain health

1.3.1 Overview

The role of the cerebrovasculature is to effectively distribute blood to the cerebral tissue so that homeostasis can be maintained, or higher energy demands can be met. Inadequate delivery of blood to the cerebral tissue will induce cellular dysfunction and ultimately atrophy because the brain lacks intracellular energy stores (Öz et al., 2007; Zimmerman et al., 2021). This is why CBF and its proxy, cerebral artery blood velocity (Willie et al., 2011), are two of the most extensively studied markers of cerebrovascular health. Indeed, a lower resting CBF has been associated with numerous markers of impaired brain health, including grey matter atrophy (Zonneveld et al., 2015), myelin content (Kiely et al., 2023), cerebral tissue integrity (Bouhrara et al., 2022), cerebral artery stiffness (Jefferson et al., 2018), cerebral artery stenosis (Bokkers et al., 2009; Han et al., 2019; Leeuwis et al., 2020; Yu et al., 2022), and amyloid deposition (Ebenau et al., 2023; Sojkova et al., 2008). However, it should be considered that more may not always mean better. Some research suggests that a higher CBF (i.e., hyperperfusion) can be indicative of a compensatory response to dysfunction of the cerebrovasculature or the cerebral tissue. For example, although global CBF reductions are evident in neurodegenerative diseases, there can be regional hyperperfusion, particularly in the earlier stages of clinical cognitive impairment (Beason-Held et al., 2013; Camargo and Wang, 2021; Swinford et al., 2023; T. Tang et al., 2022).

Given that CBF is ultimately what keeps the cerebral tissue alive, it understandably receives the most attention within the literature. However, more novel measures of cerebral haemodynamics exist, including ATT, which also appears to have value as a marker of brain vascular health. For example, alongside age-related lengthening of ATT (Damestani et al., 2023), a prolonged ATT is present in those with higher blood pressure (Yetim et al., 2023), cerebral artery stenosis (Bokkers et al., 2009; Yu et al., 2022), lower cerebrovascular reactivity (Takata et al., 2023), greater white

matter hyperintensities (Gyanwali et al., 2022), multiple sclerosis (Paling et al., 2014), vascular cognitive impairment (Gyanwali et al., 2022), and Alzheimer's disease (Gyanwali et al., 2022; M. Sun et al., 2022), and has been associated with clinical cognitive impairment (Mak et al., 2012; M. Sun et al., 2022). Furthermore, there is evidence to indicate that ATT experiences more substantial age-related changes than CBF (Damestani et al., 2023), and thus may have greater prognostic value as a marker of brain health. Moreover, there are examples that, compared with CBF, ATT is superior at identifying abnormal perfusion post-stroke and in cerebrovascular disease (Haller et al., 2016). Although existing research generally suggests a longer ATT is indicative of worse brain health (like lower CBF), this should not simply be assumed. For example, there have been tentative links made between longer ATT and increases in cerebral oxygen extraction in patients with cerebrovascular disease (Hara et al., 2022; Takeuchi et al., 2022).

1.3.2 Cerebral haemodynamics and cognitive function

Cognitive function unavoidably worsens with age, even in healthy ageing (Gorbach et al., 2017; Rönnlund et al., 2005; Schaie, 1994; Zaninotto et al., 2018). These longitudinal investigations reveal that considerable cognitive decline starts only after the age of sixty (approximately) (Figure 1.4A, 1.4B, 1.4D, and 1.5A), contrary to cross-sectional studies showing progressive decline from young adulthood onwards (Park et al., 2002; Salthouse, 2010). Interestingly, there is large individual variation in age-related cognitive changes (Figure 1.4C, 1.5A, 1.5B), with some ageing much better or worse than the average decline (Gorbach et al., 2017; Habib et al., 2007; Josefsson et al., 2012). As previously mentioned, cerebral haemodynamics also show age-related declines (Bakker et al., 2004; Damestani et al., 2023; Vernooij et al., 2008) and have thus been hypothesised to contribute to the cognitive declines experienced by older adults (de la Torre, 2018; Mokhber et al., 2021). Given the variability observed in both these brain health outcomes, it seems reasonable to suggest that the considerable variability in age-related changes to cerebral

haemodynamics (Figure 1.1 and 1.2) may explain the large individual variability in cognitive performance observed in older adults. The following two sections will discuss the evidence linking changes in CBF and ATT (the two primary outcome measures of this thesis) with cognitive function in older adults.

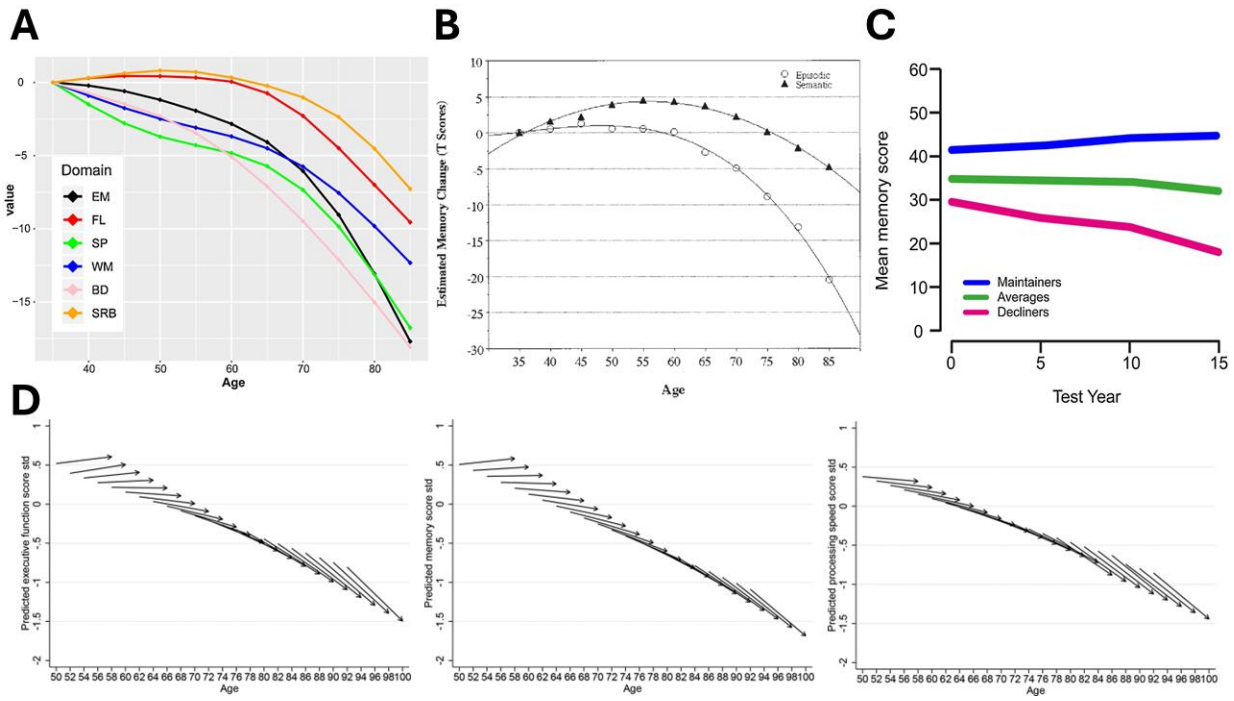

Figure 1.4: Longitudinal changes in cognitive function across the life span.

A: Average age-related cognitive decline in episodic memory (EM), word fluency (FL), speed of processing (SP), working memory (WM), block design (BD), and vocabulary (SRB). From Nyberg et al. (2020).

B: Average age-related decline in episodic (white) and semantic (black) memory (n=967). From Rönnlund et al. (2005).

C: Based on memory performance assessed over 15-years, participants were categorised as either maintainers, averagers, or decliners (n=1558). From Josefsson et al. (2012).

D: Changes in executive function (left), memory (middle), and processing speed (right) measured over eight-years. Cognitive decline is limited until the age of 60 (approximately), which then accelerates in later life. From Zaninotto et al. (2018).

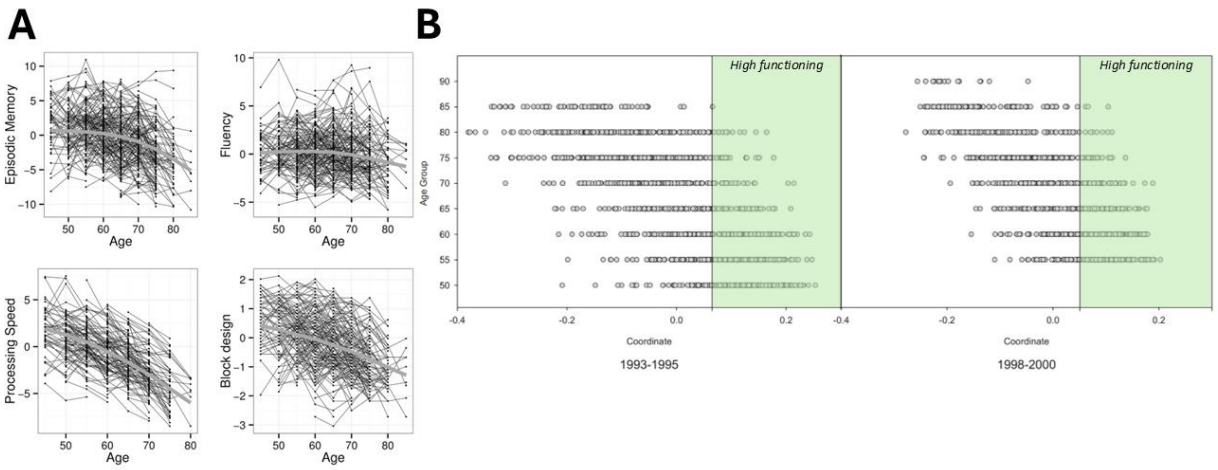

Figure 1.5: Individual variability of longitudinal changes in cognitive function across the life span.

A: Individual trajectories for age-related changes in different cognitive domains (n=264). From Gorbach et al. (2017).

B: Principal coordinates scores were based on cognitive, health, lifestyle, and socio-economic variables. The left panel shows large variability in cross-sectional scores categorised by age (n=1463), with participants in the green region deemed high functioning. The right panel shows scores from a sub-sample of these participants assessed five-years later (n=1078). Of those deemed high functioning elderly at baseline (n=51, ≥70 years), only 35% remained high functioning five-years later. Adapted from Habib et al. (2007).

1.3.4 Cerebral blood flow and cognitive function

Chronic cerebral hypoperfusion is associated with various risk factors and pathophysiological processes involved in clinical vascular cognitive impairment and has therefore been hypothesised to be the underpinning mechanism that ultimately explains the observed declines in cognitive function (Figure 1.6A) (Rajeev et al., 2023). Similarly, vascular risk factors and subsequent changes to cerebral haemodynamics, including hypoperfusion, are thought to be at the heart of more general cognitive declines and dementia experienced during ageing (Figure 1.6B) (de la Torre, 2013). The brain lacks intracellular energy stores and thus the end result of chronic cerebral hypoperfusion is reductions in cellular energy availability (Öz et al., 2007; Zimmerman et al., 2021), likely contributing to observed links between low CBF and cerebral atrophy (Zonneveld et al., 2015), blood-brain barrier disruptions (Shima et al., 1994; Xu et al., 2022; Zhang et al., 2022), neuroinflammation (Jakimovski et al., 2024), and oxidative stress (Liu and Zhang, 2012; Mracskó et al., 2010) (Figure 1.7). Furthermore, low CBF may also increase

amyloid deposition (Ebenau et al., 2023; Sojkova et al., 2008). A culmination of these adverse effects associated with cerebral hypoperfusion are likely to explain cognitive declines. Interestingly, research has suggested that reductions in whole-brain CBF and grey matter volume explain nearly all of the age-related variance in cognitive function, but that CBF has greater unique variance (Steffener et al., 2012). Indeed, a lower CBF is commonly associated with poorer cognitive function in those with dementia (Swinford et al., 2023; Zhang et al., 2021).

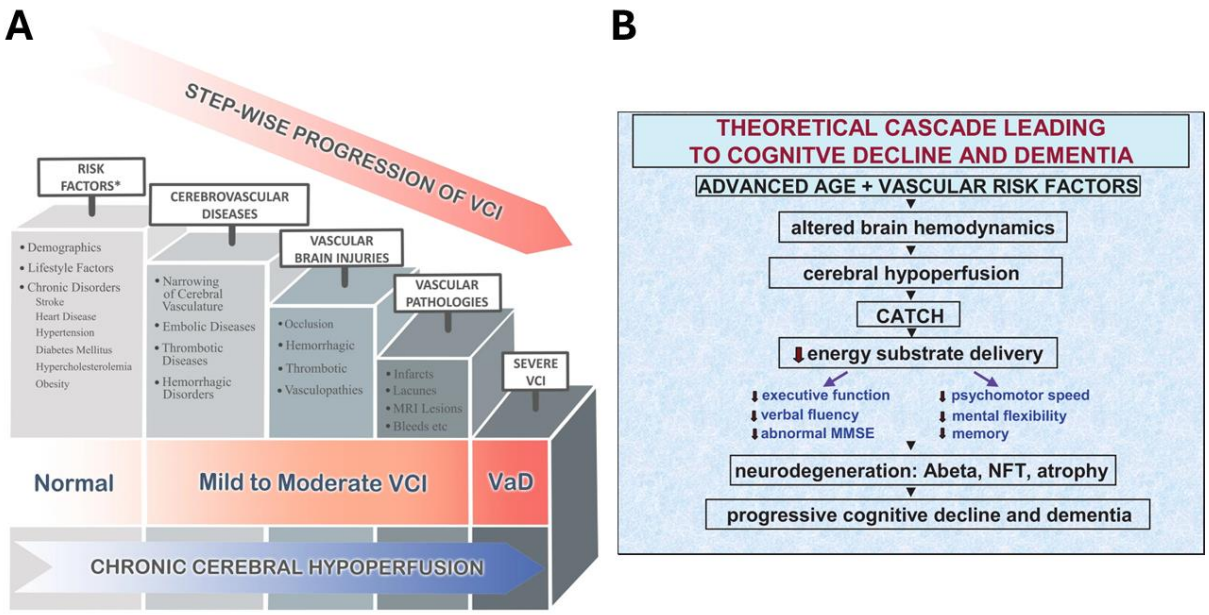

Figure 1.6: Theoretical models explaining the role of cerebral blood flow for cognitive decline and dementia.

A: Chronic cerebral hypoperfusion is associated with risk factors and pathophysiological processes involved with vascular cognitive impairment (VCI) and is thus hypothesised to be the underlying cause of progression through the stages of VCI, ultimately ending with vascular dementia (VaD). From Rajeev et al. (2023).

B: Ageing and the associated vascular risk factors are hypothesised to induce cerebral hypoperfusion, subsequently reducing energy availability of the cerebral tissue and inducing cognitive declines. From de la Torre (2013).

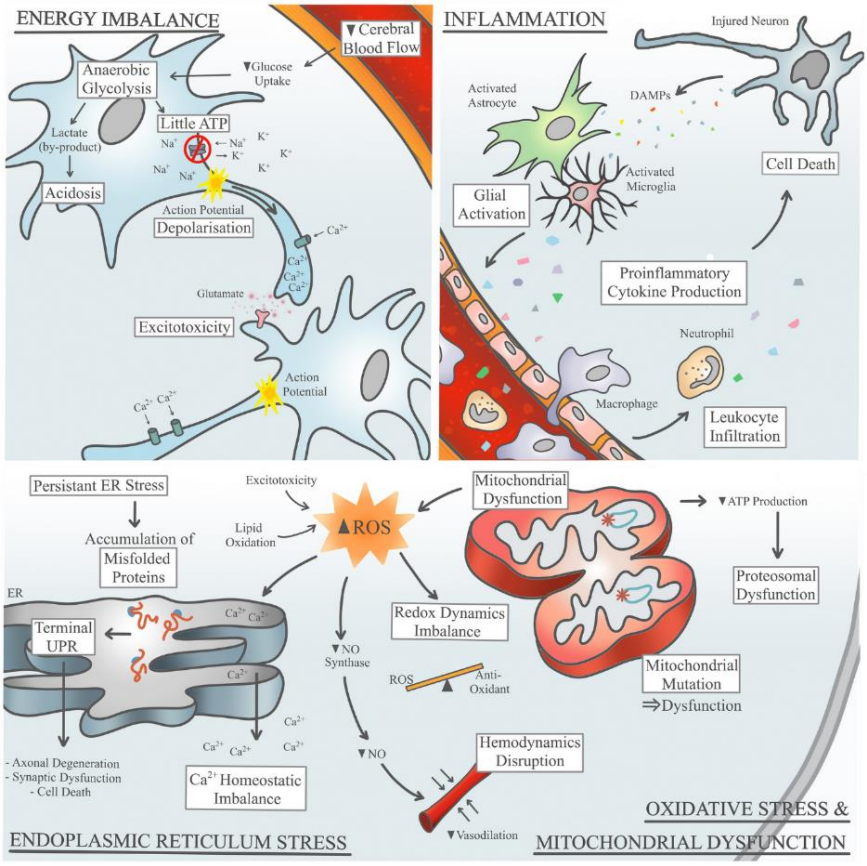

Figure 1.7: Potential mechanisms by which chronic cerebral hypoperfusion could adversely affect cellular and molecular pathways within the cerebral tissue. Notably, these involve an energy imbalance, excessive production of reactive oxygen species (ROS), neuroinflammation, and mitochondrial dysfunction that detrimentally affect neuronal integrity and function. From Rajeev et al. (2023).

Reductions in CBF are thought to occur early in the development of vascular cognitive impairment, which worsen further upon development to vascular dementia (Rajeev et al., 2023), the second most common type of dementia (Cao et al., 2020). Furthermore, although the most common type of dementia (Alzheimer's disease) is primarily categorised by amyloid deposition and neurofibrillary tangles (Alzheimer et al., 1995; Bloom, 2014), the vascular hypothesis of Alzheimer's disease (de la Torre, 1999) highlights how systemic and cerebral vascular function is also impaired within this population, and that cerebral hypoperfusion is likely an important factor in the pathophysiology of Alzheimer's Disease (de la Torre, 2018). Indeed, intra-brain vascular

dysregulation has been suggested to be an early pathological event in late-onset Alzheimer's disease progression (Iturria-Medina et al., 2016). Furthermore, longitudinal studies in those with Alzheimer's disease show accelerated CBF decline compared with healthy controls, and these CBF declines are associated with greater rates of cognitive decline (Weijs et al., 2023).

Importantly, CBF also appears to be predictive of cognitive decline in cognitively normal, community-dwelling populations. For example, a ~7 year follow-up study in 4759 Dutch older adults (64±11 years) found a lower resting global volumetric CBF (normalised for brain volume) at baseline was associated with a 7–60% higher risk of developing dementia (80% Alzheimer's) (Wolters et al., 2017). Furthermore, the same study also reported that a lower baseline CBF was associated with greater declines in global cognitive function after ~6 years (n=3700), particularly executive function and memory, even when excluding those who developed dementia (Wolters et al., 2017). The predictive capacity of lower resting global grey matter CBF on global cognitive decline has also been documented in cognitively normal older adults (n=89, 66±7 years) after just two years follow-up, driven primarily by the attention/psychomotor speed and memory domains (van Dinther et al., 2023). In contrast to Wolters et al. (2017), CBF_{GM} was not associated with executive function, and specifically frontal and temporal CBF_{GM} were most important for memory declines (van Dinther et al., 2023). Interestingly, these longitudinal findings from van Dinther et al. (2023) built on previous cross-sectional research within the same participant cohort which found no association between contemporaneous measures of CBF_{GM} and cognitive function (Leeuwis et al., 2020), agreeing with other cross-sectional research (Hshieh et al., 2017; Leeuwis et al., 2018; Poels et al., 2008). Similarly, regarding cerebral artery blood velocity, a greater blood velocity has been associated with less cognitive decline over the ~6.5 years prior to imaging in community dwelling older adults (n=1716, 71±6 years) (Ruitenberg et al., 2005).

Collectively, these data suggest that it is important to consider the temporal component when assessing associations between cerebral haemodynamics and cognitive function. Understanding may be lost if these variables are analysed just at the same timepoint, instead of investigating changes over time. Importantly, longitudinal investigations can determine whether changes in cerebral haemodynamics precede changes in cognitive function to infer causality. Nevertheless, it should be noted that cross-sectional associations between CBF and cognitive function have been documented in older adults. For example, higher global volumetric CBF (normalised for brain volume) was associated with superior cognitive function and to a lower risk of mild cognitive impairment and dementia (n=2489, 80±5 years) (Moonen et al., 2021). Similar cognitive benefits were seen with arterial spin labelling-derived whole-brain CBF, but these effects were limited to older adults (71±5 years) that were European (n=215), and were not present within South Asian (n=151) or African Caribbean (n=87) cohorts (Leeuwis et al., 2018).

In contrast to the majority of this aforementioned research, there is evidence indicating that greater blood flow does not always equate to better cognitive outcomes. The fact that widespread CBF reductions are present in those with dementia is not disputed (Swinford et al., 2023; Zhang et al., 2021). These CBF reductions are also present in mild cognitive impairment, but to a lesser extent, which then worsen upon conversion to dementia (Swinford et al., 2023; Zhang et al., 2021). However, meta-analyses indicate there are certain regions of the brain in mild cognitive impairment that show increased CBF relative to healthy controls (Swinford et al., 2023; T. Tang et al., 2022), including frontal, temporal, and basal ganglia regions, which does not concur with the hypothesis that cerebral hypoperfusion worsens overall brain health and thus cognitive function. These findings are supported by longitudinal research in originally cognitively normal older adults (n=121, 56–86 years) that show both regional CBF reductions (frontal lobe and anterior cingulate cortex) and CBF increases (parietal lobe, insula, and thalamus), measured over ~7 years, were

present in participants who went on to develop clinical cognitive impairment ~11 years after their first CBF measurement (n=22) (Beason-Held et al., 2013).

These region-specific increases appear to occur early along the pathway of clinical cognitive impairment and are thus hypothesised to be indicative of compensation, whereby a specific region is struggling to meet metabolic demands, and thus more blood is delivered in order to try and maintain normal function of the tissue and thus cognitive function (Beason-Held et al., 2013; Dai et al., 2009; X. Wang et al., 2020). Therefore, it is possible that similar regional CBF increases are present in individuals that are currently cognitively normal but may soon begin to show signs of preclinical cognitive impairment. If so, this could contribute to the inconsistent findings from cross-sectional research investigating CBF and cognitive function. Furthermore, research also reports that healthy older adults with lower CBF_{GM} had faster reaction times during an alertness task, possibly linked to superior neural efficiency and thus lower blood flow requirements (Bertsch et al., 2009).

Collectively, these data illustrate a complex relationship between CBF and cognitive function both in cognitively normal and cognitively impaired populations. Nevertheless, generally, higher CBF is associated with better cognitive outcomes, including a slower rate of cognitive decline and a lower risk of dementia in healthy older adults (van Dinther et al., 2023; Wolters et al., 2017). However, regional hyperperfusion is present in mild cognitive impairment (Swinford et al., 2023; T. Tang et al., 2022), possibly indicative of dysfunctional or inefficient tissue that requires compensatory blood flow to maintain normal function. It is likely that this regional hyperperfusion also exists in cognitively normal populations, whereby their neurovascular reserve is used to maintain or slow declines in cognitive function. Indeed, longitudinal regional CBF increases have been shown in healthy older adults (Figure 1.3) (Beason-Held et al., 2007). Similarly, hypoperfusion should not always be assumed to be indicative of worse brain health because it

could be a consequence of improved efficiency of the cerebral tissue (e.g., oxygen extraction and/or utilisation) that would lower blood flow requirements, which could actually benefit cognitive function. Cross-sectional findings are undoubtedly confounded by the heterogeneity in the pre-existing cerebrovascular health status of individuals, influenced by a variety of genetic and lifestyle factors. For example, two individuals could both have low CBF, but one individual has high cognitive function due to superior vascular and/or metabolic efficiency whereas the other individual has poorer cognitive function and will soon develop mild cognitive impairment because of the presence of preclinical (and thus unknown) cerebrovascular disease and cerebral atrophy associated with lower CBF. Therefore, longitudinal studies are required to develop understanding regarding the importance of CBF (and other cerebral haemodynamics) for cognitive function in healthy older adults.

1.3.5 Arterial transit time and cognitive function

In comparison to CBF, limited research has investigated the importance of ATT to cognitive function in older adults. Although ATT lengthens with age (Damestani et al., 2023) and could therefore be considered a marker of vascular brain health, a mechanistic link between ATT and cognitive function is less obvious than with CBF. Whereas CBF reflects the rate of delivery of oxygenated blood to the cerebral tissue, ATT simply represents the time taken for the oxygenated blood to reach the cerebral tissue from large cerebral arteries in the neck. In theory, individuals could have identical CBF, but the time taken for the blood to travel to the tissue could differ. Although a linear, inverse relationship between CBF and ATT is often reported (i.e., higher CBF is accompanied with a shorter ATT, or vice versa) (Pizzini et al., 2024; Yu et al., 2022), ATT prolongation without changes in CBF has been observed in those with vascular cognitive impairment, dementia, and cerebrovascular disease (Gyanwali et al., 2022; Haller et al., 2016), and a lack of CBF-ATT correlation has been reported in healthy adults (Paling et al., 2014).

Furthermore, although CBF experiences considerable age-related changes across the brain, changes in ATT appear to be even more widespread than CBF (Figure 1.1B) (Damestani et al., 2023), and thus may have stronger links with cognitive function.

Any mechanistic links between ATT and cognitive function are likely to relate to the two primary determinants of ATT, cerebral blood velocity and the vascular path length (i.e., the physical distance travelled). Regarding the vascular path length, for example, cerebral artery stenosis could induce a longer ATT by forcing recruitment of other blood vessels to deliver blood to the region downstream of the affected artery, increasing the vascular path length. Even if CBF to that region had since normalised, it is likely that the tissue experienced some degree of ischaemia before alternative vessel recruitment was achieved, possibly resulting in cell death or dysfunction that could influence behavioural outcomes and accelerate normal age-related declines in cognitive function (i.e., longer ATT associated with impaired cognitive function). In contrast, a possible mechanism involving cerebral blood velocity could evoke a positive relationship between ATT and cognitive function (i.e., longer ATT associated with superior cognition). For example, a larger network of small vessels within the brain will increase total vessel cross-sectional area and thus slow blood velocity within these regions of the cerebral vascular tree, ultimately lengthening ATT. In terms of how this could benefit cognitive function, slower blood velocities at the cerebral tissue could increase the time available for gaseous exchange and thus improve cerebral oxygen extraction (Østergaard, 2020). Similarly, if a longer ATT is indicative of a larger network of smaller cerebral vessels, likely including capillaries, reductions in diffusion distance could also aid oxygen extraction (Dunn et al., 2016). Interestingly, there have been tentative links made between a longer ATT or its proxy, a greater spatial coefficient of variation in ASL signal (sCoV), and improved cerebral oxygen extraction in patients with cerebrovascular disease (Hara et al., 2022; Takeuchi et al., 2022). Furthermore, a similar marker to ATT is PET-derived tracer delay time, that also shows a positive association with cerebral oxygen extraction

in patients with cerebrovascular disease (Islam et al., 2017). However, in these cases, a longer ATT is likely induced by adverse structural cerebral changes (e.g., collateral vessel recruitment or cerebral artery stenosis-induced resistance) rather than by a larger network of small cerebral vessels. Thus, the greater oxygen extraction observed in these populations is likely a compensatory response of the tissue to chronic cerebrovascular impairment. These hypotheses are speculative but highlight that if ATT is important for cognitive function, the pathological cause of ATT changes will likely dictate the direction of the relationship.

Based upon the existing, limited, literature investigating ATT and cognitive function, it is generally observed that a longer ATT is associated with poorer cognitive function. For example, a prolonged ATT_{GM} is present in populations with vascular dementia and Alzheimer's disease (Gyanwali et al., 2022; M. Sun et al., 2022). Interestingly, ATT_{GM} prolongation was not observed in individuals with mild cognitive impairment (Gyanwali et al., 2022; M. Sun et al., 2022) but was present in those specifically with vascular cognitive impairment (Gyanwali et al., 2022), indicating that these cerebrovascular changes may occur at a later stage in Alzheimer's disease but manifest early in vascular dementia. Furthermore, cross-sectional data indicate that the degree of ATT_{GM} prolongation in the hippocampus, olfactory, cingulate, inferior frontal, and inferior parietal regions were associated with the severity of clinical cognitive impairment (Mak et al., 2012; M. Sun et al., 2022). However, the cross-sectional nature of these studies means they cannot decipher whether changes in ATT precede and thus dictate changes in cognitive function. Furthermore, no research has investigated the importance of ATT to more conventional measures of cognitive function in healthy older adults (e.g., memory, attention, or processing speed).

Taken together, there is theoretical motivation for investigating relationships between ATT and cognitive function. Namely, that ATT prolongation could be indicative of prior (or sustained) cerebral ischaemia or indicative of changes to cerebral oxygen extraction. Furthermore, age-

related changes to ATT appear to be more widespread than CBF and thus may have stronger links or could be an early marker of potential cognitive decline. Currently, there is limited research on this topic, apart from that a prolonged ATT appears to be evident in neurodegenerative diseases. No attempts have been made to assess the causality of these observations, nor has any research considered the importance of ATT within healthy ageing or explored potential associations between ATT and brain function using standard behavioural testing measures (e.g., memory, attention, or processing speed).

1.4 Methodological approaches to measure cerebral haemodynamics

CBF is typically defined as the blood volume that flows per unit of cerebral tissue mass per unit of time, expressed in mL/100 g/min. The first CBF measurements were based on the Fick principle which states that blood flow to an organ can be calculated by dividing the uptake (i.e., consumption) of a substance by the arteriovenous difference in the amount of the substance present (Fick, 1870). Seminal work measured whole-brain CBF by examining the dynamics of arterial and venous nitric oxide concentrations across the brain in response to nitric oxide inhalation, a physiologically inert substance (Kety and Schmidt, 1945). The rate at which the venous concentration of the inert gas approaches the arterial concentration is proportional to CBF. This work laid the foundations for quantitative CBF measurements in humans, but the technique was limited by its inability to assess CBF in specific regions, the required invasive arterial catheterisation, and some methodological assumptions (Fantini et al., 2016). The same methodological concept was later used with intra-arterial injections of radioactive tracers (^{33}Xe or ^{85}Kr) and assessment of their clearance curves from cerebral tissue in different regions to determine regional CBF measurements (Høedt-Rasmussen et al., 1966). Here, the most commonly used modern techniques to measure cerebral haemodynamics *in vivo* in humans will be discussed.

1.4.1 Duplex Doppler ultrasound

Duplex Doppler ultrasound is a non-invasive technique that can be used to simultaneously measure the blood velocity and diameter of a cerebral artery (Gill, 1985; Hoskins, 1990), commonly the internal carotid and vertebral arteries. A Doppler probe emits sound waves that are reflected by structures in the tissue and moving red blood cells. B-mode imaging provides a two-dimensional longitudinal image of a vessel (i.e., an upper and lower vessel wall separated by the vessel lumen), used to measure vessel diameter with edge-detection software (Hoskins, 1990). Pulse-wave Doppler is used to measure blood velocity, calculated using the Doppler-shift in sound waves reflected from moving red blood cells within the vessel lumen (Hoskins, 1990). Volumetric CBF can then be calculated from blood velocity and vessel diameter measurements using the following equation (Blanco, 2015; Gill, 1985):

$$\text{CBF (mL/min)} = \text{time-averaged mean blood velocity} \times ((\text{diameter}/2)^2 \times \pi) \times 60$$

Volumetric CBF measurements can be made across several pairs of bilateral cerebral arteries and then summed to estimate total volumetric CBF (Liu et al., 2014; Tomoto et al., 2023a). Furthermore, these total volumetric CBF measurements can be normalised for total brain volume by completing an additional T1-weighted structural MRI scan (Liu et al., 2014; Tomoto et al., 2023a). Although, unlike other techniques such as PET or ASL, segmentation of blood flow specifically to grey matter is not possible and the measurements represent blood flow through large cerebral arteries rather than the rate of blood flow at the cerebral tissue. Duplex ultrasound is relatively cheap and accessible, but the quality of measurements are dependent on sonographer experience (Pinto et al., 2013). Regional blood flow analysis with this technique is limited to anterior and posterior territories (i.e., measuring blood flow through arteries which feed these respective regions).

1.4.2 Transcranial Doppler ultrasound (TCD)

Transcranial Doppler (TCD) is another ultrasound technique which, unlike duplex Doppler, can only measure blood velocity and not vessel diameter. Using TCD, Doppler probes are attached to a headset that can insonate intracranial cerebral arteries when orientated through one of three temporal acoustic windows, most commonly imaged is the middle cerebral artery. Cerebral blood velocity, typically measured in cm/s, is used as a proxy for volumetric CBF (i.e., mL/min) because the two variables are theoretically proportional, assuming a constant vessel diameter during the measurement process (Willie et al., 2011). TCD requires relatively simple, low-cost equipment that requires limited training, has high repeatability (Kaczynski et al., 2018), allows measurement over longer time periods, and can be used during exercise. Middle cerebral artery blood velocity measured with TCD is shown to have a moderate correlation (r=0.75) with CBF_{GM} of the middle cerebral artery territory (Rijbroek et al., 2009), but it appears to lack correlation with global CBF_{GM} (r=0.03; Figure 1.8A) and is actually more closely correlated with global ATT_{GM} (r=0.63; Figure 1.8B) (Burley et al., 2021).

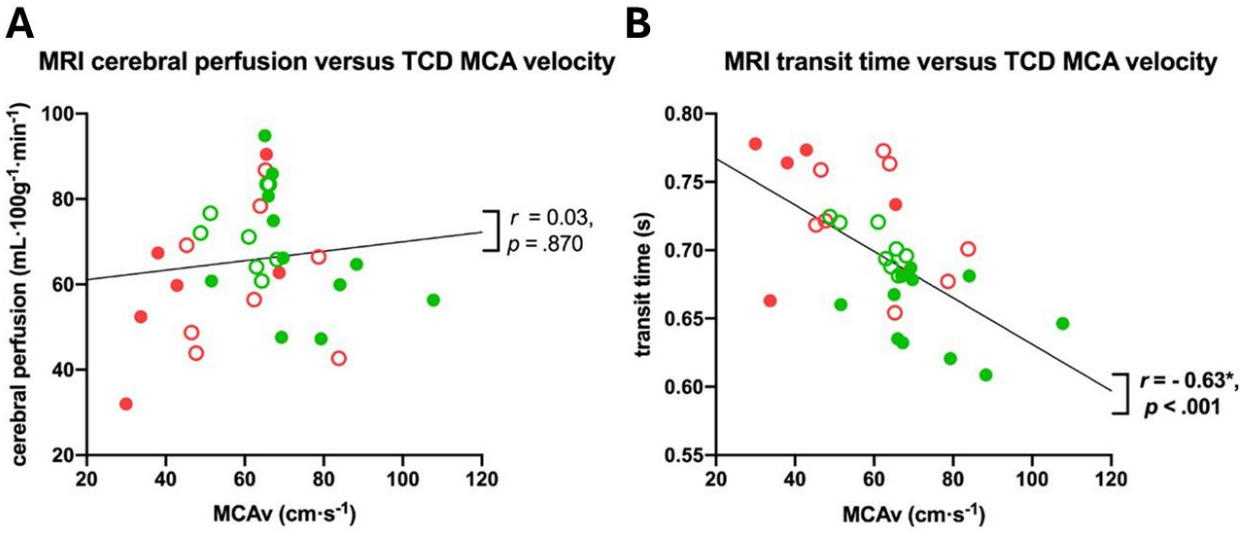

Figure 1.8: Spearman correlations between transcranial Doppler (TCD) measured middle cerebral artery blood velocity (MCA_V) and global grey matter cerebral blood flow (CBF; panel A) or arterial transit time (ATT; panel B). From Burley et al. (2021).

1.4.3 Near-infrared spectroscopy (NIRS)

Near-infrared spectroscopy (NIRS) is a validated non-invasive technique to estimate *in vivo* local tissue oxygenation and perfusion (Mancini et al., 1994). It was first used to detect *in vivo*, real time changes in haemoglobin concentrations in humans in 1977 (Jöbsis, 1977). Human tissue is relatively transparent to near-infrared light emitted by a NIRS probe, but any absorption is oxygen-dependant and determined by the chromophores oxyhaemoglobin (O_2Hb) or deoxyhaemoglobin (HHb) (Mancini et al., 1994). Absolute values of [O_2Hb], [HHb] and [Total Haemoglobin] (tHb) cannot be measured, but the relative change from baseline is detectable based on an application of the modified Beer-Lambert law and the usage of spatially resolved spectroscopy (Al-Rawi et al., 2001). Changes in overall oxygen saturation of the tissue represents the balance between oxygen delivery and consumption (Mancini et al., 1994), whereas assessing changes in total haemoglobin (i.e., both O_2Hb and HHb) offers an index of perfusion. However, absolute measurements of local CBF require tracer tracking techniques, including endogenous oxyhaemoglobin or exogenous indocyanine green (Fantini et al., 2016). Specifically, a bolus of endogenous oxyhaemoglobin delivered to the cerebral tissue is induced by first lowering inspired oxygen fraction and then rapidly increasing it, whereas indocyanine green is injected intravenously (Fantini et al., 2016). Advantages of NIRS include that the relative perfusion metrics are non-invasive and can measure cerebral perfusion metrics over long periods of time, as well as during exercise. Furthermore, modern systems can be wearable and wireless meaning measurements can be performed in a natural environment without restricting movement. However, the NIRS signal can be contaminated by extracerebral tissue and, because typical NIRS systems only have a depth sensitivity of ~1.5 cm, CBF measurements are limited only to the outer cortex (Russo and Senese, 2023). Furthermore, absolute CBF quantification requires gas inhalation or an intravenous tracer injection.

1.4.4 Phase-contrast MRI (PC-MRI)

Whole-brain CBF can be measured using phase-contrast MRI (PC-MRI). The process can be visualised in Figure 1.9. Like duplex Doppler ultrasound, this MRI technique measures blood velocity and the diameter of cerebral arteries, commonly the internal carotid and vertebral arteries (Han et al., 2022; Khan et al., 2017). A preceding three-dimensional time-of-flight magnetic resonance angiographic image is required to locate the desired vessels and ensure perpendicular positioning of the phase-contrast imaging plane relative to the vessels (Figure 1.9B). Using these measures, blood flow through these arteries can be calculated and then summed to give total whole-brain CBF (i.e., mL/min). These values are then paired with an accompanying structural MRI scan that measures total tissue volume, allowing CBF values to be normalised to brain volume (i.e., mL/100 g/min). However, specific segmentation of CBF to grey and white matter regions is not possible with this technique. Appropriate positioning of the imaging slice is essential for accurate estimation of blood velocity and vessel diameter and therefore blood flow measurements are operator dependent, like duplex Doppler.

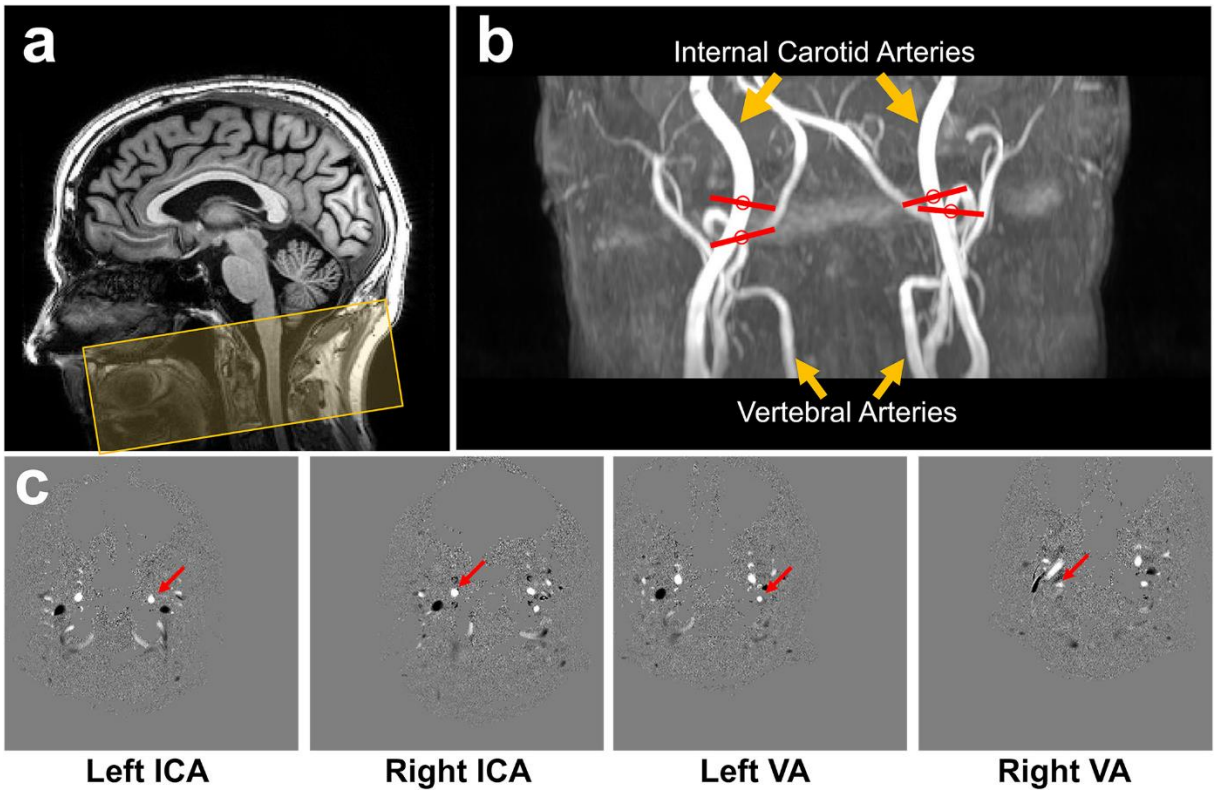

Figure 1.9: Measuring cerebral blood flow using phase-contrast MRI. From Han et al. (2022).

A: Slice position of the three-dimensional time-of-flight angiographic scan performed to visualize the desired arteries.
B: Slice positions of phase-contrast scans overlaid on the angiographic image (red bars). Scans are placed perpendicular to the desired arteries, based on the maximum intensity projection image of the angiogram.
C: Phase images from the phase-contrast scans providing a cross-sectional view of the desired arteries. Vessel cross-sectional area and blood velocity within the vessels can be calculated from these images.

1.4.5 Positron emission tomography (PET)

PET is generally considered the 'gold-standard' technique for direct CBF measurements in humans. It requires intravenous injection or inhalation of ^{15}O-labeled water radiotracers, including measurement of the arterial concentration of the tracer. CBF calculations apply the Kety-Schmidt model (see above) and are based upon the delivery, rather than the clearance, of the tracer to the cerebral tissue, measured in counts per unit tissue mass over the scan duration. Tracer delivery is primarily dictated by arterial inflow whereas clearance can also be affected by diffusion effects and multiple tissue compartments (Fantini et al., 2016). Unlike Doppler or PC-MRI techniques, PET can provide regional CBF quantification. Furthermore, PET can also be used

to calculate cerebral blood volume, cerebral metabolic rate of oxygen or glucose, and oxygen extraction fraction (Wintermark et al., 2005). However, PET has some important limitations, including the need for an on-site cyclotron, exposure to a dose of radiation, high economic costs, and the need for invasive arterial measurements.

1.4.6 Single-photon emission computed tomography (SPECT)

Similarly to PET, SPECT requires the intravenous injection or inhalation of a radiopharmaceutical. Whilst PET uses positron-emitters, SPECT uses gamma-emitter radiotracers, such as ^{133}Xenon, [^{99m}Tc]hexamethylpropylenamine oxime (^{99m}Tc-HMPAO), ^{99m}Tc-Bicisate (ethyl cysteine dimer [ECD]), or ^{123}I inosine–5'-monophosphate (Wintermark et al., 2005). Retention radiotracers pass the blood-brain barrier, are metabolised, and are retained intracellularly by the cerebral tissue. The SPECT system determines the temporal evolution of the radiotracers spatial distribution within the brain (Fantini et al., 2016). Like PET, the Kety-Schmidt model is used to calculate CBF maps, but calculations also need to consider the microsphere principle for the radiotracer used (Wintermark et al., 2005). Similarly to PET, the key limitations of this method are the invasive nature and radiation exposure involved. Given these key limitations, other non-invasive and radiation free approaches are more commonly used in healthy ageing brain imaging research to study cerebral haemodynamics.

1.4.7 *Arterial spin labelling (ASL)*

The MRI sequence arterial spin labelling (ASL) uses MRI scanners to estimate CBF and ATT, which can be segmented in to grey and white matter using an accompanying T1-weighted structural MRI scan. ASL allows both global and regional (region-of-interest or voxel-wise) analyses. It is non-invasive and does not require an exogenous tracer, but instead uses endogenous blood water to estimate resting cerebral haemodynamics. Since its inception over 30 years ago (Detre et al., 1992), many technical advancements have been made and now a variety of labelling schemes,

readout options, and post-processing methods are available (Alsop et al., 2015). ASL measurements are reproducible and agree well with values obtained using [^{15}O]-water positron emission tomography (PET) (Fan et al., 2016).

The ASL technique uses radiofrequency pulses that transiently magnetically label arterial blood water in large cerebral arteries in the neck. The labelled blood then travels into the brain before labelled images are taken at a single or multiple post-labelling delay(s) (Figure 1.10A). Labelling techniques can be pulsed (PASL), continuous (CASL), or pseudo-continuous (PCASL). PASL uses a single or limited number of short radiofrequency pulses (10-20 ms) that labels a slab of tissue, including arterial blood water, that is 10-20 cm thick (Alsop et al., 2015; Iutaka et al., 2023; Wolf and Detre, 2007). In contrast, CASL and PCASL techniques labels blood as it passes through a thinner labelling plane using either a single long radiofrequency pulse (1-3 s) or at least 1000 single radiofrequency pulses at a rate of approximately one per millisecond (Alsop et al., 2015; Wolf and Detre, 2007). Guidelines recommend pseudo-continuous labelling due to superior signal-to-noise ratio and hardware accessibility (Alsop et al., 2015). Each labelled image is paired with a control image for comparison. Alongside magnetically inverting blood water, the radiofrequency pulses used for labelling also cause direct off-resonance effects on image intensity that vary with distance from the labelling plane (Alsop and Detre, 1998; Wolf and Detre, 2007). Therefore, the control image also needs to be exposed to these direct effects, but without labelling the arterial blood water. Originally, this was achieved by applying a radiofrequency pulse distal to the imaged region or by using separate radiofrequency pulses at two planes in the neck (one applied slightly distal to the other) to invert the blood water flowing through twice, thus having no net effect on blood labelling (Alsop and Detre, 1998). More recent PCASL techniques acquire the control image by using identical radiofrequency pulses to the labelling protocol, but every second pulse is phase-shifted by 180°, neutralising the labelling effect on the blood water (Haller et al., 2016). Multiple pairs of control and labelled images (i.e., images with or without out prior

labelling) are taken at each post-labelling delay and are used to estimate CBF through pairwise subtraction of these images (Figure 1.10A), thus removing the static tissue signal and providing a signal difference image that is proportional to CBF (Wolf and Detre, 2007). Although CBF can be estimated from single post-labelling delay ASL, ATT can only be estimated with multiple post-labelling delays, as this allows identification of when the labelled blood arrives to the cerebral tissue.

It is important to choose an appropriate timing when using a single post-labelling delay because the time taken for the blood to reach the cerebral tissue (i.e., ATT) lengthens with age (Damestani et al., 2023) and in clinical populations (Z. Sun et al., 2022; Yu et al., 2022). Therefore, CBF estimations may be underestimated if the single post-labelling delay is too early or too late because the labelled blood may have not yet reached or already passed through the tissue, respectively. The appearance of ASL signal follows an inverted-U response, which can be visualised in Figure 1.10B, meaning CBF will be underestimated when using a post-labelling delay that does not capture the peak ASL signal appearance. Resultant CBF maps from several different single post-labelling delays are shown in Figure 1.10C, with evidence of underestimation in regions where the post-labelling delay is less than ATT. It is recommended that single post-labelling delays of 1500 ms, 1800 ms, or 2000 ms are used for children, healthy adults <70 years old, and healthy adults >70 years old or clinical populations, respectively (Alsop et al., 2015). However, even when choosing a population-appropriate single post-labelling delay, CBF estimation accuracy is lost because it does not account for regional (Dai et al., 2017; Mutsaerts et al., 2015) or individual (Burley et al., 2021; Liu et al., 2012) differences in the time for labelled blood to arrive at the tissue (i.e., ATT). Multiple post-labelling delays can be used to adjust CBF for these regional and individual differences, improving CBF estimation accuracy (Dai et al., 2017; Woods et al., 2024, 2019). An example of an ATT-corrected CBF map produced using multiple post-labelling delays is shown in Figure 1.10C. Both the number and timing of these delays will

affect ATT estimation accuracy. A greater number of delays will improve accuracy but will increase acquisition time and processing requirements. An optimal multiple post-labelling delay range of 200, 975, 1425, 1850, 2025, 2150, 2250, and 2300 ms has been suggested for populations with expected ATT of ≤2000 ms (Woods et al., 2019). When using multiple-delay protocols, it is essential to collect greater volumes of ASL data at the longest delay (i.e., 2300 ms) due to the lower signal-to-noise ratio at longer delays, resulting from the magnetic relaxation time of arterial blood (typically 1300-1750 ms) and that some of the labelled blood has left the brain tissue (Alsop et al., 2015). The aforementioned post-labelling delays should be suitable for most populations, although longer delays may be needed for very elderly populations or those with severe cerebrovascular diseases where ATT could be longer than 2000 ms.

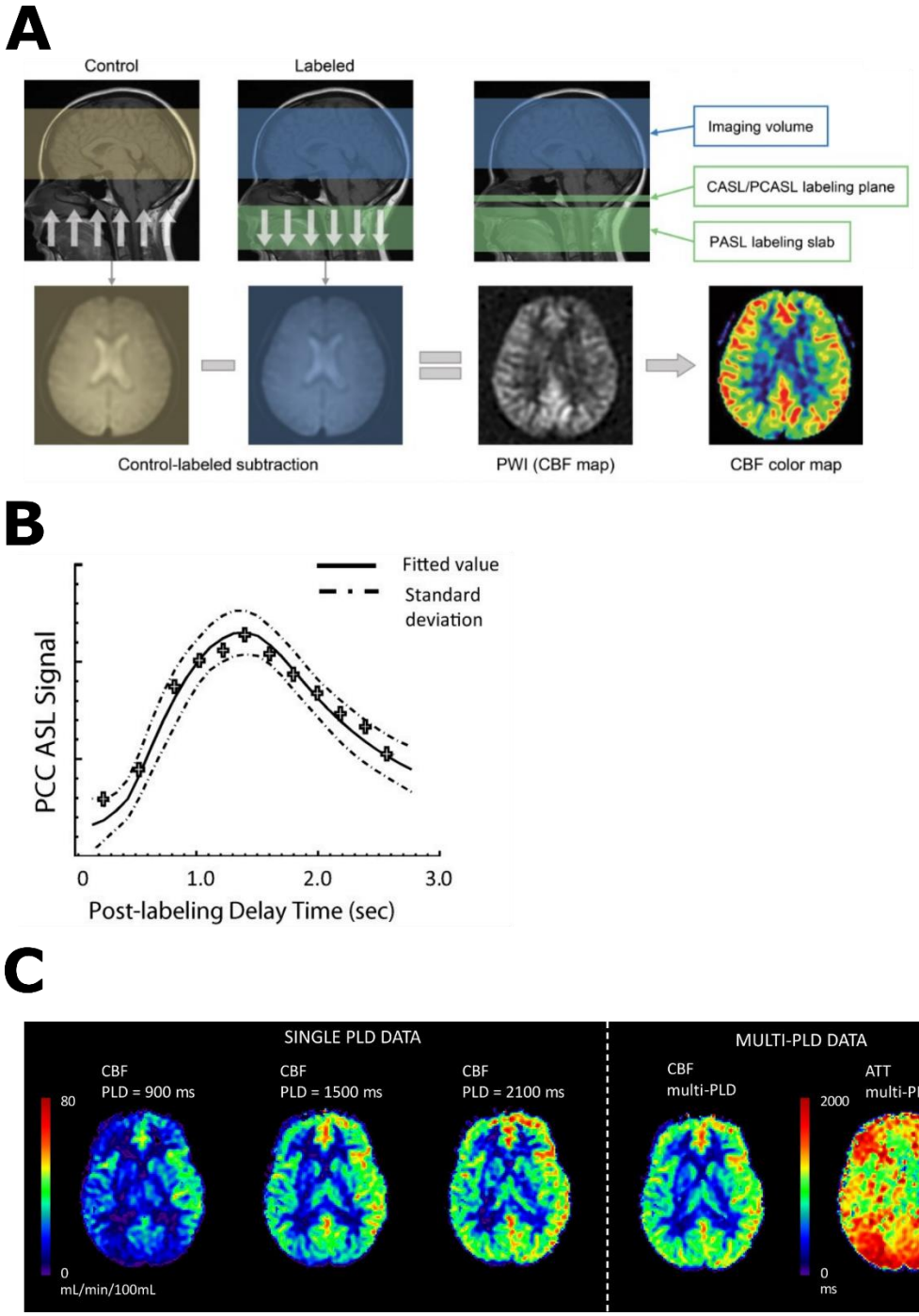

Figure 1.10: Overview of how arterial spin labelling (ASL) is used to estimate cerebral blood flow (CBF) and arterial transit time (ATT).

A: Arterial blood is magnetically labelled in a slab (PASL) or plane (CASL/PCASL) before travelling up to the imaging volume. Control and labelled images are acquired (arrows indicate inversion of arterial blood water in the labelled images) and are then subtracted to estimate CBF. From Iutaka et al. (2012).

B: An example of how the quantity of ASL signal within a region of the brain differs depending on the post-labelling delay used. PCC; posterior cingulate cortex. From Liu et al. (2012).

C: An example of how CBF is underestimated in regions of the brain where the single post-labelling delay (PLD) is shorter than the ATT, including an ATT-corrected CBF map produced using multiple PLDs. From Woods et al. (2024).

Although single post-labelling delay ASL cannot be used to estimate ATT, it can identify the spatial coefficient of variation in ASL signal (sCoV), which is used as a proxy of ATT (Mutsaerts et al., 2017). A spatially heterogeneous ASL signal occurs when ATT exceeds the post-labelling delay, resulting in the presence of high-intensity macro-vascular artefacts (i.e., labelled blood is still within the vasculature, not the tissue). Grey matter sCoV and ATT_{GM} have a strong correlation (r=0.85) (Mutsaerts et al., 2017).

The ASL signal is also susceptible to partial volume error effects, whereby voxels in the brain are not exclusively one type of tissue, but instead contain a mixture of grey and white matter or cerebrospinal fluid. This is particularly relevant for older populations that will have experienced significant brain atrophy, leading to increases in tissue heterogeneity of voxels (Tanna et al., 1991). These partial volume effects can cause underestimation of CBF measurements even when analysing only voxels containing >80% grey matter (Asllani et al., 2008) because blood flow to white matter is far less than to grey matter (Dijsselhof et al., 2023). An algorithm has been developed to correct for ASL images for partial volume error effects (Asllani et al., 2008), which is particularly important for research involving older adult populations (Asllani et al., 2009).

1.4.8 Summary

Although all of these aforementioned techniques have the same aim, to assess resting cerebral haemodynamics *in vivo*, they do so in very different ways and target very different regions of the cerebrovascular tree. For example, duplex Doppler ultrasound and PC-MRI measure blood flow within the largest intra-cranial arteries at the base of the cerebrovascular tree, such as the internal carotid or vertebral arteries. Although both of these techniques ultimately measure the same variables in the same arteries (i.e., cerebral blood velocity and vessel cross-sectional area) to calculate CBF, they do so by using very different techniques and it is less common to normalise Doppler measurements for brain volume as is done using PC-MRI. TCD also uses Doppler

ultrasound, but measures blood velocity (not flow) within vessels downstream of the larger intra-cranial arteries, such as the middle or posterior cerebral artery. The difference between these techniques and ASL or PET is more stark, because ASL and PET estimate CBF at the cerebral tissue (typically grey matter) and is therefore imaging the microvasculature instead of larger cerebral arteries. Furthermore, highly specific regional measurements are possible using ASL or PET, whereas volumetric blood flow measurements within intra-cranial arteries can only give an indication of the flow being delivered to the region of the cerebrovascular tree that the vessel supplies (e.g., anterior or posterior circulation).

Given these inherent methodological differences and the choice for researchers to investigate a selection of different arteries or regions of the brain, comparing results from studies assessing resting cerebral haemodynamics is problematic. Some interesting work has been done comparing agreement between the measurements provided by some of these techniques (Table 1.1) (Tomoto et al., 2023a). Correlations between ASL-derived cortical CBF and normalised whole-brain volumetric CBF measured either with duplex Doppler or PC-MRI-derived were 0.42 and 0.62, respectively. Regarding middle cerebral artery blood velocity (TCD), this had correlations of 0.50, 0.52, or 0.32 with total/cortical CBF (mL/100 g/min) measured using duplex Doppler, PC-MRI, or ASL, respectively. Interestingly, right middle cerebral artery blood velocity (TCD) only had weak correlations with blood velocity and flow within the right internal carotid artery (0.22 and 0.24, respectively). Moderate-to-strong correlations between measures of CBF (mL/100 g/min) indicate that some similarities do exist between the metrics provided by different techniques, but they are not completely comparable. Correlations between these measures, particularly ASL-derived, and TCD-derived blood velocity are however weaker. It also appears that blood velocity measurements from different cerebral arteries may lack comparability. However, it should be considered that Tomoto et al. (2023a) used ASL with a single post-labelling delay of 1525 ms, which is likely too short for the older adults that made up ~35% of the sample, resulting

in CBF underestimations. A smaller study has used multiple post-labelling delay ASL to compare grey matter CBF and TCD-derived middle cerebral artery blood velocity, reporting no correlation (Figure 1.8) (Burley et al., 2021).

Table 1.1: Correlations between CBF measurements by CDUS, MRI, and TCD. From Tomoto et al. (2023a).

	CDUS		PC-MRI		ASL		TCD
	Total CBF	nCBF	Total CBF	nCBF	Cortex	Global brain	CBF velocity
CDUS							
Total CBF		0.819	0.629	0.400	0.300	0.271	0.461
(mL/min)		(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
nCBF			0.497	0.565	0.423	0.427	0.503
(mL/100 g/min)			(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
PC-MRI							
Total CBF				0.872	0.536	0.520	0.515
(mL/min)				(<0.001)	(<0.001)	(<0.001)	(<0.001)
nCBF					0.624	0.637	0.515
(mL/100 g/min)					(<0.001)	(<0.001)	(<0.001)
ASL							
Cortex						0.990	0.318
(mL/100 g/min)						(<0.001)	(<0.001)
Global brain							0.311
(mL/100 g/min)							(<0.001)

Data are Pearson's product-moment correlation coefficients and (P-values). CDUS: color-coded duplex ultrasonography; CBF: cerebral blood flow; nCBF: normalised CBF; PC-MRI: phase contrast magnetic resonance imaging; ASL: arterial spin labelling; TCD: transcranial Doppler. Global brain perfusion was calculated by grey matter and white matter perfusion.

Although not completely comparable, the haemodynamic measurements provided by these techniques are equally insightful because they can give information about a different region, vessel, or metric (i.e., blood flow, blood velocity, or vessel diameter). This is particularly true when comparing techniques measuring blood flow at the cerebral tissue versus within cerebral arteries (i.e., PET or ASL vs. Doppler or PC-MRI, respectively). These techniques should be seen as complimentary and when used simultaneously could allow for greater understanding of how cerebral haemodynamics differ between populations, over time, or in response to a given intervention. Therefore, future studies should consider using a multi-modal approach when assessing cerebral haemodynamics to gain a more comprehensive picture of the cerebrovasculature. Although combining these imaging modalities has the potential to improve

understanding, caution is also needed when comparing research using differing modalities to investigate a similar research question, such as the effects of age, cardiorespiratory fitness, obesity, or blood pressure on resting cerebral haemodynamics. Indeed, conflicting between-study findings regarding such effects can, in part, be due to the imaging modality used. The following section will review this literature, specifically focussing on the impact of cardiorespiratory fitness on resting cerebral haemodynamics in healthy older adults.

1.5 Cardiorespiratory fitness and brain health in older adults

This section aims to provide an overview of the literature regarding the relationship between cardiorespiratory fitness and cerebral haemodynamics, specifically in healthy older adults. First, age-related changes in cardiorespiratory fitness will be discussed before outlining how cardiorespiratory fitness affects both general and brain health outcomes, including the rationale for investigating its relationship with cerebral haemodynamics. Next, the state of existing cross-sectional and intervention research will then be described and summarised, highlighting how differences in study design and measurement techniques may contribute to observed results. The section will conclude by identifying gaps in the literature and suggesting potential options for future research.

1.5.1 Age-related changes to cardiorespiratory fitness

Cardiorespiratory fitness, most commonly defined as peak oxygen consumption ($\dot{V}O_{2peak}$), declines as we age (Letnes et al., 2023). Longitudinal research highlights how this decline is non-linear and accelerates in later life (Figure 1.11A), particularly for males (Jackson et al., 2009; Letnes et al., 2020). This decline is inevitable and is contributed to by age-related declines in muscle mass (Lexell, 1995) and the accumulation of damage to our cardiovascular and metabolic systems (Ferrari et al., 2003; Grevendonk et al., 2021; Singam et al., 2019). It is also true that our physical activity patterns change as we get older, whereby we spend less time engaging with

moderate-to-vigorous intensity physical activity and more time engaging with sedentary behaviours (Hallal et al., 2012), although changes to these behaviours do not appear to change the rate at which cardiorespiratory fitness declines (Fleg et al., 2005). However, a higher cardiorespiratory fitness level for any given age can be achieved by maintaining higher physical activity levels (Figure 1.11B) (Fleg et al., 2005; Letnes et al., 2020), participating in exercise training (Fosstveit et al., 2024a), or through dietary habits that prevent adverse metabolic changes (i.e., metabolic syndrome) (Letnes et al., 2020). Taken together, these data highlight how age-related cardiorespiratory fitness declines are unavoidable and actually accelerate as we get older. Crucially, however, modifiable lifestyle factors can augment cardiorespiratory fitness across the lifespan, which may be particularly important to a healthy ageing process.

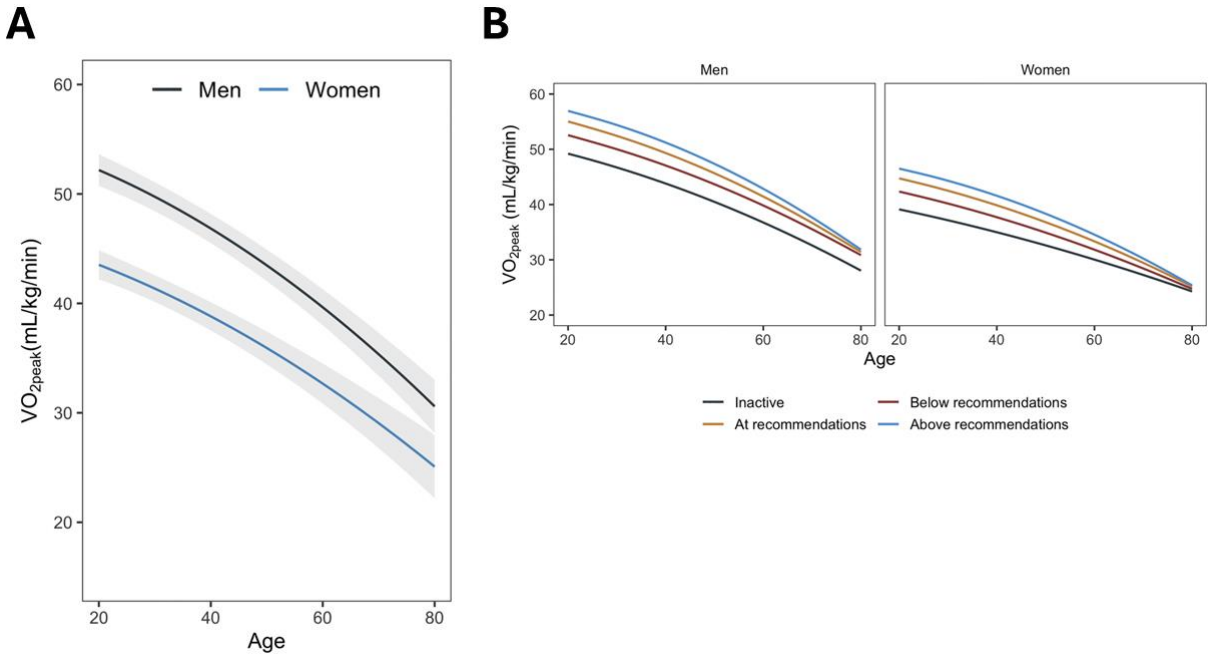

Figure 1.11: Longitudinal changes in cardiorespiratory fitness ($\dot{V}O_{2peak}$) over the lifespan.

A: Cardiorespiratory fitness decreases with age, with greater rate of decline in later life. This acceleration is more prominent in males. From Letnes et al. (2020).

B: Physical activity levels have minimal impact on the rate of age-related cardiorespiratory fitness declines, but higher physical activity levels help maintain higher cardiorespiratory fitness for a given age. From Letnes et al. (2020).

1.5.2 Benefits of cardiorespiratory fitness to general and brain health

Unsurprisingly, higher levels of cardiorespiratory fitness are beneficial to practically all aspects of health. For example, those with higher fitness have a lower risk of cardiovascular and neurodegenerative diseases, as well as overall mortality (Lang et al., 2024; Tari et al., 2019). These broader health benefits result from a myriad of physiological adaptations that are associated with higher fitness levels through engagement with regular physical activity or exercise training, such as lower blood pressure (Edwards et al., 2023), lower arterial stiffness (Tanaka, 2019), greater endothelial function (Green et al., 2017; Smith et al., 2021), angiogenesis (Green et al., 2017), erythropoiesis (Mairbäurl, 2013), improved body composition and blood lipid profile (Katzmarzyk et al., 2001; Westerterp, 2018), mitochondrial biogenesis and efficiency (Memme et al., 2021; Steiner et al., 2011; Trevellin et al., 2014), lower inflammation (Cerqueira et al., 2020), greater grey matter volume and neuronal integrity (Erickson et al., 2014, 2012), and greater white matter integrity (Maleki et al., 2022). The physiological and disease reducing benefits of maintaining higher cardiorespiratory fitness levels are indisputable and, importantly, engagement with exercise training also improves not only the physical, but also the psychological component of quality of life (Raafs et al., 2020).

1.5.3 Effects of cardiorespiratory fitness and exercise training on cognitive function

Although there is large individual variability (Gorbach et al., 2017; Habib et al., 2007; Nyberg et al., 2020), most aspects of cognitive function decline with age (Gorbach et al., 2017; Nyberg et al., 2020; Rönnlund et al., 2005; Schaie, 1994), which has a detrimental impact on quality of life and the ability to live independently in later life (Salthouse, 2012; Stites et al., 2018). Therefore, it is important to identify modifiable lifestyle factors that can improve or maintain cognitive function, enabling a healthy ageing process. Cardiorespiratory fitness has understandably received a lot of attention because of the aforementioned central, peripheral, and cerebral physiological

adaptations associated with cardiorespiratory fitness gains portray an enticing, and often assumed, link between cardiorespiratory fitness and superior cognitive function. The cardiorespiratory fitness hypothesis states that any cognitive benefits of engaging with physical activity are mediated by the vascular, metabolic, and pulmonary physiological adaptions associated with cardiorespiratory fitness gains (Figure 1.12) (Voss and Jain, 2022). Therefore, specifically cardiorespiratory fitness, rather than the volume or intensity of exercise, is suggested to be most predictive of cognitive benefits.

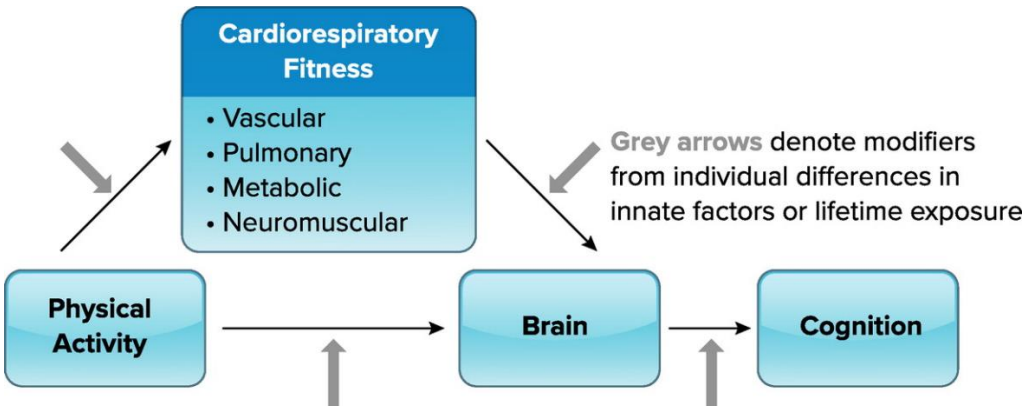

Figure 1.12: The cardiorespiratory fitness hypothesis posits that the benefits of physical activity on cognitive aging are mediated by physiological adaptations associated with cardiorespiratory fitness. An extension of the hypothesis is that the benefits of fitness on the aging brain are mediated through a direct effect on brain cerebrovascular health, metabolism, structure, and neuronal function. From Voss and Jain (2022).

Indeed, there is evidence from several meta-analyses indicating that exercise training in healthy older adults has beneficial effects on cognitive function (Barha et al., 2017; Colcombe and Kramer, 2003; Ludyga et al., 2020; Northey et al., 2018; Sanders et al., 2019; Zhang et al., 2023), particularly for executive function and memory domains (Colcombe and Kramer, 2003; Zhang et al., 2023). Furthermore, it appears that potential benefits are not restricted to just aerobic exercise, with similar or even greater effect sizes reported in response to resistance exercise training (Ludyga et al., 2020; Northey et al., 2018; Zhang et al., 2023). In support of the cardiorespiratory fitness hypotheses, there is also evidence highlighting the specific importance

of cardiorespiratory gains, rather than just simply exercise training participation. For example, the magnitude of cardiorespiratory fitness gains has been associated with the magnitude of cognitive improvements both in studies with (Kovacevic et al., 2020; Vidoni et al., 2015) and without (Brown et al., 2021; Maass et al., 2015; Voss et al., 2012) group-level differences in cognitive performance (i.e., control vs. exercise intervention). However, from these meta-analyses it is still unclear as to what exercise intensity or intervention length produces the best cognitive benefits. Moreover, these cognitive benefits may only be small (Sanders et al., 2019) whilst other meta-analyses in older adults have reported no benefits of aerobic exercise on cognitive performance (Kelly et al., 2014; Young et al., 2015).

Both the within- and between-study variability in exercise-induced cognitive function changes is likely explained by the individual characteristics of the sample, including, but not limited to, their health and education status, baseline cardiorespiratory fitness level, and their genetics. For example, those who achieve higher educational attainment tend to experience less age-related cognitive declines (Lövdén et al., 2020). It is therefore likely that highly educated older adults will experience less, or even no, cognitive benefits from exercise training because their cognitive function has been relatively well maintained (i.e., ceiling effect). Similarly, cardiorespiratory fitness levels are higher in older adults who have healthier lifestyle patterns (i.e., physical activity and diet) (Letnes et al., 2020) or even just more favourable genetics (Bouchard et al., 1999). Both baseline cardiorespiratory fitness level (Fuentes Artiles et al., 2023; Laddu et al., 2018; Pihlainen et al., 2020) and genetics (Bouchard et al., 1999) have been shown to influence the fitness gains experienced in response to exercise training, which are hypothesised to be a crucial instigator of cognitive benefits (Voss and Jain, 2022). Therefore, older adults with individual characteristics that predispose them to experience larger cardiorespiratory fitness gains in response to a given training stimulus may also be predisposed to experience larger benefits in cognitive function. It should also be considered that frequent engagement with cognitively demanding activities during

leisure time (e.g., socialising, reading, musical instrument) is associated with superior cognitive function in older adults (Christelis and Dobrescu, 2020; Kelly et al., 2017; H.-X. Wang et al., 2013; Wang et al., 2022), and thus older adults who engage with these types of activities may not experience any further benefits from exercise training, even if they are currently physically inactive. Finally, it is possible that inconsistencies between studies may be due to differences in the baseline brain health of the sample studied, irrespective of any cardiorespiratory fitness gains. For example, worse baseline brain integrity, assessed as periventricular white matter lesion severity, was associated with less improvement in working memory and processing speed following a six-month intervention, despite comparable fitness gains between high and low lesion severity groups (von Cederwald et al., 2023).

Collectively, these data do indicate that exercise training and cardiorespiratory fitness can have beneficial effects of cognitive function, but these links are not as robust or linear as is commonly assumed. Any potential cognitive benefits of exercise training are likely dependant on a variety of individual characteristics, whereby those with the greatest capacity to improve cardiorespiratory fitness and/or brain health will experience the largest benefits. Furthermore, the potential underlying mechanisms explaining any exercise training-induced cognitive improvements in older adults are poorly understood and will be discussed in the following section.

1.5.4 Mechanisms explaining potential exercise-induced cognitive benefits

Broadly speaking, there are two primary hypotheses regarding how exercise may improve cognitive function, the vascular hypothesis and the neurogenesis hypothesis (Barnes et al., 2021). The vascular hypothesis was based on the prevalence of vascular dysfunction in those with cognitive impairment, whereby deterioration in any region of the cerebrovascular tree will ultimately affect neuronal health and thus cognitive function (de la Torre, 2018, 2004). The neurogenesis hypothesis suggests that neurotrophic factors released in response to exercise

have beneficial effects on neurogenesis and neuroplasticity (Stillman et al., 2020; van Praag et al., 1999). It is unlikely that only one of these hypotheses is true, but instead that both hypotheses work in tandem to explain exercise-induced cognitive benefits. The cardiorespiratory fitness hypothesis combines these two hypotheses in a more general manner, stating that cognitive benefits are mediated by physiological adaptions associated with cardiorespiratory fitness gains (Voss and Jain, 2022), thus encompassing both vascular and neural components. Cardiorespiratory fitness is most commonly defined as the maximal capacity of skeletal muscle to take in and use oxygen and is therefore not specific to brain health. Cardiorespiratory fitness is primarily dictated by systemic cardiovascular and pulmonary adaptations, as well as skeletal muscle-specific adaptations, including skeletal muscle capillarisation, mitochondrial biogenesis, and increased glycogen storage (Hughes et al., 1993; Liu et al., 2022; Memme et al., 2021; Meredith et al., 1989). Although these skeletal muscle-specific adaptations are essential for increasing maximal oxygen consumption, they are unlikely to be key contributors to exercise-induced cognitive benefits. Similar cerebral-specific vascular, metabolic, and structural adaptations are generally considered to be more important (Voss and Jain, 2022), which occur alongside systemic cardiovascular adaptations (e.g., arterial stiffness, endothelial function, erythropoiesis). Thus, brain-specific exercise-induced adaptations could work to improve the delivery of oxygenated blood to the cerebral tissue, the extraction of oxygen from the blood by the cerebral tissue, or the utilisation of oxygen within the cerebral tissue.

Regarding exercise training-induced cerebral vascular adaptations, rodent studies show that exercise training increases cerebral capillarisation (Morland et al., 2017; Stevenson et al., 2020). In humans, exercise training has been shown to promote small vessel cerebral angiogenesis (Bullitt et al., 2009), reduce vessel tortuosity (Bullitt et al., 2009), increase cerebrovascular reactivity (Bailey et al., 2013; Marley et al., 2020), and reduce cerebral artery stiffness (Tarumi et al., 2013; Tomoto et al., 2023b). Similar metabolic adaptations to those that occur within skeletal

muscle are also evident in the brain, with rodent studies demonstrating that exercise training increases both the number and efficiency of mitochondria within the cerebral tissue (Braga et al., 2021; Dominiak et al., 2022; Steiner et al., 2011). Regarding structural cerebral adaptations, cardiorespiratory fitness has been associated with superior neuronal integrity (Erickson et al., 2012; Gonzales et al., 2013), grey matter volume (Erickson et al., 2014), and white matter integrity (Maleki et al., 2022). Furthermore, cardiorespiratory fitness has been associated with functional connectivity in frontal, temporal, and parahippocampal gyri (Voss and Jain, 2022).

Signalling factors including brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) support angiogenesis, neurogenesis, and synaptic plasticity, and are thus hypothesised to mediate some of the aforementioned exercise-induced cerebral adaptations (Cotman et al., 2007; Voss et al., 2013). Indeed, peripheral increases in these signalling factors occur following an acute exercise bout in both younger (Dinoff et al., 2017; Hashimoto et al., 2018; Kujach et al., 2020; Rasmussen et al., 2009; Weaver et al., 2021) and older adults (Babaei et al., 2014; Coelho et al., 2014, 2012; Tsai et al., 2021; Walsh et al., 2016). In older adults, lower levels of peripheral BDNF are associated with hippocampal atrophy and cognitive decline (Erickson et al., 2010) and therefore exercise-induced increases may be particularly important to offset age-related BDNF declines (Erickson et al., 2010; Lommatzsch et al., 2005).

Interestingly, in humans, evidence indicates that exercise-induced lactate exposure may be involved with the peripheral BDNF response to acute exercise (Ferris et al., 2007; Kujach et al., 2020). Lactate can cross the blood-brain barrier and act on its receptor, HCAR1, that are highly expressed within the brain (Lauritzen et al., 2014). Lactate is thought to support myelination (Ichihara et al., 2017) as well as acting as a substrate for neurons (Quistorff et al., 2008; van Hall et al., 2009). The uptake of lactate by the brain is increased by exercise and is dependent on the

arterial lactate concentration (Hashimoto et al., 2018; Quistorff et al., 2008; van Hall et al., 2009). Larger exercise-induced increases in blood lactate concentration have been associated with larger increases in peripheral BDNF and VEGF (Ferris et al., 2007; Kujach et al., 2020), and even simply injecting lactate into humans stimulates can increase peripheral BDNF levels (Schiffer et al., 2011). Furthermore, larger exercise-induced lactate exposures have been associated with superior cognitive performance post-exercise (Ballester-Ferrer et al., 2022; Jacob et al., 2023; Skriver et al., 2014). Moreover, in rodents, exercise training-induced increases in cerebral capillary density were mediated by lactate-dependant VEGF stimulation, with identical effects occurring in non-exercising rodents that were just injected with lactate (Morland et al., 2017). Similarly, exercise training in rodents increased hippocampal lactate levels which mediated increases in BDNF expression and memory performance (El Hayek et al., 2019). Collectively, these data indicate that repeated lactate exposure may partially mediate some of the cerebral adaptations observed in response to exercise training, which could consequently lead to cognitive benefits. As such, high-intensity exercise training may elicit stronger cerebral adaptations because of greater lactate exposures.

As previously mentioned, cerebral haemodynamics are inherently crucial to brain health because the brain lacks intracellular energy stores, meaning that substrate and thus energy availability is dictated by blood flow to the cerebral tissue (Öz et al., 2007; Zimmerman et al., 2021). Given the potential links between cerebral haemodynamics and cognitive function (de la Torre, 2018; Rajeev et al., 2023; van Dinther et al., 2023; Wolters et al., 2017), there has been speculation regarding the involvement of exercise training-induced changes to cerebral haemodynamics explaining potential cognitive improvements (Barnes et al., 2021; Bliss et al., 2023; Cabral et al., 2019; Davenport et al., 2012). Indeed, some of the aforementioned central and cerebral adaptations to exercise training have the theoretical capacity to influence cerebral haemodynamics. For example, changes in central and cerebral arterial vessel diameters are essential for CBF

regulation (Claassen et al., 2021) and thus training-induced increases in vessel elasticity and endothelial function may improve CBF regulatory efficiency. Similarly, training-induced cerebral angiogenesis and capillarisation may increase the capacity of the cerebrovascular system to deliver blood to the tissue. Indeed, indices of capillarisation have been associated with greater resting skeletal muscle perfusion (Betz et al., 2023; Weber et al., 2007, 2006). Furthermore, changes in cerebral haemodynamics may underpin some of the other training-induced cerebral adaptations, such as to grey and white matter integrity (Bouhrara et al., 2022; Kiely et al., 2023; Zonneveld et al., 2015) or increased functional connectivity (Galiano et al., 2020; Huang et al., 2021; Wu et al., 2023). Despite all of these theoretical links between exercise-training induced adaptations and cerebral haemodynamics, the impact of exercise training on cerebral haemodynamics in healthy older adults, and whether any changes mediate cognitive improvements, is poorly understood. The following sections will summarise existing cross-sectional and intervention literature investigating whether cerebral blood velocity, CBF, and ATT are associated with cardiorespiratory fitness and how these haemodynamic markers respond to exercise training, including whether any changes are important mediators of cognitive improvements.

1.6 Effects of cardiorespiratory fitness and exercise training on cerebral haemodynamics

1.6.1 *Cross-sectional studies*

Due to its practicality and non-invasive nature, transcranial Doppler ultrasound has been commonly used to assess associations between resting cerebral blood velocity and cardiorespiratory fitness (summarised in Figure 1.13). One of the most commonly cited papers on this topic was published in 2008, reporting that endurance trained adults (n=154) had ~17% greater middle cerebral artery blood velocity (MCA_V) compared to sedentary peers (n=153) across the lifespan (Figure 1.13A) (Ainslie et al., 2008). Furthermore, a positive association between

cardiorespiratory fitness and MCA_V was evident in the combined sample when controlling for age (Ainslie et al., 2008). A subsequent study showed similar differences in MCA_V between sedentary and trained adults, reporting a positive simple correlation between cardiorespiratory fitness and MCA_V in older adults (n=42, 68±5 years; Figure 1.13B) (Bailey et al., 2013). However, a significant limitation of both of these studies was that all participants were male whereas sex differences are known to exist in cerebral haemodynamics (Alwatban et al., 2021; Damestani et al., 2023; Lu et al., 2011). More recent studies have been conducted including female participants, but these report less conclusive findings. For example, across the life span (n=153, 18–90 years), there was only a positive correlation between cardiorespiratory fitness and MCA_V in females, not in males or the combined sample (Figure 1.13C) (Zeller et al., 2022). Furthermore, this positive correlation did not survive adjustment for age, and there was actually a negative age-adjusted association in the combined sample (Zeller et al., 2022). In another mixed-sex sample (n=157, 18–72 years), positive cardiorespiratory fitness-MCA_V correlations were observed in male, female, and combined groups (Figure 1.13D), but only the female association survived adjustment for age, and none of the associations survived further adjustment for sex (combined only), age, BMI, and $\dot{V}O_{2max}$ test modality (Lefferts et al., 2022).

Collectively, the reported findings from these cross-sectional studies make it difficult to make definitive conclusions regarding associations between cardiorespiratory fitness and resting cerebral blood velocity, primarily because of the differing age ranges and sex-compositions of the samples used, but also differing statistical controls. Early work appeared to robustly show positive associations in males (Ainslie et al., 2008; Bailey et al., 2013), including after statistical adjustment for age (Ainslie et al., 2008). These findings were somewhat replicated in both males and females, but associations did not survive statistical adjustment for age (Lefferts et al., 2022; Zeller et al., 2022) or were in fact negative in direction after age-adjustment (Zeller et al., 2022).

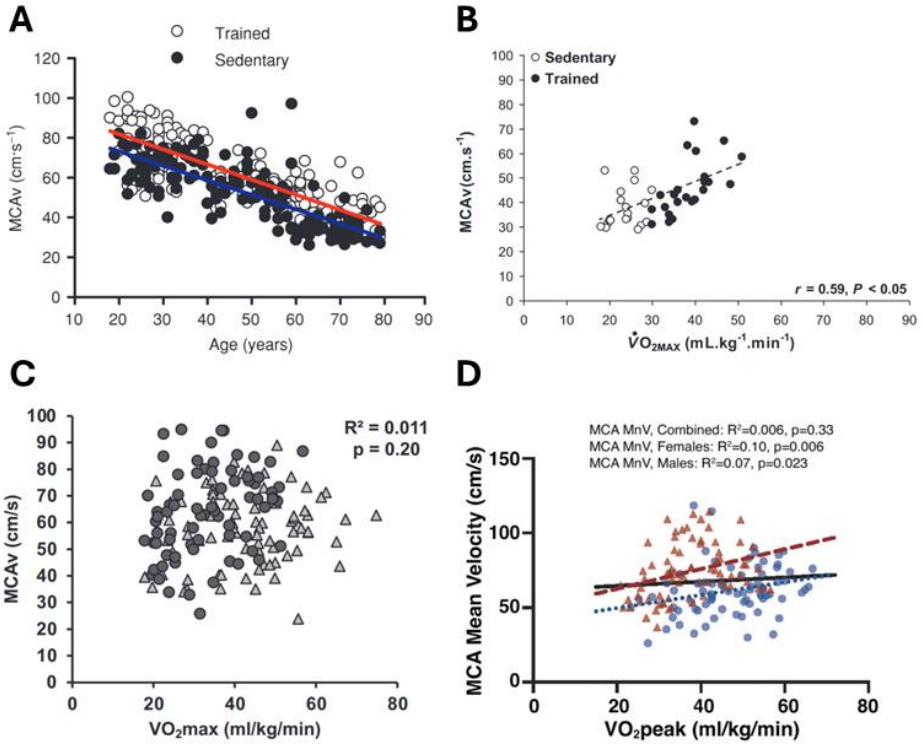

Figure 1.13: Cross-sectional associations between cardiorespiratory fitness ($\dot{V}O_{2max/peak}$) and middle cerebral artery blood velocity (MCA_V).

A: Endurance trained male adults have greater MCA_V across the life span compared to sedentary peers. Including linear regression lines (n=307, red; trained, blue; sedentary). From Ainslie et al. (2008).
B: Older adult males with greater $\dot{V}O_{2max}$ have greater MCA_V (n=43). From Bailey et al. (2013).
C: No correlation between $\dot{V}O_{2max}$ and MCA_V in a combined sample of males and females (n=153, triangles and circles, respectively), but a positive correlation was evident in females only. Adjusting for age revealed a negative association in the combined sample. From Zeller et al. (2022).
D: Positive correlation between $\dot{V}O_{2peak}$ and MCA_V in males (circles), females (triangles), and the combined sample (n=157). Only the female association survived adjustment for age, but none survived further adjustment for sex (combined only), age, BMI, and $\dot{V}O_{2peak}$ test modality. From Lefferts et al. (2022).

Cerebral artery blood velocity (including MCA_V), measured with Doppler ultrasound, is commonly used as a proxy for CBF, but cross-sectional studies specifically investigating CBF using other imaging modalities in healthy adults are also available. Practically all available studies have used arterial spin labelling (ASL) to estimate resting CBF (summarised in Table 1.2). Several studies have demonstrated positive associations between cardiorespiratory fitness and regional CBF in older adults. For example, one study used a composite relative CBF measure, including the inferior temporal gyri, angular gyri, and posterior cingulate regions (Figure 1.14A); however, the

positive association was only evident in female participants (Dougherty et al., 2020). Similarly, a positive association has been reported in the default mode network (Figure 1.14B) (Johnson et al., 2016). Specifically grey matter CBF (CBF_{GM}) has also demonstrated positive associations with cardiorespiratory fitness in frontal and parietal regions, as well as globally (Figure 1.14C); however, fitness was only estimated from an equation using simple demographic information (Zimmerman et al., 2014). Interestingly, this study also reports that reductions in estimated cardiorespiratory fitness fully explain the relationship between age and CBF_{GM} (Zimmerman et al., 2014). Other studies have also compared groups of older adults based on training status (i.e., long-term endurance trained vs. sedentary). For example, there were no differences in absolute CBF_{GM} between sedentary and endurance trained older adults globally or in any lobular region, but voxel-wise analysis revealed endurance trained older adults had higher relative CBF_{GM} in posterior cingulate and precuneus regions (Thomas et al., 2013). Furthermore, absolute CBF_{GM} of the posterior cingulate and precuneus was significantly lower than a younger adult control group for sedentary older adults, but not in the trained older adults (Figure 1.14D); indicating that endurance training may reduce age-related declines within these regions (Thomas et al., 2013). A similar study in middle-aged/older adults also reports mixed results, whereby CBF_{GM} in the occipitoparietal area was greater in those who were endurance trained compared to sedentary, but there were no differences in the hippocampus, precuneus, or posterior cingulate (Tarumi et al., 2013). More recently, it has been demonstrated that only sedentary middle-aged/older adults, and not those who are in endurance trained, have significantly lower CBF across the whole cerebral cortex in comparison to a young control group (Figure 1.14E) (Sugawara et al., 2020), extending previous (more isolated) findings (Thomas et al., 2013) that endurance training may limit age-related CBF reductions.

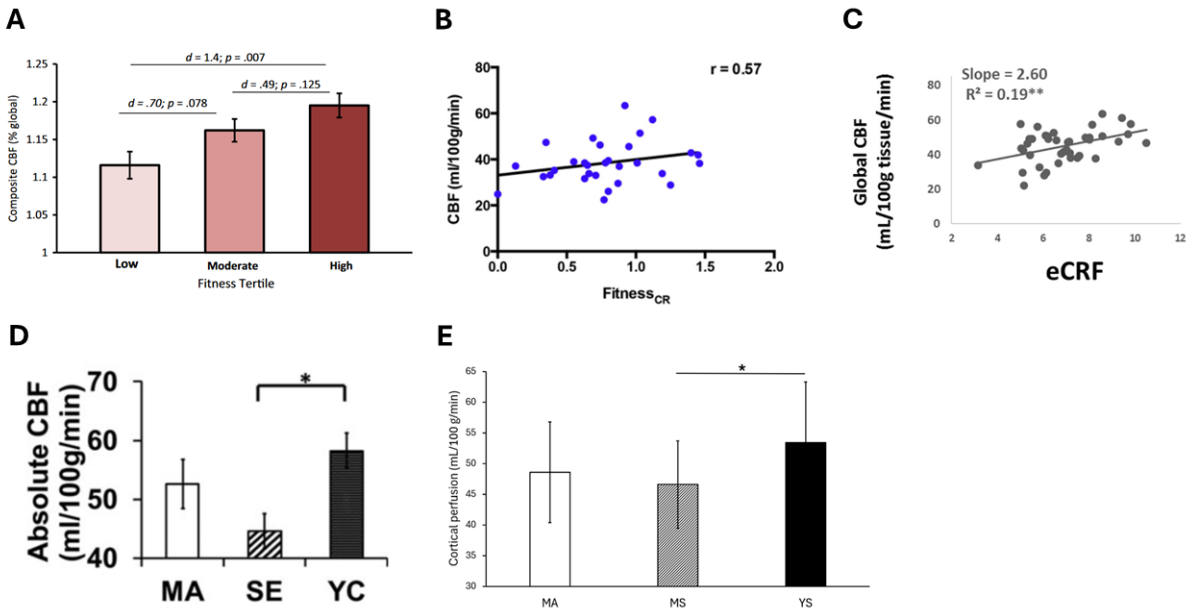

Figure 1.14: Cross-sectional studies showing a positive association between cardiorespiratory fitness and cerebral blood flow (CBF) in healthy older adults.

A: A positive association using composite relative CBF in female older adults, but not males (male data not shown). From Dougherty et al. (2020).

B: A positive association in the default mode network CBF. From Johnson et al. (2016).

C: A positive association between grey matter global CBF and estimated cardiorespiratory fitness (eCRF). From Zimmerman et al. (2014).

D: Sedentary (SE), but not endurance trained (MA), older adults show significant grey matter CBF declines compared to young controls (YC) in posterior cingulate and precuneus regions. From Thomas et al. (2013).

E: Sedentary (MS), but not endurance trained (MA), middle-aged/older adults show significant CBF declines compared to young sedentary controls (YS) across the cerebral cortex. Figure made using data in Table 2 from Sugawara et al. (2020).

However, in contrast to the reported positive cross-sectional associations between cardiorespiratory fitness and CBF, numerous studies have reported the opposite (summarised in Table 1.2 and Figure 1.15). For example, negative associations were observed in regions that demonstrated significant relationships between cardiorespiratory fitness and cerebrovascular reactivity (including large portions of temporal, parietal cortices and smaller amounts of the frontal lobes) (Figure 1.15A); however, there were no significant relationships identified globally or from general voxel-wise analysis (Intzandt et al., 2020). Furthermore, a significant negative association was reported with global CBF_{GM}, but this did not survive adjustment for age and sex

(*P*=0.08) (Olivo et al., 2021). Voxel-wise analysis from the same study revealed a negative association in the left hippocampus (adjusted for age and sex) (Olivo et al., 2021). Further adding to the disparities, other research also reports a lack of cross-sectional association between cardiorespiratory fitness and global or regional CBF in healthy older adults (Figure 1.15B and 1.15C) (Burley et al., 2021; Flodin et al., 2017; Krishnamurthy et al., 2022).

Regarding ATT, only one of these aforementioned studies used ASL with multiple post-labelling delays (Burley et al., 2021), allowing estimation of ATT. In this small sample of older adults (n=14), there was no correlation between ATT and cardiorespiratory fitness (Figure 1.15D). These findings do agree with other (more recent) cross-sectional research reporting no robust associations between cardiorespiratory fitness and cerebral blood velocity (Lefferts et al., 2022; Zeller et al., 2022), given that ATT and MCAv are correlated (Figure 1.15B) (Burley et al., 2021).

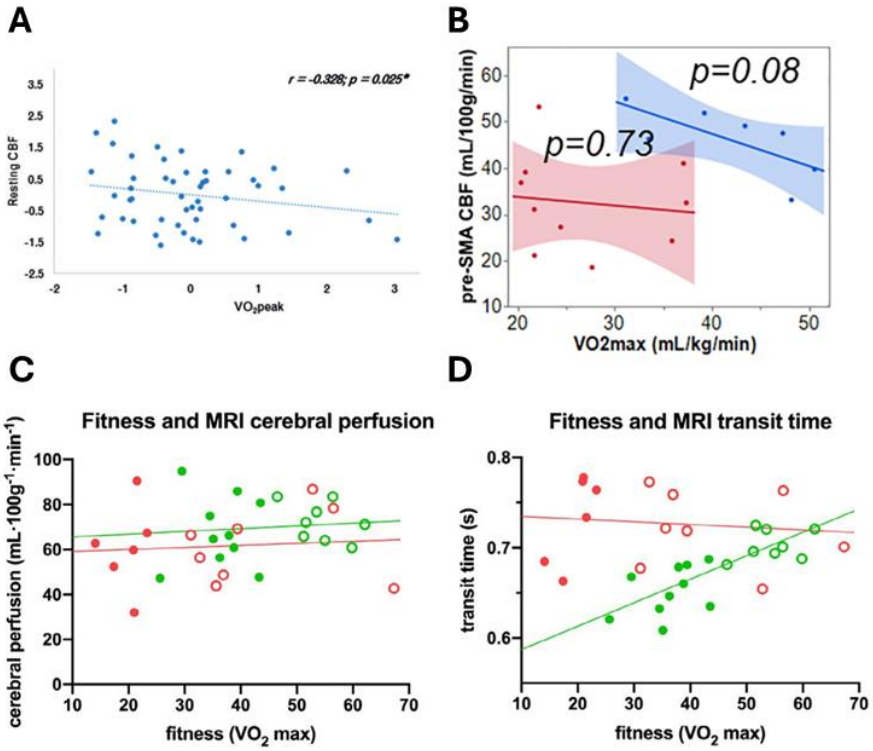

Figure 1.15: Cross-sectional studies showing a negative or lack of association between cardiorespiratory fitness ($\dot{V}O_{2max/peak}$) and cerebral blood flow (CBF) or arterial transit time (ATT) in healthy older adults.

A: A negative association in a region of interest including large portions of temporal, parietal cortices and smaller amounts of the frontal lobes. From Intzandt et al. (2020).

B: No significant association within the pre-supplementary motor area (red; older adults, blue; younger adults). From Krishnamurthy et al. (2022).

C: No significant association with global or regional grey matter CBF (regional data not shown - red circles; older adults, green circles; younger adults). From Burley et al. (2021).

D: No significant association with global or regional grey matter ATT (regional data not shown - red circles; older adults, green circles; younger adults). From Burley et al. (2021).

Table 1.2: Summary of findings from cross-sectional studies investigating associations between cardiorespiratory fitness and cerebral blood flow and arterial transit time in healthy older adults.

Publication	Sample	Imaging modality	CBF region	CRF	Results
Burley et al. (2021)	n=15 10M:5F 67±7 years	PASL *PLDs:* *400, 600, 800, 1000, 1200, 1400, 1600, 1800 ms*	▪ Global ▪ Frontal lobe ▪ Parietal lobe ▪ Occiptal lobe ▪ Motor lobe ▪ Cingulate gyrus (grey matter) *ATT was also estimated for the same regions*	$\dot{V}O_{2peak}$ *Choice of cycle ergometer or treadmill*	No association between $\dot{V}O_{2peak}$ and CBF or ATT (any region). No differences in CBF or ATT between high and low fit older adults (in any region).
Dougherty et al. (2020)	n=100 27M:63F 64±6 years	PCASL *PLD: 2025 ms*	▪ Composite ROI - Inferior temporal gyri (L/R) - Angular gyri (L/R) - Posterior cingulate	$\dot{V}O_{2peak}$	Postitive association between $\dot{V}O_{2peak}$ and composite CBF in females, but not in male participants.
Flodin et al. (2017)	n=55 25M:30F 69±3 years	PCASL *PLD: 2000 ms*	▪ Global ▪ Voxel-wise	$\dot{V}O_{2peak}$	No association between $\dot{V}O_{2peak}$ and CBF from global or voxel-wise analyses.
Intzandt et al. (2020)	n=50 17M:33F 63±5 years	PCASL *PLD: 900 ms*	▪ Global ▪ Voxel-wise ▪ Composite ROI - Includes regions with a significant $CVR_{CO_2}/\dot{V}O_{2peak}$ relationship - Large portions of temporal and parietal cortices, and smaller amounts of the frontal lobe (grey matter)	$\dot{V}O_{2peak}$	No association between $\dot{V}O_{2peak}$ and CBF from global or voxel-wise analyses. Negative association between $\dot{V}O_{2peak}$ and composite CBF.

Johnson et al. (2016)	n=30 9M:21F 64±3 years	Q2TIPS PASL	▪ Default mode network	$\dot{V}O_{2peak}$	Postitive association between $\dot{V}O_{2peak}$ and CBF in the default mode network.
Krishnamurthy et al. (2022)	n=12 6M:6F 67±5 years	PCASL *PLD: 1800 ms*	▪ Pre-supplemntary motor area (pre-SMA) (grey matter)	Estimated $\dot{V}O_{2max}$ *Sub-maximal cycle ergometer test (YMCA)*	No association between estimated $\dot{V}O_{2peak}$ and CBF in the pre-SMA.
Olivo et al. (2021)	n=49 25M:24F 70±3 years	PCASL *PLD: 2000 ms*	▪ Global ▪ Voxel-wise (grey matter)	$\dot{V}O_{2peak}$	Negative association between $\dot{V}O_{2peak}$ and global CBF. - *P*=0.08 after correcting for age and sex. Voxel-wise analyses identified negative association between $\dot{V}O_{2peak}$ and left hippocampus CBF.
Sugawara et al. (2020)	*Young* n=21 10M:11F 29±6 years *Sedentary* n=21 11M:10F 53±5 years *Trained* n=21 11M:10F 53±4 years	PCASL *PLD: 1525 ms*	▪ Cortical	$\dot{V}O_{2peak}$	No between-group difference in cortical CBF between sedentary and endurance trained middle-aged/older adults. Compared with young adults, cortical CBF was significantly lower in sedentary middle-aged/older adults, but there was no difference with endurance trained older adults.
Tarumi et al. (2013)	n=36 13M:23F	ASL labelling approach and	▪ Hippocampus ▪ Precuneus	$\dot{V}O_{2peak}$	Endurance trained middle-aged/older adults had greater occipitoparietal CBF than sedentary peers.

	52±2 years	PLD were not reported	▪ Posterior cingulate ▪ Occipitoparietal (grey matter)		No between-group differences in hippocampal, precuneus, or posterior cingulate CBF.
Thomas et al. (2013)	*Young* n=9 5M:4F 27±4 years *Sedentary* n=10 8M:2F 74±6 years *Trained* n=10 7M:3F 75±6 years	PCASL *PLD: 1525 ms*	▪ Global ▪ Frontal lobe ▪ Temporal lobe ▪ Parietal lobe ▪ Occipital lobe ▪ Insula ▪ Cerebellum ▪ Subcortical ▪ Entire cerebrum (grey matter)	$\dot{V}O_{2peak}$	No difference in global absolute CBF or relative CBF of any region between sedentary and endurance trained older adults. Voxel-wise analyses identified that endurance trained older adults had greater relative CBF in posterior cingulate and precuneus regions than sedentary peers. Compared with young adults, absolute CBF in posterior cingulate and precuneus regions was significantly lower in sedentary older adults, but there was no difference with endurance trained older adults.
Zimmerman et al. (2014)	n=41 19M:22F 69±8 years	TILT PCASL *PLD: 500 ms*	▪ Global ▪ Frontal ▪ Parietal (grey matter)	Estimated $\dot{V}O_{2max}$ *Equation (demographics/resting HR/self-reported activity)*	Postitive association between estimated $\dot{V}O_{2peak}$ and global frontal, and parietal CBF.

ATT; arterial transit time, CBF; cerebral blood flow, CRF; cardiorespiratory fitness, CVR_{CO2}; cerebrovascular reactivity to carbon dioxide, HR; heart rate, L; left, R; right, M:F; male:female, PASL; pulsed arterial spin labelling, PCASL; pseudo-continuous arterial spin labelling, PLD; post-labelling delay, Q2TIPS; thin slice periodic saturation sequence, ROI; region-of-interest, TILT; transfer insensitive labelling technique, $\dot{V}O_{2peak/max}$; peak/maximal oxygen consumption.

Evidently, based on research to date, there is no clear consensus regarding the cross-sectional associations between cardiorespiratory fitness and CBF in older adults. Studies that do show associations (positive or negative) only do so in small regions, apart from two studies that report positive (Zimmerman et al., 2014) or negative (Intzandt et al., 2020) global effects. However, Zimmerman et al. (2014) estimated cardiorespiratory fitness using an equation and did not control for age, and when Intzandt et al. (2020) statistically controlled for age, the relationship was no longer significant (P=0.08). Indeed, variations in the statistical controls used and the demographics of the sample studied may partly explain between-study differences. Furthermore, differences in ASL acquisition parameters are also likely to contribute to the disparities between findings. For example, for single post-labelling delay ASL, which all studies used besides Burley et al. (2021), the recommended delay timing is 1800 or 2000 ms in healthy adults aged less or greater than 70 years, respectively (Alsop et al., 2015). However, some studies, including two reporting global effects, used delays shorter than this recommendation (Intzandt et al., 2020; Sugawara et al., 2020; Thomas et al., 2013). Moreover, Tarumi et al. (2013) failed to report the labelling technique or post-labelling delay used. As discussed in Section 1.4.7 above, post-labelling delays that are too short will systematically underestimate CBF because the magnetically labelled blood has not yet fully arrived at the imaged tissue (Dai et al., 2017). Assuming all the individuals within a given sample have the same ATT, this systematic underestimation would not affect the validity of any observed associations with cardiorespiratory fitness. However, as outlined earlier in this chapter, ATT differs based upon age, sex, blood pressure, and stenosis prevalence (Damestani et al., 2023; Yetim et al., 2023; Yu et al., 2022), and potentially other factors such as BMI or indeed cardiorespiratory fitness. As such, these individual variations in ATT mean making robust conclusions from single-delay ASL data problematic. The one study using multiple post-labelling delay ASL (which can estimate ATT) reported no associations between ATT_{GM} and cardiorespiratory fitness in older adults (Burley et al., 2021), but

the sample was small (n=15) and the longest delay used was only 1800 ms, which may still not be long enough to capture the full spectrum of ATT observed in older adult populations (i.e., >2000 ms) (Dai et al., 2017; Woods et al., 2024, 2019).

Finally, an important limitation of all these studies is their cross-sectional design, meaning that it is impossible to infer causality from any of the observed associations. The following section will discuss results from randomised controlled trials that investigated the effects of exercise training on cerebral haemodynamics in healthy older adults.

1.6.2 *Intervention studies*

There is a considerable amount of literature investigating how resting cerebral blood velocity responds to exercise training; however, similarly to the aforementioned Doppler-based cross-sectional research, the findings are also inconsistent. This is evident in results from a meta-analysis including eight studies (six randomised controlled trials) in middle-aged and older adults (Smith et al., 2021). Pooled data indicated exercise training has no significant effect on MCA_V. Four of these studies specifically included healthy adult participants, which also had mixed results. The two healthy adult participant studies that reported increases in MCA_V used non-randomised control groups and exclusively female cohorts (Akazawa et al., 2012; Bailey et al., 2016), and one only found differences in peak MCA_V (not mean) (Akazawa et al., 2012). Another systematic review that included fifteen studies with a mixture of designs and populations (i.e., young and older participants, healthy and non-healthy participants, randomised and non-randomised controlled trials, or pre-post studies) found only three reported significant changes (increases) in MCA_V following exercise training (Kleinloog et al., 2023). The meta-analysis (Smith et al., 2021) and systematic review (Kleinloog et al., 2023) on this topic highlights the need for more high quality studies (i.e., randomised controlled trials) in order to better understand how cerebral blood velocity responds to exercise training. Moreover, many different populations (e.g., age range and

health status) and exercise interventions (e.g., short-, medium-, or long-term, exercise intensity, aerobic and/or resistance exercise) have been studied meaning between-study comparisons are limited. The following paragraph will give a brief summary of key results from the randomised controlled trials in heathy older adults included within these two aforementioned review articles, as well as more recently published studies that are not part of these reviews.

A 28-week intervention of approximately three sessions per week involving a mixture of aerobic walking exercise and circuits (50–70% of maximum heart rate) was conducted in generally healthy but sedentary older adults (control; n=21, exercise; n=22) (Vicente-Campos et al., 2012). This intervention had no effect on MCA_V in the right or left artery; however, changes in cardiorespiratory fitness (i.e., $\dot{V}O_{2max}$) were not measured. Intervention efficacy was instead assessed with a 2.4-kilometre walking test, whereby velocity during the final two minutes of the test increased in the exercise group. Similarly, no changes were observed in mean, systolic, or diastolic MCA_V in healthy older adults following 16-weeks of taekwondo training (control; n=18, exercise; n=19) (Cho and Roh, 2019). Five taekwondo sessions were completed per week at 50–80% of maximum heart rate, which did improve performance in the chair stand test and two-minute step test, but changes in $\dot{V}O_{2max}$ were not measured. Another study investigated both MCA_V and PCA_V following 24-weeks of land or water-based walking exercise at 50–65% of heart rate reserve, three times per week (control; n=22, land exercise; n=19, water exercise; n=22) (Green et al., 2021). Despite increases in cardiorespiratory fitness ($\dot{V}O_{2max}$) within both exercise groups, no differences in MCA_V or PCA_V were observed. A pilot study has also been done in female breast cancer survivors who were free from other cancers or cardiovascular/cerebrovascular diseases (Northey et al., 2019). Participants completed either 12-weeks of moderate-intensity continuous training (MICT; 55–65% peak power) or high-intensity interval training (HIIT; 30 s x 4–7 intervals at ~105% peak power separated by 2-minute active recovery) three times per week (control; n=6, MICT; n=5, HIIT; n=6). There were no significant changes in MCA_V within either group, but this is likely confounded by the

small sample size of the groups (pre-to-post MCA_V changes of ~-0.2, ~1.9, and ~3.5 cm/s for control, MICT, and HIIT, respectively). Additionally, a longer, one-year, exercise intervention in older adults, involving 3–5 sessions per week at an intensity of 75–85% or 85–95% of maximum heart rate, showed no differences in mean, systolic, or diastolic MCA_V (control; n=22, exercise; n=21) (Sugawara et al., 2022). Duplex Doppler ultrasound was also used to measure blood velocity within the right and left internal carotid and vertebral arteries (control; n=27, exercise; n=28), but again no changes were observed in the exercise group (Tomoto et al., 2023b)

Collectively, these randomised controlled trials do not indicate that exercise training induces changes to cerebral blood velocity in healthy older adults. Although these studies were randomised controlled trails, only three reported adherence to the exercise intervention (Green et al., 2021; Northey et al., 2019; Sugawara et al., 2022), with ~80% attendance, which could have a large impact on subsequent cerebrovascular adaptations. Furthermore, of these three, only two reported the adherence to the prescribed exercise intensity (Northey et al., 2019; Sugawara et al., 2022), rather than simply session attendance. Interestingly, the majority of these studies used continuous, moderate intensity exercise, but the only study to report notable non-significant differences in MCA_V was a pilot study involving just 12-weeks of HIIT, where there was a large effect size when comparing changes between the control and HIIT groups (Northey et al., 2019). Furthermore, in this pilot study, HIIT induced larger cardiorespiratory fitness improvements than MICT (~3.5 vs. ~1.3 mL/kg/min, respectively). These data support the cardiorespiratory fitness hypothesis of cerebral adaptations to exercise training (Voss and Jain, 2022) and indicate that high-intensity exercise may elicit greater cerebral adaptations.

Of the Doppler-based studies described above, none assessed associations between individual changes in cerebral blood velocity and cardiorespiratory fitness. Further, in general, the sample sizes used have been relatively small, and exercise interventions relatively short (e.g., 12-weeks).

Differences in cerebral blood velocity may be apparent following longer-term exercise training (i.e., a decade), as indicated by data from cross-sectional studies comparing trained older adults with their sedentary peers (Ainslie et al., 2008; Bailey et al., 2013). Moreover, apart from two studies (Green et al., 2021; Tomoto et al., 2023b), only blood velocity within the middle cerebral artery has been investigated, potentially missing effects in other regions. Given the inverse association between cerebral blood velocity and ATT (Burley et al., 2021), these findings would indicate that exercise training has minimal impact on ATT in older adults. However, currently no research has explicitly investigated ATT, and as discussed above, it is possible that exercise training could change the path length of the cerebrovasculature (e.g., angiogenesis or tortuosity reductions), another key determinant of ATT.

Compared with cross-sectional studies investigating associations between cardiorespiratory fitness and CBF, there are fewer studies assessing how specifically CBF responds to exercise training in healthy older adults. Similarly to these cross-sectional (and Doppler-based blood velocity) studies, the available randomised controlled trials also have mixed and conflicting results (summarised in Table 1.3). For example, voxel-wise analysis in sedentary older adults (control; n=19, exercise, n=18) found increased relative CBF within the anterior cingulate cortex following 12-weeks of moderate-intensity continuous training (thrice weekly at 50–75% of maximum heart rate) (Figure 1.16A) (Chapman et al., 2013). However, relative hippocampal CBF and absolute global CBF were unchanged. Furthermore, cardiorespiratory fitness gains were relatively small (~5.7%), despite the participants in this study's having low baseline levels (~19.3 mL/kg/min) (Chapman et al., 2013). Another 12-week moderate-intensity continuous training programme (thrice weekly at 65–80% of maximum heart rate) also found no significant group × time interaction for hippocampal CBF in older adults (control; n=16, exercise, n=16) (Maass et al., 2015). Separate within-group ANOVAs were also reported, identifying no changes in the control group but *reductions* to hippocampal CBF in the exercise group. However, in contrast to apparent

group-level CBF reductions, there was a positive partial correlation between changes in cardiorespiratory fitness (assessed as oxygen consumption at ventilatory threshold) and CBF within the hippocampus and global grey matter within the exercise group (Figure 1.16B) (Maass et al., 2015). Regional differences in CBF have also been reported following only an 8-week exercise intervention in generally healthy, but overweight, males (n=17, cross-over), involving 30-min, thrice weekly sessions at 70% of maximal power (Kleinloog et al., 2019). Specifically, global grey matter CBF was unchanged but voxel-wise analysis found increased CBF (~26–28%) within two clusters located approximately within the subcallosal cortex or anterior cingulate cortex. However, another cluster, located approximately within the temporal fusiform gyrus or parahippocampal gyrus, experienced CBF reductions (~19%) (Figure 1.16C).

Only a minority of studies have used longer exercise interventions, which theoretically allow more time for physiological adaptations to occur that may influence cerebral haemodynamics. However, a six-month, thrice weekly aerobic training programme (40–80% of maximum heart rate) in heathy, but sedentary, older adults induced no changes in global or regional CBF (control; n=25, exercise, n=30), despite considerable increases in cardiorespiratory fitness (~30%) (Flodin et al., 2017). Furthermore, changes in cardiorespiratory fitness were not associated with changes in CBF within the exercise group, or when combining with the control group, who also experienced (smaller) cardiorespiratory fitness increases (~20%) (Flodin et al., 2017). In contrast, increases to hippocampal CBF have been reported in a sample of generally healthy, but inactive, older adults (control; n=15, exercise; n=29) in response to a one-year exercise intervention (150 mins/week at 40–85% of heart rate reserve) (Kaufman et al., 2021). However, these hippocampal CBF increases were only present in participants who were apolipoprotein E4 (APOE4) carriers (n=14) (Figure 1.16D), a genetic risk factor for Alzheimer's disease. Furthermore, another one-year exercise intervention (control; n=27, exercise; n=28) that involved 3–5 sessions per week of combined moderate- and high-intensity continuous exercise (75–85% or 85–95% of maximum heart rate,

respectively) induced global volumetric CBF increases in older adults (Figure 1.16E), measured with duplex Doppler (Tomoto et al., 2023b). Interestingly, changes in volumetric CBF were positively associated with changes in cardiorespiratory fitness (Figure 1.16F) and negatively associated with changes in carotid artery stiffness within the exercise group.

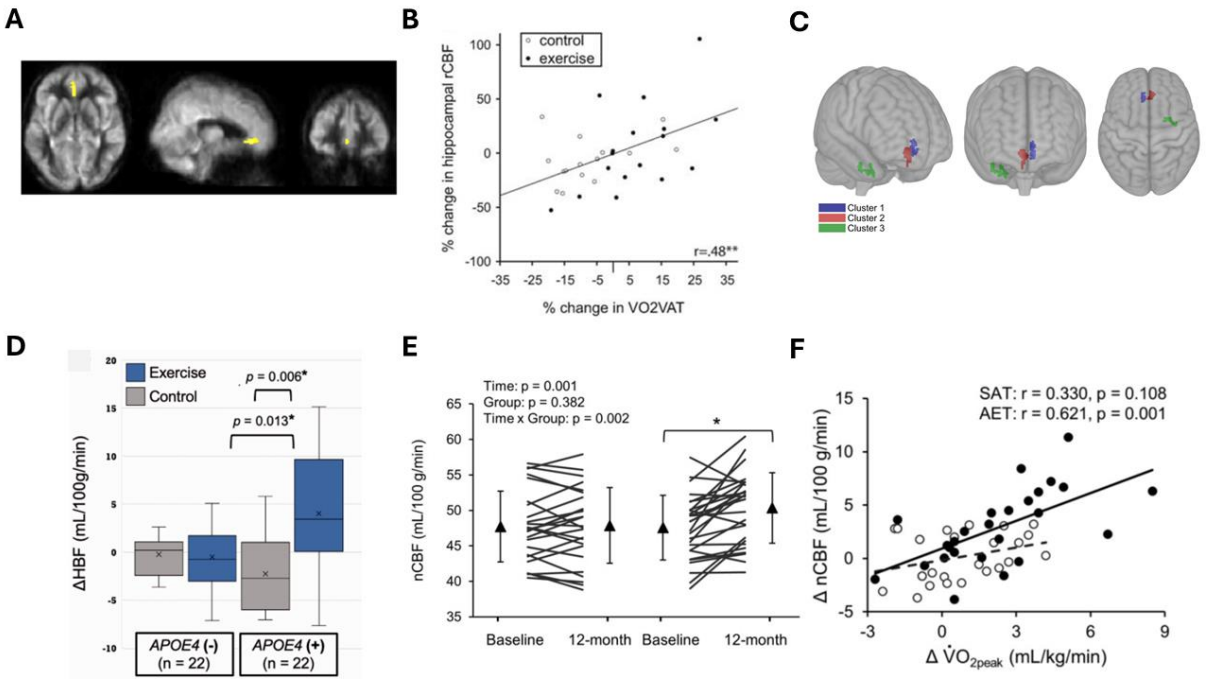

Figure 1.16: Randomised controlled trials investigating cerebral blood flow (CBF) responses to exercise training in healthy older adults.

A: Voxel-wise analysis finds increased relative CBF in the anterior cingulate cortex (yellow highlighted region) following 12-weeks exercise training. No changes were observed in absolute global CBF or relative hippocampal CBF. From Chapman et al. (2013).

B: A positive association between pre-to-post intervention (12-weeks) changes in hippocampal CBF and changes in cardiorespiratory fitness (VO2VAT; oxygen consumption at ventilatory threshold) in control and exercise participants. The relationship is also significant within the exercise group only, and with global grey matter CBF. At group-level, hippocampal CBF within the exercise group appeared to decrease (6.5 mL/100 g/min). From Maass et al. (2015).

C: Voxel-wise analysis finds cluster 1 and 2 to increase following 8-weeks exercise training in males (6.4 and 7.0 mL/100 g/min, respectively), but decreases in CBF within cluster 3 (4.4 mL/100 g/min). From Kleinloog et al. (2019).

D: Hippocampal CBF increased following a one-year exercise intervention, but only in apolipoprotein E4 (APOE4) carriers (n=14). From Kaufman et al. (2021).

E: Global volumetric CBF, normalised for total brain volume (nCBF), increased following one-year exercise training. Volumetric blood flow through the internal carotid and vertebral arteries were summed to calculate global volumetric CBF. From Tomoto et al. (2023b).

F: A positive association between pre-to-post intervention changes (one-year) in global volumetric CBF, normalised for total brain volume (nCBF), and cardiorespiratory fitness ($\dot{V}O_{2peak}$) within the exercise group, but not the control group. From Tomoto et al. (2023b).

Table 1.3: Summary of findings from studies investigating the cerebral blood flow responses to exercise training in healthy older adults.

Publication	Sample	Imaging modality	CBF	CRF	Intervention	Results
Chapman et al. (2013)	*Control* n=19 5M:14F 64±4 years *Exercise* n=18 5M:13F 64±4 years	PCASL *PLD: 1525 ms*	▪ Global ▪ Hippocampus ▪ Voxel-wise	$\dot{V}O_{2max}$	▪ 12-weeks ▪ Thrice-weekly ▪ 50 mins at 50-75% HR_{max} *All participants >90% attendance*	$\dot{V}O_{2max}$ increased ~1.1 from 19.3±0.9 mL/kg/min. No change in absolute global CBF or relative hippocampal CBF. Voxel-wise analyses identified increased relative CBF in the anterior cingulate cortex.
Flodin et al. (2017)	*Control* n=25 11M:14F 69±3 years *Exercise* n=30 14M:16F 68±3 years	PCASL *PLD: 2000 ms*	▪ Global ▪ Voxel-wise	$\dot{V}O_{2peak}$	▪ 6-months ▪ Thrice weekly ▪ 30-60 mins at 40-80% HR_{max} *Attendance ~85%*	$\dot{V}O_{2peak}$ increased ~6.5 from 21.4±3.8 mL/kg/min. - Control group also increased 4.0 from 19.6±3.3 mL/kg/min. No group-level changes in global or voxel-wise CBF. No association between $\Delta\dot{V}O_{2peak}$ and ΔCBF within control, exercise, or combined groups.
Kaufman et al. (2021)	*Control* n=15 5M:10F 72±5 years *Exercise* n=29 12M:17F 71±5 years	PASL *PLD: 700 ms*	▪ Hippocampus	$\dot{V}O_{2max}$	▪ 1-year ▪ 150 mins/week ▪ 40-85% HRR *Adherence not clearly reported*	$\dot{V}O_{2max}$ increased ~2.7 from ~22 mL/kg/min. Hippocampal CBF increased in APOE4 carriers (4.1±1.3 mL/100 g/min) No change in non-carriers (-0.5±1.2 mL/100 g/min).

Kleinloog et al. (2019)	n=17 17M:0F 67±2 years (cross-over)	PCASL *PLD: 2000 ms*	▪ Global ▪ Voxel-wise (grey matter)	$\dot{V}O_{2peak}$	▪ 8-week ▪ Thrice weekly ▪ 30-mins at 70%W_{max} *All participants >92% attendance*	$\dot{V}O_{2peak}$ increased 262±236 mL/min. No group-level change in global CBF. Voxel-wise analyses - Increased CBF in two clusters (26–28%; subcallosal cortex/ACC/frontal medial cortex). - Reduced CBF in one cluster (19%; temporal fusiform gyrus/parahippocampal gyrus).
Maass et al. (2015)	*Control* n=19 8M:11F 68±4 yrs *Exercise* n=21 10M:11F 69±5 years	Gadolinium contrast-based MRI	▪ Global ▪ Hippocampus (grey matter)	$\dot{V}O_2$ at ventilatory threshold (VAT)	▪ 12-weeks ▪ Thrice weekly ▪ 30 mins at 65-80% HR_{max} *Adherence not reported*	*Only n=16 from each group had CBF data (some analyses used imputed data).* $\dot{V}O_2$ at VAT increased ~10.4%. No between-group changes in hippocampal CBF. Within-group analyses indicate reductions in hippocampal CBF within the exercise. - –6.5 mL/100 g/min Positive association between $\Delta\dot{V}O_2$ at VAT and Δglobal CBF or Δhippocampus CBF within the exercise group and combined (exercise and control) group.
Tomoto et al. (2023b)	*Control* n=27 8M:19F 68±5 years *Exercise* n=28 7M:21F 68±5 years	2D duplex ultrasound to measure volumetric blood flow through cerebral arteries Structural MRI scan allowed normalisation	▪ Global (ICA+VA) ▪ ICA ▪ VA	$\dot{V}O_{2peak}$	▪ 1-year ▪ 2-3 sessions per week at 75–85% HR_{max} ▪ 1-2 sessions per week at 85–95% HR_{max} ▪ 25-40 mins each *~80% sessions completed at the prescribed intensity*	$\dot{V}O_{2peak}$ increased ~2.2 from 22.7±3.7 mL/kg/min. Global volumetric CBF increased ~2.8 mL/100 g/min (6±7%). Total volumetric blood flow (ICA+VA) increased ~26 from 585 mL/min - Specifically, volumetric flow in the bilateral ICA, but not the VA, increased. MCA_V did not change.

	of blood flow (mL/min) to total brain tissue volume (mL/100 g/min)		Positive $\Delta\dot{V}O_{2peak}$–Δglobal CBF association in the exercise group, but not controls. Negative $\Delta\dot{V}O_{2peak}$–Δcarotid stiffness association in the exercise group, but not controls. Negative Δglobal CBF–Δcarotid stiffness association in the exercise group, but not controls. Positive Δglobal CBF–ΔMCA_V association in the exercise group, but not controls.

ACC; anterior cingulate cortex, APOE4; apolipoprotein E4, ATT; arterial transit time, CBF; cerebral blood flow, CRF; cardiorespiratory fitness, CVR_{CO2}; cerebrovascular reactivity to carbon dioxide, HR_{max}; maximum heart rate, HRR; heart rate reserve, ICA; internal carotid artery, MCA_V; middle cerebral artery blood velocity, M:F; male:female, MRI; magnetic resonance imaging, PASL; pulsed arterial spin labelling, PCASL; pseudo-continuous arterial spin labelling, PLD; post-labelling delay, VA; vertebral artery, $\dot{V}O_{2peak/max}$; peak/maximal oxygen consumption, W_{max}; maximum wattage, 2D; two-dimensional.

Evidently, there is a lack of high-quality research investigating how CBF responds to exercise training in healthy older adults. Moreover, the research that is available from randomised controlled trials by no means supports the commonly assumed and cited notion that exercise training increases CBF. Studies that have reported increases in CBF do so in small regions identified by voxel-wise analysis (Chapman et al., 2013; Kleinloog et al., 2019) or within a singular *a priori* region (hippocampus) (Kaufman et al., 2021). Moreover, the CBF increases reported by Chapman et al. (2013) are relative instead of absolute values, which are more sensitive to change, and the hippocampal increases evident in Kaufman et al. (2021) were limited only to APOE4 carriers. Regarding global CBF, studies using ASL do not report any changes (Chapman et al., 2013; Flodin et al., 2017; Kleinloog et al., 2019). Furthermore, alongside regional CBF increases, decreases in CBF have been documented within the hippocampus (Maass et al., 2015) and a cluster within the temporal fusiform gyrus or parahippocampal gyrus (Kleinloog et al., 2019). It should be considered that the group-level hippocampal CBF reductions were evident from a within-group ANOVA, despite analyses revealing no significant group × time interaction (Maass et al., 2015). Confusingly, Maass et al. (2015) also reported that greater increases in cardiorespiratory fitness (assessed as oxygen consumption at ventilatory threshold) within the exercise group were associated with greater increases in hippocampal and global grey matter CBF, but it appears that 7/17 exercise participants (41%) either experienced reductions or no considerable change to their fitness levels (Figure 1.16B) (Maass et al., 2015).

The only study to report global CBF changes assessed volumetric CBF, measured with duplex Doppler ultrasound (i.e., the summation of blood flow through the internal carotid and verterbral arteries, normalised for total brain volume) (Tomoto et al., 2023b). All other studies used single-delay ASL to measure CBF, apart from Maass et al. (2015) which used gadolinium contrast-based perfusion imaging. Interestingly, Tomoto et al. (2023b) used the longest intervention (one-year) and was the only to intergrate high-intensity sessions within the exericse intervention, which may

be one potential explanation for the observed global CBF increases. Tomoto et al. (2023b) also highlights the potential importance of cardiorespiratory fitness to CBF changes, with an apparent positive association between the two variables' change scores, supporting the cardiorespiratory fitness hypothesis of cerebral adaptations to exercise training (Voss and Jain, 2022). However this finding was not replicated following 6-months moderate-intensity training (Flodin et al., 2017). Although the global nature of the findings from Tomoto et al. (2023b) are the first of their kind within this area, the measurement technique used (duplex Doppler ultrasound) does not allow determination of whether blood flow arriving at the cerebral tissue increases, including which tissue type (grey or white matter) or which specific region of the brain receives increases in blood flow. Between-study differences in where CBF was measured (i.e., large cerebral arteries, whole-brain, or grey matter) are likely to contribute to the varied results observed.

A key limitation of all the ASL studies discussed is that single-delay ASL was used (Chapman et al., 2013; Flodin et al., 2017; Kaufman et al., 2021; Kleinloog et al., 2019), and two studies (Chapman et al., 2013; Kaufman et al., 2021) used a shorter than recommended post-labelling delay for older adult populations (i.e., <2000 ms) (Alsop et al., 2015). As previously mentioned, single-delay ASL is unable to correct for individual or regional differences in ATT, sacrificing CBF estimation accuracy and ultimately underestimating CBF. Furthermore, this underestimation may be affected by potential exercise-training induced cerebrovasculature changes that could alter ATT (discussed in Section 1.5.4), making any pre-to-post intervention comparisons problematic if using the single-delay approach. Therefore, it is difficult to make definitive conclusions regarding training-induced CBF changes using data from single-delay ASL studies. It is also important to consider adherence to the exercise interventions. All studies, except one (Maass et al., 2015), do report a measure of adherence, but this was primarily only to the proportion of sessions attended, with most studies reporting >85% attendance (Chapman et al., 2013; Flodin et al., 2017; Kleinloog et al., 2019). Kaufman et al. (2021) reported that 21% of participants completed <80% of the

prescribed intervention, but this figure refers to a larger participant sample from a parent study and does not clarify whether it relates to adherence to exercise session number or intensity, and thus the adherence of the sub-group with CBF data is unclear. Only one study expressed attendance as successful if the target intensity (heart rate) was met during the session (Tomoto et al., 2023b). Future studies should endeavour to include objective adherence metrics that not only describe the number of sessions completed but also the intensity and/or volume of exercise completed within those sessions, because this information could help explain any observed or lack of effects in relevant outcome measures.

To summarise, there is not enough robust evidence to make definitive conclusions regarding how exercise training affects CBF in healthy older adults. The one study reporting considerable effects was longer in duration (one-year) and included high-intensity exercise sessions (Tomoto et al., 2023b), in comparison to others that were much shorter in duration (8–12 weeks) (Chapman et al., 2013; Kleinloog et al., 2019; Maass et al., 2015) and included only moderate-intensity continuous exercise sessions. It is difficult to compare findings between the available studies due to key differences in measurement techniques (e.g., ASL, gadolinium contrast-based MRI, or duplex Doppler), population characteristics (e.g., age, body mass, cardiorespiratory fitness, sex), intervention characteristics (e.g., length, intensity, or volume), or statistical controls. Future studies should attempt to unpick individual differences within their sample, evident in Figure 1.16B, 1.16E, and 1.16F, to try and identify characteristics of individuals that do and do not experience changes, through which a better understanding of how exercise training could be tailored or prescribed to certain individuals that may benefit most can be gained. Furthermore, as outlined in Section 1.5.4, given that lactate appears to have a potential positive role for brain health (Jacob et al., 2023; Morland et al., 2017) and that the only study reporting global CBF changes incorporated high-intensity exercise sessions (Tomoto et al., 2023b), future studies should use high-intensity exercise training to confirm whether exercise training can influence CBF

in older adults, before attempting to fine-tune the optimal exercise dose required to instigate a change for a given individual. Finally, studies may opt to use ASL to quantify CBF changes because it is non-invasive but offers both global and regional analyses with cerebral tissue segmentation. However, single-delay ASL should be avoided because of the potential impacts of ATT on CBF estimation. As mentioned, the older adult population will naturally experience larger variation in ATT, and it is possible that ATT may change as a result of the intervention, meaning ATT should be corrected for by using multiple post-labelling delay ASL. Aside from improving CBF estimation accuracy, it will also improve understanding of how exercise training affects ATT, which has not yet been studied.

1.7 Exercise training-induced changes in resting cerebral haemodynamics and cognitive function

As previously discussed in Section 1.5.3, exercise training has been shown to have beneficial effects on cognitive function in older adults (Barha et al., 2017; Colcombe and Kramer, 2003; Ludyga et al., 2020; Northey et al., 2018; Sanders et al., 2019; Zhang et al., 2023), although these effects are not as robust as commonly assumed. Exercise training-induced changes in resting cerebral haemodynamics have been hypothesised to contribute to these cognitive benefits, particularly CBF. The focus on CBF stems from the fact that the brain lacks intracellular energy stores and thus blood flow to the cerebral tissue dictates energy availability and potentially the overall functionality of the tissue (Öz et al., 2007; Zimmerman et al., 2021). Therefore, theoretically, cerebral haemodyanics have the potential to underpin other commonly cited mechanisms that may contribute to the cognitive benefits of exercise, such as neurogenesis or functional connectivity (Erickson et al., 2014; Voss and Jain, 2022). However, as discussed in the previous section, the impact of exercise training on resting cerebral haemodynamics is far from clear. The purpose of the following section is to outline results from studies that investigated both

cognitive and cerebral haemodynamics responses to exercise training in older adults, and then assessed whether there were associations between the changes in these variables.

Several studies have reported positive associations between changes in CBF and cognitive function. For example, participants in Chapman et al. (2013) completed a 12-week aerobic exercise intervention that induced improvements in memory function, but not in executive function or attention. This study reported a positive association between changes in relative hippocampal CBF and changes in memory function. However, there were no group-level differences in relative hippocampal CBF following the intervention, and these correlation analyses used the change in relative hippocampal CBF from pre-intervention to the intervention-midpoint (i.e., 6-weeks), rather than the post-intervention measures. Similarly, the 12-week aerobic exercise intervention from Maass et al. (2015) induced no between-group differences in three memory tests, but changes in hippocampal CBF and global grey matter CBF were positivley associated with changes in early recall and recognition scores for the complex figure test within the exercise group. Furthermore, structural equation modelling indicated that exercise-training induced increases in cardiorespiratory fitness increased hippocampal CBF, which increased hippocampal volume, leading to increased memory recognition. However, there were no between-group differences in hippocampal or global CBF following the intervention, and hippocampal CBF actually tended to decrease within the exercise group (59% experienced no considerable change or decreased). Moreover, 67% of exercise participants experienced either no considerable change or decreases in complex figure test recognition scores.

The one-year aerobic intervention by Kaufman et al. (2021) also found no between-group differences in visuospatial functioning, executive functioning, or verbal memory; however, a positive association was observed between changes in hippocampal CBF and changes in verbal memory, specifically for APOE4 carriers within the exercise group (who experienced elevations in

hippocampal CBF). Even the one-year combined moderate- and high-intensity exercise intervention by Tomoto et al. (2023b) did not induce any between-group differences within a comprehensive cognitive test battery spanning inductive reasoning, long-term episodic memory, working memory, processing speed, and verbal ability domains. Despite increased global volumetric CBF in the exericse group, changes in CBF were not correlated with changes in any of the eleven cognitive function measures. Interestingly, however, reductions in carotid stiffness and cerebrovascular resistance, which were associated with CBF increases, were associated with improved immediate recall scores in the exercise group.

The six-month aerobic intervention used by Flodin et al. (2017) did result in group-level improvements in global composite cognition, reported in another publication using a largely shared participant cohort (Jonasson et al., 2017). However, the absence of group-level changes in global or voxel-wise CBF indicates that changes to haemodyanics were not an important factor for these cognitive improvements, although this was not directly compared. Instead, this study found some evidence indicating that increases in thickness of the prefrontal cortex were associated with the cognitive improvements in the exercise group; however, these structural changes were not present at group-level nor were they associated with cardiorespiratory fitness gains (Jonasson et al., 2017). Interestingly, the same group also found that, in the same sample, worse baseline brain integrity was associated with less improvement in working memory and processing speed at follow-up (von Cederwald et al., 2023). This association appeared to be driven by the active control group, who also experienced significant cardiorespiratory fitness gains (but to a lesser extent).

Although not an exercise training study, it has been shown that endurance trained middle-aged/older adults have greater occipitoparietal CBF_{GM} than sedentary peers, and, in the pooled

sample, there was a positive assocaition between occipitoparietal CBF_{GM} and global composite cognition, though it did not reach statistical significance (r=0.31, *P*=0.08).

Collectivley, these studies provide limited evidence for associations between exercise training-induced changes to resting CBF and improvements in cognitive function in healthy older adults. This is somewhat unsurprising given the general lack of group-level training-induced changes to either CBF or cognitive fucntion. However, several studies have noteable limitations, such as to analytical methods (Chapman et al., 2013), data interpretation (Maass et al., 2015), ASL-measured CBF methodology (Chapman et al., 2013; Kaufman et al., 2021), limited sample sizes (Chapman et al., 2013; Maass et al., 2015), or poor exercise adherence reporting (Kaufman et al., 2021; Maass et al., 2015). Therefore, further high-quality studies are required to meaningfully improve understanding of whether cerebral haemodynamics are important for training-induced changes in cognitive function. However, it is interesting that all of the reported effects from these studies relate specifically to the memory domain of cognition. Specifically frontal and temporal CBF are reported to have the largest effect on memory function in older adults (van Dinther et al., 2023). Consequently, future studies should consider making this domain and these regions a focus of their research.

In addition, it should also be considered that the length of these interventions (i.e., 12-weeks, six-months, or one-year) may be too short for any exercise-training induced adaptations to have a tangible effect on behavioural outcomes. For example, the one-year intervention by Tomoto et al. (2023b) increased global volumetric CBF, but did not induce group-level changes in cognitive function. It is possible that the observed CBF increases were not present for long enough to have a considerable impact on the structure and/or function of the cerebral tissue that would manifest in detectable cognitive improvements. Previous research reporting that lower resting CBF predicts greater cognitive decline in healthy older adults did so over a two or six year follow-up

period (van Dinther et al., 2023; Wolters et al., 2017), whereas contemporaneous associations were absent (Leeuwis et al., 2020). Therefore, studies within this area potentially need to use longer follow-up periods to properly assess whether training-induced changes in CBF affect cognitive function in older adults.

Furthermore, following findings that baseline structural brain integrity may influence cognitive outcomes following an exercise intervention (von Cederwald et al., 2023), it should be considered that the status of baseline cerebral haemodyanics could also affect cognitive changes and subsqequent interpreations. For example, an acute exercise bout in older adults has been shown to cause an immediate reduction in CBF which then normalises or even rebounds over baseline levels after ~30-mins (Palmer et al., 2022). The hippocampus was one of the regions that rebounded, with further analyses revealing that this rebound effect was limited only to participants with low baseline CBF, and authors suggested this could be indicative of a greater vascular plasticity capacity. Theoretically, repeated exposure to this CBF rebound throughout an exercise intervention could induce more substantial cerebrovascular or strucutral cerebral changes that could benefit cognitve function.

Finally, it may be that the literature actually needs to move away from the concept that regular exercise can improve cognitive function in older adults, but instead focus on whether regular exercise can prevent or lessen age-related declines in cognitive function, and assess whether changes to cerebral haemodynamics are important for this. Indeed, higher baseline physical activity levels in older adults have been shown to reduce cognitive decline over the following 14-years (Ottenbacher et al., 2014), and cognitive function was maintained in older adults that increased their physical activity levels over 10-years, in comparison to step-wise cognitive declines in those who only maintained or reduced their activity levels (van Gelder et al., 2004). Studies similar to these that incorporate periodic measurements of cerebral haemodynamics are

needed to fully understand the relationships between regular exercise, cerebral haemodynamics, and cognitive function.

1.8 Physical activity behaviours and brain health

1.8.1 Overview

This section will first explain what physical activity behaviours are, how they are measured, and how they could possibly be more predictive of brain health metrics than cardiorespiratory fitness (i.e., $\dot{V}O_{2max}$). Next, common age-related changes in physical activity behaviours and their impacts on both general and brain health will be outlined. Finally, research investigating the impact of physical activity behaviours on resting cerebral haemodynamics will be discussed.

Physical activity is defined as any bodily movement induced by skeletal muscle contraction that raises energy expenditure above resting rates (2018 Physical Activity Guidelines Advisory Committee, 2018). This is in contrast to exercise, which specifically refers to physical activity that is planned, structured, and performed for the purpose of maintaining physical fitness (2018 Physical Activity Guidelines Advisory Committee, 2018). Sedentary behaviour is also an important physical activity behaviour, defined as any waking behaviour performed in a seated, reclined, or lying posture at an energy expenditure of ≤1.5 metabolic equivalents (i.e., sitting or lying down) (2018 Physical Activity Guidelines Advisory Committee, 2018). Sedentary behaviour is a distinct concept from physical activity. For example, an individual may be both physically active (i.e., meeting activity guidelines) and highly sedentary due to occupational- and/or leisure-related sedentary behaviour (Gómez-Redondo et al., 2022; Hamilton et al., 2008; Thivel et al., 2018; Tremblay et al., 2010; van der Ploeg and Hillsdon, 2017). Physical activity behaviours can be measured subjectively using questionnaires, which is ideal for large scale and fast data collection. These questionnaires generally have reasonable reliability and repeatability (Craig et al., 2003; Helmerhorst et al., 2012); however, they can lack validity. Objective assessment of

physical activity levels is also possible by wearing accelerometers that measure the frequency of activity counts. Compared with accelerometer-derived physical activity behaviours, self-report questionnaires can overestimate and underestimate levels of physical activity and sedentary behaviour, respectively (Ferrari et al., 2020), and the self-report measures can lack association with other health metrics (Ferrari et al., 2020; Guo et al., 2019). Accelerometers are typically worn on the wrist or hip for seven consecutive days to capture an individual's habitual physical activity behaviours, which are then used to determine average daily values. It is common for physical activity levels to be classed according to intensity, including light physical activity (LPA), moderate physical activity (MPA), vigorous physical activity (VPA), or combined moderate-to-vigorous physical activity (MVPA), based upon the number of activity counts recorded per minute. For accuracy, objective determination of physical activity behaviours using accelerometers is often preferred; however, it is still possible that participants may modify their habitual physical behaviours levels because they know they are being observed (Clemes et al., 2008).

Understandably, given its associations with cardiovascular disease, dementia, and overall mortality risk reduction (Lang et al., 2024; Tari et al., 2019), cardiorespiratory fitness (i.e., peak oxygen consumption) has received a lot of attention within research investigating strategies to optimise healthy brain ageing. However, the HERITAGE Family Study reports that both baseline cardiorespiratory fitness (Figure 1.17A) and the response of cardiorespiratory fitness to exercise training (Figure 1.17B) are largely dictated by genetics (~47% heritability) (Bouchard et al., 1999, 1998). This means that for a given physical activity stimulus, there will be large inter-individual variability in cardiorespiratory fitness responses, even with perfect adherence from all individuals. Importantly, however, subsequent research has shown that the proportion of individuals exhibiting a low cardiorespiratory response can be reduced by increasing the volume or intensity of physical activity (Montero and Lundby, 2017; Ross et al., 2015).

For physical activity to improve cardiorespiratory fitness, it needs to be performed at higher intensities (i.e., MVPA), although the cardiorespiratory fitness response will vary between-individuals completing equivalent MVPA, and maintaining higher MVPA levels may not largely affect the rate of age-related decline in cardiorespiratory fitness (Fleg et al., 2005). Interestingly, data from ~90,000 participants indicates that only ~5% of cardiorespiratory fitness variation is explained by MVPA (Raichlen et al., 2020). This means that cardiorespiratory fitness may not accurately reflect an individual's physical health status or lifestyle behaviours, consequently leading to erroneous conclusions. Furthermore, although exercise predominantly involves MVPA, MVPA can be achieved outside of structured exercise, in normal daily life, such as by brisk walking, climbing stairs, or gardening. In older adults, these types of activities, rather than exercise, will predominantly contribute to their MVPA levels and will reflect a greater relative intensity in older adults who have lower cardiorespiratory fitness levels. Therefore, the existing focus on how cardiorespiratory fitness impacts brain health in older adults, rather than levels physical activity, may explain some of the previously discussed mixed findings regarding cerebral haemodynamics and cognitive function. More specifically, levels of MVPA, which are not affected by genetics or exclusively dictated by exercise participation, may be more highly predictive of changes in brain health in older adults.

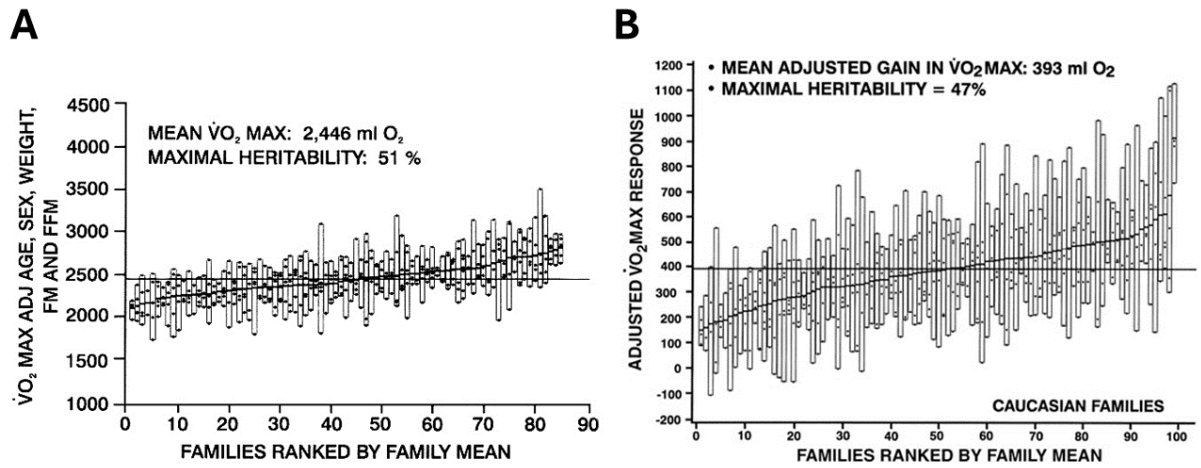

Figure 1.17: Inter-individual variation and heritability of cardiorespiratory fitness from The HERITAGE Family Study.

A: Heritability of baseline cardiorespiratory fitness ($\dot{V}O_{2max}$). Each family is enclosed within a bar, with dots representing individual data points (n=429). From Bouchard et al. (1998).

B: Inter-individual variation and heritability of the cardiorespiratory fitness ($\dot{V}O_{2max}$) response to a standardised, 20-week aerobic exercise training protocol. Each family is enclosed within a bar, with dots representing individual data points (n=481). From Bouchard et al. (1999).

Furthermore, it should be considered that modifying physical activity behaviours at the lower end of the intensity spectrum (i.e., reducing sedentary behaviour and increasing LPA) may have more considerable impacts on brain health because, in older adults >70 years, these two behaviours are typically engaged with for ~95% of each day (sedentary; ~65%, LPA; ~30%), compared with MVPA that makes up only ~5% of each day (Figure 1.18D) (Husu et al., 2016). Sedentary behaviour and LPA are closely intertwined and thus should not be investigated in isolation (Collins et al., 2023). Naturally, reductions in sedentary behaviour (i.e., sitting or lying down) will most likely be replaced with behaviours such as standing or light ambulation, categorised as LPA (and vice versa). Indeed, a strong inverse association between sedentary behaviour and LPA has been reported, whereas this was only weak with MVPA (Healy et al., 2008). Compared with MVPA, changes to sedentary behaviour and LPA only have a minimal effect on cardiorespiratory fitness. For example, of all the physical activity behaviours, research indicates that only increases in VPA benefits fitness, whereas replacing sedentary behaviour with LPA (or vice versa) has no effect on

fitness (O'Brien et al., 2022). Moreover, a lifestyle intervention (not exercise training) that reduced sedentary behaviour by 40-min/day did not result in significant changes to cardiorespiratory fitness (Norha et al., 2023). However, given the daily time spent engaging with these behaviours, they could have a larger impact on total energy expenditure and thus body composition, outcome variables that have been shown to affect cerebral haemodynamics and grey matter volume (Birdsill et al., 2013; Leidhin et al., 2021; Pflanz et al., 2022).

1.8.2 Age-related changes in physical activity behaviours

It is known that, within the general population, overall physical activity levels decline as we get older. In UK, Finnish, and American populations, these declines are predominantly limited to adults aged >65 years, which further worsen in those aged >75 years (Figure 1.18A, 1.18B, 1.18C) (Doherty et al., 2017; Schrack et al., 2014; Wennman et al., 2019). In terms of changes within specific activity behaviour classifications, increases in sedentary time and reductions in MVPA appear to be driving the overall physical activity declines in older adults (Figure 1.18D) (Berkemeyer et al., 2016; Husu et al., 2016; van Schooten et al., 2018). Global guidelines from the World Health Organisation aimed at improving health outcomes recommend that adults should complete at least 150 mins/week of MVPA or 75 mins/week of VPA (Bull et al., 2020). However, globally, the estimated proportion of all adults (not just older adults) that do not meet these guidelines is nearly 30%, and this proportion increases with age (WHO, 2022). For example, in European older adults aged >70 years, this proportion rises to ~45% (WHO, 2022), with similar values reported specifically in English older adults (NHS England, 2023). Understandably, these global and national-level studies use self-reported questionnaire data to classify individuals; however, compared with accelerometers (i.e., objective assessment), questionnaire data can misclassify individuals as meeting physical activity guidelines (Downs et al., 2014; Garriguet et al., 2015; Nelson et al., 2019) and therefore the global status of physical activity levels are actually

likely to be worse than reported above. For example, 90% of a sample met guidelines according to the commonly used International Physical Activity Questionnaire (IPAQ), whereas this fell to 70% or 29% according to total or 10-min bouts of accelerometer-derived MVPA, respectively (Garriguet et al., 2015). In contrast to the highly specific physical activity guidelines, the recommendations regarding sedentary behaviour are far more vague, simply stating that sedentary behaviour should be limited, and replaced with physical activity of any intensity (Bull et al., 2020). Collectively, these data indicate that sedentary behaviour and physical activity levels are relatively well-maintained in middle-aged adults, but these significantly, and continue to worsen in later-life. These age-related changes may be relevant to the deterioration in brain health of older adults.

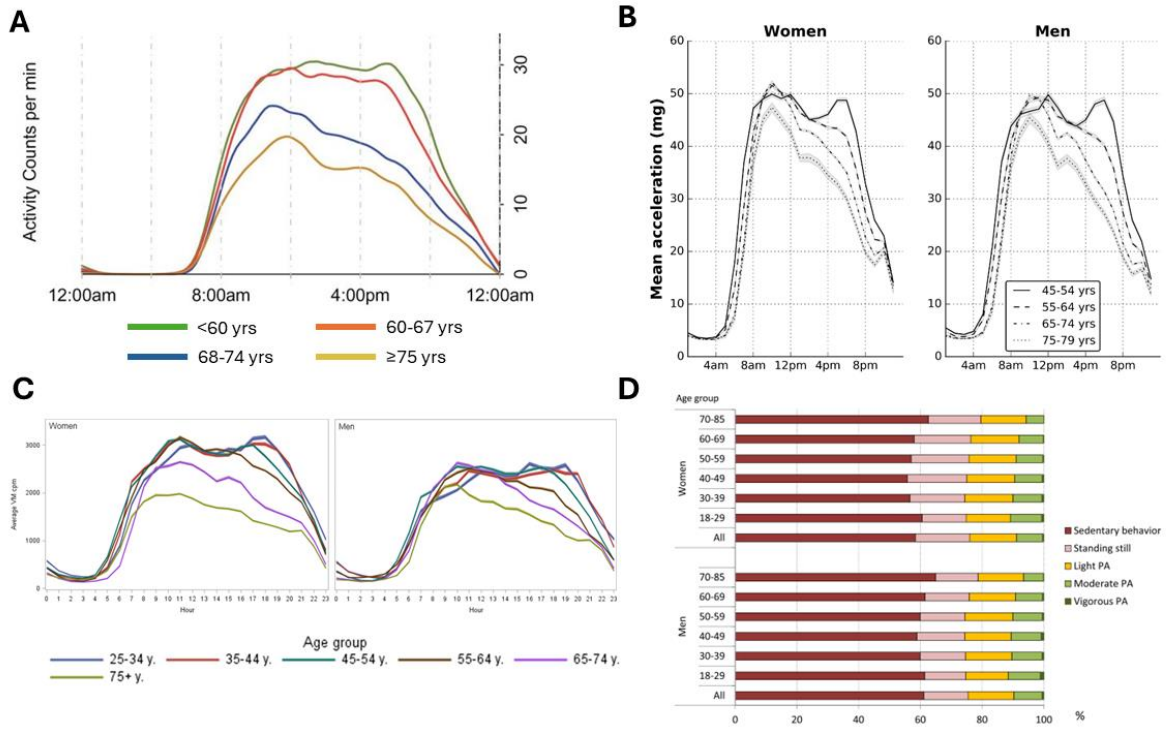

Figure 1.18: Cross-sectional studies demonstrating changes in physical activity behaviours across the lifespan.

A: Mean activity counts per minute across a day are similar in American adults (n=611) aged <60 years (green) and 60–70 years (orange), but stepwise reductions are evident in adults aged 68–74 years (blue) and ≥75 years (yellow). Baltimore Longitudinal Study of Aging from Schrack et al. (2014).

B: Mean acceleration across a day is similar in UK adults (n=915, females; left, males; right) aged 45–54 years and 55–64 years, but stepwise reductions are evident in adults aged 65–74 years and ≥75 years. FinnHealth 2017 Survey from Wennman et al. (2019).

C: Mean vector magnitude counts per minute across a day is similar in Finnish adults (n=96,600, females; left, males; right) aged 25–64 years, but stepwise reductions are evident in adults aged 65–74 years and 75–79 years. UK Biobank from Doherty et al. (2017).

D: Mean proportions of the day spent engaging in sedentary behaviour, standing behaviour, light physical activity, moderate physical activity, and vigorous physical activity across the lifespan split by decade in Finnish adults (n=1587, females; top, males; bottom). From Husu et al. (2016).

1.8.3 The impact of physical activity behaviours on general health

Similarly to cardiorespiratory fitness, physical activity behaviours have been associated with various health outcomes. For example, greater sedentary behaviour increases mortality risk (Katzmarzyk et al., 2009; Patterson et al., 2018), but this risk can be reduced or even ameliorated by engaging with higher levels of physical activity (Biswas et al., 2015; Ekelund et al., 2020, 2016). Moreover, replacing both LPA or MVPA with sedentary behaviour increases mortality risk, but the

risk increase is greater where MVPA is altered (Millard et al., 2021). Interestingly, research indicates that how you accumulate MVPA (i.e., sporadic vs. 5- or 10-min bouts) does not affect its beneficial effects on mortality (Jefferis et al., 2019; Millard et al., 2021; Saint-Maurice et al., 2018). Research has also investigated the impact of not meeting activity guidelines (i.e., physical inactivity) on disease risk. Globally, physical inactivity is thought to cause ~6% and ~10% of the burden from coronary heart disease and type-2-diabetes, respectively (Lee et al., 2012). Sedentary behaviour of >10 hrs/day has also been shown to increase cardiovascular disease risk (Pandey et al., 2016). Similarly to mortality risk, regular engagement with high physical activity levels appears to reduce the impact sedentary behaviour on cardiovascular disease risk (Lee et al., 2020).

Regarding obesity, higher levels of MVPA have been associated with lower BMI and waist circumference, but the associations were strongest when only MVPA in ≥10-min bouts was considered (Strath et al., 2008). Similarly, large population-based data from the UK Biobank indicate that greater overall physical activity levels improve indices of adiposity, such as BMI, waist circumference, and body fat percentage (Guo et al., 2019). These detrimental effects of increased sedentary behaviour and low MVPA on cardiometabolic risk appear to be independent of one another (Knaeps et al., 2018). Furthermore, adverse cardiovascular effects such as increases in blood pressure (Adams et al., 2024), endothelial dysfunction (Morishima et al., 2016; Restaino et al., 2016), and arterial stiffness (Ahmadi-Abhari et al., 2017) are evident with prolonged sedentary behaviour. Similarly, higher physical activity levels appear to benefit blood pressure (Biswas et al., 2023), endothelial function (Maurer et al., 2022), and arterial stiffness (particularly MVPA) (Collings et al., 2023; Gómez-Sánchez et al., 2023; Vandercappellen et al., 2020). Collectively, these data highlight the unequivocal importance of physical activity behaviours for general health outcomes, including those related to vascular health (i.e. arterial stiffness, endothelial function).

1.8.4 The impact of physical activity behaviours on brain health

There is also evidence that physical activity behaviours can affect brain health, including cognitive function and dementia risk. A meta-analysis of nine longitudinal studies specifically investigating older adults (n=20,326) found that physically active older adults had a reduced risk of developing Alzheimer's disease (Beckett et al., 2015). Eight of these studies used self-report questionnaires to assess physical activity, but objective, accelerometer-derived physical activity levels have also been longitudinally associated with reduced Alzheimer's disease risk (Buchman et al., 2012) and incidence of cognitive impairment (Middleton et al., 2011). More recently, large population-based studies from the UK Biobank have investigated whether accelerometer-derived or self-reported measures of physical activity behaviours predict dementia incidence (~7–9-year follow-up). These findings showed that for self-reported activity (n=431, 924), for every additional hour per day of sedentary behaviour (over 3 hrs/day), dementia risk was increased by 6%, whereas high overall physical activity levels were associated with a 15% risk reduction (Huang et al., 2022). Accelerometer analyses (n=90,320) also find that sedentary behaviour and lower overall physical activity levels are associated with increased dementia risk (Zhong et al., 2023). These effects were independent from one another, but a combination of the two further worsened the risk of developing dementia (Zhong et al., 2023). Furthermore, higher baseline levels of both objectively measured LPA and MVPA have been independently associated with less cognitive decline over two-years (Stubbs et al., 2017).

Aside from neurodegenerative disease risk and clinical cognitive impairment, the impact of physical activity behaviours on cognitive function (i.e., task-related performance) has also been investigated extensively and subsequently meta-analysed. For example, a meta-analysis including exclusively longitudinal studies using self-reported measures of physical activity in older adults (n=30,331) reports robust findings that higher physical activity levels reduce cognitive

decline over 1–12 years, and that these effects were similar between high and moderate physical activity levels (Sofi et al., 2011). Similarly, a more recent meta-analysis reports that higher physical activity levels reduce age-related declines in global cognitive function and episodic memory; however, associations were weak with no dose-response association (Iso-Markku et al., 2024). Interestingly, another meta-analysis focussing on both self-report and accelerometer-derived sedentary behaviour found opposite associations between the two assessment methods, and generally large heterogeneity of results (Dillon et al., 2022). Specifically, higher levels of self-report and accelerometer-derived sedentary behaviour were associated with superior or worse cognitive function, respectively. The strongest effects were seen for global cognitive function and processing speed (Dillon et al., 2022). Furthermore, a systematic review also highlights mixed results of longitudinal studies using objective assessment of physical activity behaviours in this area, reporting significant effects of MVPA in 2/4 articles, of LPA in 2/3 three articles, and of sedentary behaviour in 1/3 articles (Rojer et al., 2021). The wide variety of techniques used to assess both physical activity behaviours and cognitive function make between-study comparisons challenging. Furthermore, studies use different statistical controls and not all account for the interplay between sedentary behaviour and physical activity levels. Nevertheless, overall, the literature does indicate that spending less time sedentary and more time physically active will have beneficial effects of cognitive brain health outcomes.

Structural and functional cerebral changes associated with physical activity behaviours (Domingos et al., 2021a; Erickson et al., 2022; Sexton et al., 2016; Stillman et al., 2016; Zou et al., 2024) are hypothesised to explain these broader brain health benefits (i.e., reduced risk of cognitive decline and neurodegenerative disease). The majority of research in this area has used self-report measures of physical activity behaviours, although cross-sectional research using accelerometers in older adults has identified associations between physical activity levels and brain volumes. For example, LPA and MVPA were associated with temporal lobe white matter

integrity and a lower volume of white matter hyperintensities, respectively (Burzynska et al., 2014). Grey matter volumes also appear to benefit from greater overall physical activity levels (Dougherty et al., 2016; Fox et al., 2022; Halloway et al., 2019), but evidence indicates this effect may be more pronounced in females (Varma et al., 2016, 2015). Interestingly, research has indicated that specifically higher levels of LPA benefit total cerebral volume, whereas MVPA had no additional benefit (Spartano et al., 2019). However, despite being measured and being closely correlated with LPA, sedentary behaviour was not controlled for in analysis and thus it is unclear as to which behaviour is driving these effects (Spartano et al., 2019). Additionally, other research shows the contrary, that MVPA, but not LPA or sedentary behaviour are associated with hippocampal volume (Machida et al., 2022). Moreover, it has been demonstrated that higher overall physical activity levels are associated with only total grey, not white, matter volumes, and these associations were limited to older, and not younger adults (i.e., 40–59 vs. ≥60 years) (Hamer et al., 2018). Sedentary behaviour, LPA, and MVPA have also been associated with superior functional connectivity (Domingos et al., 2021b); however, statistical analyses did control for the impact of these behaviours on one-another, and other research suggests that only cardiorespiratory fitness, not physical activity, has beneficial effects for functional connectivity (Voss et al., 2016).

Longitudinal studies using accelerometers are limited, but available data generally support the beneficial effects of favourable physical activity behaviours on structural brain health. For example, over approximately eight-years follow-up, participants with higher overall physical activity levels at baseline experienced less frontal, but not temporal, lobe atrophy (Yuki et al., 2012). Similarly, greater declines in total grey and white matter volumes over five-years were associated with lower overall physical activity at follow-up, whereas sedentary behaviour (adjusted for lifestyle PA) was only associated with white matter atrophy over the same period (Arnardottir et al., 2016). Interestingly, it has been shown that, over 10-years, the change in

physical activity level (self-reported) over that period was the best predictor of changes in brain volumes, grey and white matter integrity, and cognition when compared to just using the baseline or even the five-year change in physical activity levels (Best et al., 2017).

Collectively, cross-sectional and longitudinal data do support that favourable physical activity behaviours can benefit brain structure and function, although this is by no means replicated in all investigations (Kharabian Masouleh et al., 2018; Maasakkers et al., 2021; Mellow et al., 2024; Tian et al., 2014; Torres et al., 2019), with data regarding sedentary behaviour considerably more mixed (Maasakkers et al., 2022). Moreover, there is a lack of longitudinal investigations using accelerometer-derived physical activity measurements, and often investigations consider a behaviour only in isolation rather than controlling for their effects on one-another (i.e., sedentary behaviour and LPA). Furthermore, longitudinal investigations generally focus on whether baseline physical activity behaviours predict changes in brain health, whereas changes in these behaviours over time may be more predictive. It seems reasonable to assume that increasing, decreasing, or maintaining physical activity behaviours will have differential effects on brain health measures. Despite this wealth of brain health literature, very few investigations have focussed on the potential impact of physical activity behaviours on cerebral haemodynamics. As previously discussed, cerebral haemodynamics experience age-related changes and these changes are thought to contribute to cognitive declines. Cerebral haemodynamics have the potential to underpin some of the structural and functional cerebral changes described above. The following section therefore aims to summarise the existing literature investigating associations between physical activity behaviours and cerebral haemodynamics in older adults.

1.9 Physical activity behaviours and cerebral haemodynamics

Only a limited number of studies have investigated associations between resting cerebral haemodynamics and physical activity behaviours in older adults. Similar to the cardiorespiratory fitness literature, these studies also report inconsistent findings, with effects generally isolated to small regions within the brain. Regarding cerebral blood velocity, studies have investigated both the short- and longer-term effects of sitting (i.e., sedentary behaviour) in older adults. For example, prolonged uninterrupted sitting for eight-hours was shown to induce significant reductions in cerebral blood velocity (bilateral MCA_V) after four-hours, which were maintained until the end of the sitting bout (Wheeler et al., 2019). Interestingly, walking for 30-mins prior to the sitting bout did not prevent the initial decline in MCA_V, but MCA_V experienced some recovery during the second half of the sitting bout where it was not significantly different from baseline levels. Furthermore, this recovery appeared to occur earlier if the sitting bout was interrupted with short breaks of light-intensity walking every 30-mins (Wheeler et al., 2019). Acute sitting-induced declines in MCA_V failed to be replicated in a later study (Maasakkers et al., 2020); however, only a three-hour sitting bout (vs. eight) was used, but this was sufficient to increase cerebrovascular resistance. The effects of a 16-week reduced-sitting intervention on cerebral blood velocity have also been studied in older adults (Hartman et al., 2021). The intervention reduced sedentary behaviour by ~1-hr/day and subsequently increased physical activity levels (daily steps increased ~2700/day), ultimately increasing resting cerebral blood velocity from baseline levels (~3 cm/s). Evidently, there is a dearth of research investigating cerebral blood velocity and physical activity behaviours, which does not allow for robust conclusions to be made. More longer-term studies are required to assess whether repeated exposure to the potential transient adverse effects of sedentary behaviour on cerebral blood velocity contribute to longer-term changes.

Cross-sectional research investigating associations between resting CBF and physical activity behaviours in older adults has also been conducted. For example, accelerometer-derived sedentary behaviour has been associated higher hippocampal CBF in older adults (n=33) (Zlatar et al., 2014). However, this effect was limited to the left hippocampus and only in participants who were APOE4 carriers (n=9). Overall physical activity was also measured but was not significantly associated with hippocampal CBF (Zlatar et al., 2014). The same group completed another cross-sectional study in a slightly larger sample of older adults (n=52) and performed voxel-wise CBF analyses within two regions-of-interest, the frontal lobe and parahippocampus/hippocampus (Zlatar et al., 2019). Accelerometers determined sedentary behaviour, LPA, MVPA, and overall physical activity. Physical activity behaviours were not associated with CBF within the hippocampus, but analyses did identify clusters within the frontal lobe that were (Figure 1.19A). Specifically, sedentary behaviour was associated with lower CBF in the middle frontal gyrus and paracentral lobule. Consistent with this, LPA and MVPA were associated with higher CBF in the middle and inferior frontal gyrus or inferior frontal gyrus, respectively. Overall physical activity was associated with higher CBF in the superior medial gyrus, supplementary motor area, inferior frontal gyrus, and precentral gyrus. Moreover, exploratory whole-brain voxel wise analyses was performed, identifying associations (in the same direction as prior results) between CBF and sedentary behaviour (left supramarginal gyrus and right fusiform gyrus), LPA (left anterior cingulate), and overall physical activity (right lingual gyrus, right middle occipital gyrus and left insula) (Zlatar et al., 2019). Again, the same group performed a further cross-sectional study in older adults (n=43), investigating associations with CBF specifically within the rostral middle frontal gyrus, medial orbitofrontal cortex, hippocampus, and inferior temporal cortices (Bangen et al., 2023). These analyses report that MVPA was not associated with CBF in any region, but LPA was associated with higher CBF in the rostral middle frontal gyrus and inferior temporal cortices (Figure 1.19B).

Collectively, this series of studies from this group highlights that associations between CBF and physical activity behaviours are not clear. Regarding sedentary behaviour, both a negative (Zlatar et al., 2014) and positive (Zlatar et al., 2019) association with CBF was reported. The negative association was present in APOE4 carriers, potentially indicating compensatory hyperperfusion. Furthermore, MVPA was reported to be positively associated with CBF in the inferior frontal gyrus (Zlatar et al., 2019), but no associations were present in the middle frontal gyrus or orbitofrontal cortex (Bangen et al., 2023). Interestingly, their most recent publication indicates a greater importance of LPA than MVPA for CBF in frontal and temporal regions.

A slightly larger scale cross-sectional study has investigated associations between CBF in 14 regions-of-interest and everyday activities in older adults (n=118), including accelerometer-derived LPA and MVPA (Sanders et al., 2023). This study found that neither LPA or MVPA were associated with CBF more broadly across lobes (i.e., frontal, parietal, temporal, and occipital lobes), or in regions including the hippocampus, cerebellum, or thalamus. However, LPA did have weak positive associations with CBF in accumbens, cingulate, and putamen regions, and moderate positive associations within insula and pallidum regions (Figure 1.19C). In contrast, MVPA was only associated with accumbens CBF (Figure 1.19C). Interestingly, this study also assessed whether baseline physical activity levels were associated with changes in CBF over time (~500-day follow-up) in a sub-set of the sample (n=86). Weak positive associations were evident between baseline LPA and CBF changes in the frontal lobe, temporal lobe, and insula regions, whereas none were present with MVPA. These data agree with Bangen et al. (2023), which indicated a greater importance of LPA than MVPA for CBF.

Other cross-sectional research in older adults (n=495) has identified that self-reported levels of high overall physical activity, but not low or moderate levels, appear to partially ameliorate the deleterious effects of obesity (assessed using waist-to-hip ratio and waist circumference) on

global grey matter CBF (Figure 1.19D) (Knight et al., 2021). Similarly, high overall physical activity levels are suggested to reduce age-related declines in CBF within the posterior cingulate cortex, which is part of a physical activity-sensitive resting state network (Figure 1.19E) (Boraxbekk et al., 2016). In Boraxbekk et al. (2016), physical activity levels were not assessed with questionnaires or accelerometers, but instead were derived from a composite score of physiological variables that are affected by physical activity levels. This included BMI, waist-to-hip ratio, blood pressure, hand grip strength, and heart rate at rest. Furthermore, two studies have compared cerebral haemodynamics between high and low sedentary groups of older adults, assessed using accelerometers (n=718) (Maasakkers et al., 2021) or via questionnaire (n=680) (Launer et al., 2015). Both studies measured global grey matter CBF, reporting no between-group differences, and Maasakkers et al. (2021) also measured pre-frontal cortex oxygen saturation, reporting no differences at rest or in response to an orthostatic challenge.

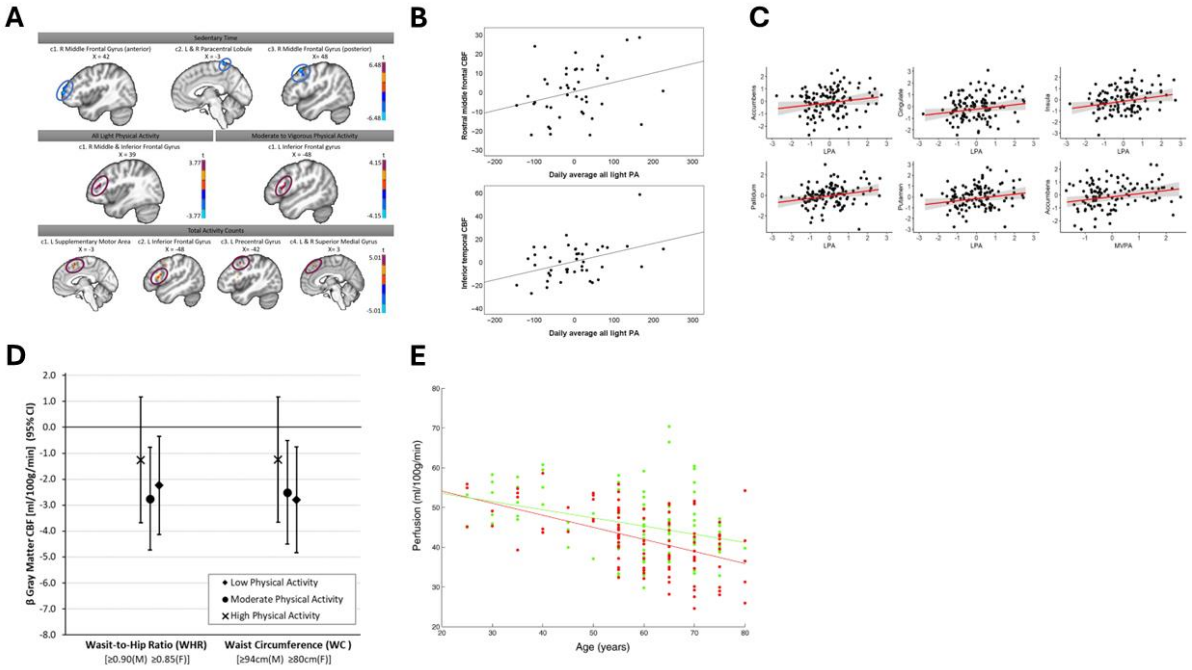

Figure 1.19: Studies investigating cross-sectional associations between physical activity behaviours and resting cerebral blood flow (CBF).

A: Positive and negative associations between accelerometer-derived physical activity levels or sedentary behaviour, respectively, with CBF in clusters within the frontal lobe (n=52). From Zlatar et al. (2019).

B: Positive associations between accelerometer-derived light physical activity (LPA) and CBF within the rostral middle frontal gyrus and inferior temporal gyrus (associations were not present in medial orbitofrontal or hippocampal regions). No associations were present with moderate-to-vigorous physical activity (MVPA) (n=43). From Bangen et al. (2023).

C: Positive associations between accelerometer-derived light physical activity (LPA) and CBF within 5/14 regions investigated, and moderate-to-vigorous physical activity (MVPA) and CBF within 1/14 regions investigated (n=118). From Sanders et al. (2023).

D: High self-reported levels of physical activity, but not low or moderate, ameliorates the significant association between obesity and low global CBF (n=495). From Knight et al. (2021).

E: Higher composite physical activity score (green), derived from body mass index, resting heart rate, blood pressure, and grip strength, is associated with higher CBF within the posterior cingulate. Green; high activity group, red; low activity group. From Boraxbekk et al. (2016).

Taken together, the available literature generally indicates that unfavourable physical activity behaviours (i.e., high sedentary behaviour and low physical activity levels) are likely to have adverse effects on cerebral haemodynamics in older adults, including to resting CBF and cerebral blood velocity. However, the exact regions affected and what intensity of physical activity has the largest impact is still unclear. Moreover, the majority of conclusions are based on cross-sectional research which cannot infer causality. Comparing results between studies is also difficult due to

differences in statistical controls, the type of accelerometer used, the accelerometer cut-points used to classify activity intensity, and CBF measurement techniques. Regarding CBF measurement techniques, all MRI-based studies to date have used single-delay ASL, which cannot adjust for regional and individual differences in ATT, sacrificing CBF estimation accuracy. Furthermore, a key limitation of all these studies is that they failed to take in to account the complex interplay between sedentary behaviour and physical activity, and thus it is not possible to isolate their effects from one another (Collins et al., 2023). As previously discussed, reducing sedentary behaviour will mean that physical activity levels increase (particularly LPA), or vice versa. Therefore, these physical activity behaviours cannot be investigated separately, but require statistical control in order to decipher which behaviour is having the effect (if any) on cerebral haemodynamics (Collins et al., 2023). For example, analyses from the discussed research involving sedentary behaviour only controlled for MVPA (Maasakkers et al., 2021; Zlatar et al., 2019) or did not control for physical activity levels at all (Launer et al., 2015; Zlatar et al., 2014). Likewise, associations between physical activity and cerebral haemodynamics were not controlled for sedentary behaviour (Bangen et al., 2023; Sanders et al., 2023; Zlatar et al., 2019, 2014). Another aspect to consider is that the type of activity engaged with whilst sedentary may influence any effect on brain health outcomes (e.g., socialising, watching television, reading, or crossword). For example, reading whilst sedentary, a cognitively demanding activity, offsets the normally deleterious effects on cognitive function (Mellow et al., 2022). Moreover, in older adults, social activities appear to partially mediate the beneficial effects of physical activity on cognition (Cohn-Schwartz and Khalaila, 2022), and both social activities and reading have been associated with greater regional CBF (Sanders et al., 2023). Therefore, longitudinal studies which track changes in individual's physical activity behaviours over the longer-term and appropriately control for the different classifications are required to make definitive conclusions about how cerebral haemodynamics may be affected by different physical activity behaviours.

1.10 Summary and aims of the thesis

Preventing severe deteriorations in brain health and cognitive function is essential for maintaining quality of life and an overall healthy ageing process. Although cognitive declines are unavoidable, there is large variation in how well cognitive function is preserved between older individuals. Identifying common characteristics of those who age cognitively well is important for promoting a healthy ageing process. The ageing process also involves changes to resting cerebral haemodynamics and, like cognitive function, there is considerable individual variation in these age-related changes. Indeed, there is evidence to indicate that resting cerebral haemodynamics are important for brain health and cognitive function in older adults. Therefore, identifying modifiable lifestyle factors that can influence cerebral haemodynamics could help to preserve cognitive function and promote a healthy ageing process.

Evidence exists indicating that modifiable factors including obesity, higher blood pressure, and lower social activity engagement could exacerbate normal age-related cerebral haemodynamic changes. Despite strong links between cardiorespiratory fitness (i.e., $\dot{V}O_{2peak}$; peak oxygen consumption) and general or brain health outcomes, including cognitive function, how cardiorespiratory fitness or exercise training affects resting cerebral haemodynamics in older adults is poorly understood. Regarding CBF, a contributing factor to heterogenous findings could be measurement technique. Arterial spin labelling (ASL) is a commonly used technique, but practically all ASL studies use a single post-labelling delay, which cannot adjust for regional and individual differences in ATT, thus sacrificing CBF estimation accuracy. The use of multiple post-labelling delay ASL may help clarify the impact cardiorespiratory fitness has on CBF, as well as uncover new insights into whether ATT is also affected. It should also be considered that the understandable focus on cardiorespiratory fitness within the existing literature may partly explain the heterogenous findings, because this has a large genetic component and may therefore not

accurately reflect an individual's lifestyle. Using accelerometers to objectively assess physical activity engagement, as well as sedentary behaviour, offers an alternative, more holistic, approach that could reveal more meaningful associations with cerebral haemodynamics.

Therefore, the aims of this thesis were to:

1. Assess cross-sectional associations between modifiable lifestyle factors (including cardiorespiratory fitness) and resting CBF or ATT in older adults (Chapter 2).
2. Assess cross-sectional associations between cognitive function and resting CBF or ATT in older adults (Chapter 2).
3. Assess the impact of exercise training on resting CBF, ATT, and cognitive function in older adults (Chapter 3).
4. Assess whether cardiorespiratory fitness influences exercise training-induced changes in resting CBF or ATT in older adults (Chapter 3).
5. Assess whether exercise training-induced changes in resting CBF or ATT influence cognitive function in older adults (Chapter 3).
6. Assess associations between accelerometer-derived physical activity behaviours and resting CBF or ATT in older adults (Chapter 4)
7. Assess the importance of exercise training adherence to subsequent changes in cardiorespiratory fitness in older adults (Chapter 5).

CHAPTER TWO

DETERIMINANTS OF CEREBRAL BLOOD FLOW AND ARTERIAL TRANSIT TIME IN HEALTHY OLDER ADULTS

The work within this chapter has been accepted for publication at *Aging US*. The accepted manuscript is followed by the accompanying Supplemental Material.

The candidate was the sole recruiter of participants included within all data chapters, was heavily involved with the health screening process to determine participant eligibility, and was primarily responsible for all participant communications and administration. The candidate completed all of the data collection relating to cardiorespiratory fitness testing and physical function testing, and was also heavily involved with the data collection relating to the cognitive function tests and the MRI scan. Under guidance from KJM, the candidate completed the MRI arterial spin labelling data analysis. The candidate completed the statistical analyses and data visualisation presented in all data chapters and was responsible for leading the writing of the presented manuscripts. KS, SJEL, HS, and SB set-up the larger project. All co-authors read and suggested edits to the manuscript. FR, KEJ, and AG also assisted data collection.

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2.1 Abstract

Cerebral blood flow (CBF) and arterial transit time (ATT), markers of brain vascular health, worsen with age. The primary aim of this cross-sectional study was to identify modifiable determinants of CBF and ATT in healthy older adults (n=78, aged 60–81 years). Associations between cardiorespiratory fitness and CBF or ATT were of particular interest because the impact of cardiorespiratory fitness is not clear within existing literature. Secondly, this study assessed whether CBF or ATT relate to cognitive function in older adults. Multiple post-labelling delay pseudo-continuous arterial spin labelling estimated resting CBF and ATT in grey matter. Results from multiple linear regressions found higher BMI was associated with lower global CBF (β=-0.35, *P*=0.008) and a longer global ATT (β=0.30, P=0.017), global ATT lengthened with increasing age (β=0.43, *P*=0.004), and higher cardiorespiratory fitness was associated with longer ATT in parietal (β=0.44, *P*=0.004) and occipital (β=0.45, *P*=0.003) regions. Global or regional CBF or ATT were not associated with processing speed, working memory, or attention. In conclusion, preventing excessive weight gain may help attenuate age-related declines in brain vascular health. ATT may be more sensitive to age-related decline than CBF, and therefore useful for early detection and management of cerebrovascular impairment. Finally, cardiorespiratory fitness appears to have little effect on CBF but may induce longer ATT in specific regions.

2.2 Introduction

Brain health worsens with age (Turrini et al., 2023) and ultimately impairs cognitive function, limiting independence in later life (Salthouse, 2012). These effects will impact many as the global population ages rapidly (United Nations Department of Economic and Social Affairs, Population Division, 2022). Alongside adverse age-related structural and functional cerebral deterioration, changes to cerebral haemodynamics occur, including widespread cerebral hypoperfusion

(Damestani et al., 2023). Evidence indicates that lower cerebral blood flow (CBF) detrimentally affects cognitive function in healthy older adults (De Vis et al., 2018; Ebenau et al., 2023; Moonen et al., 2021; van Dinther et al., 2023). Identifying strategies that limit adverse age-related changes to cerebral haemodynamics could help to promote a heathier ageing process; however, modifiable determinants of cerebral haemodynamics in older adults are currently poorly understood.

Not only CBF, but arterial transit time (ATT) also worsens with age (Damestani et al., 2023). ATT is the time taken for blood to travel from large arteries in the neck to the cerebral tissue. Prolonged ATT is associated with impaired cerebrovascular reactivity (Takata et al., 2023) and atherosclerotic risk (Hafdi et al., 2022), and is present in patients with Alzheimer's disease (M. Sun et al., 2022) or cerebral artery stenosis (Yu et al., 2022). The MRI sequence arterial spin labelling (ASL) can estimate both CBF and ATT if data are acquired at multiple post-labelling delays. Using multiple post-labelling delays also improves CBF estimation accuracy by enabling adjustment for regional and individual differences in ATT (Dai et al., 2017). Despite this, compared with single-delay ASL, only a minority of ASL studies have utilised this technique due to increased data collection time requirements, although shorter multi-delay sequences are now available (Woods et al., 2024).

Previous research in older adults has already identified some modifiable determinants of CBF. For example, greater CBF is reported in those who are physically active (Bangen et al., 2023; Zlatar et al., 2019), engage with social or leisure activities (Sanders et al., 2023), have a lower body mass index (BMI) (Birdsill et al., 2013; Knight et al., 2021), or have lower blood pressure (Leidhin et al., 2021; Yetim et al., 2023). These factors can all be addressed with simple lifestyle changes. Cardiorespiratory fitness is a modifiable factor for which its relationship with CBF is unclear, despite evidence that it benefits cognitive function (Ludyga et al., 2020; Northey et al., 2018) and

reduces dementia risk (Tari et al., 2019). Research in older adults has reported a positive relationship between cardiorespiratory fitness and CBF (Dougherty et al., 2020; Johnson et al., 2016; Tarumi et al., 2013; Zimmerman et al., 2014), and that exercise training can increase CBF (Alfini et al., 2019; Chapman et al., 2013; Kleinloog et al., 2019; Tomoto et al., 2023b; Zimmerman et al., 2014). In contrast, a negative (Intzandt et al., 2020; Olivo et al., 2021) or a lack of (Burley et al., 2021; Flodin et al., 2017; Krishnamurthy et al., 2022) association has also been reported. In summary, when observed, cardiorespiratory fitness-related CBF changes are not usually global, but confined to specific regions of the brain (Chapman et al., 2013; Intzandt et al., 2020; Kleinloog et al., 2019; Thomas et al., 2013). Regions are often small or only a portion of regions investigated show these associations (Alfini et al., 2019; Chapman et al., 2013; Kleinloog et al., 2019; Olivo et al., 2021; Tarumi et al., 2013). Furthermore, none of the aforementioned ASL studies reporting effects used multiple post-labelling delays, limiting accuracy of CBF estimation (Alsop et al., 2015). The large genetic component of cardiorespiratory fitness could also explain discrepancies in results (Bouchard et al., 1999). Given the complexity and inconsistency in the literature to date, more work is needed to understand the relationship between these variables using improved methodological approaches.

Research investigating modifiable determinants of ATT is limited, but evidence indicates a positive association with mean arterial pressure (Yetim et al., 2023) and an unexpected negative association with BMI in males with coronary artery disease (limited to two small regions within the brain) (MacIntosh et al., 2015). A proxy of ATT, the spatial coefficient of variation in ASL signal (sCoV) (Mutsaerts et al., 2017), appears to lack association with hyperlipidaemia (Gyanwali et al., 2022). Regarding cardiorespiratory fitness, one study investigated relationships with ATT, reporting a lack of or positive relationship in older (n=14) or younger (n=18) adults, respectively (Burley et al., 2021). Blood velocity within a cerebral artery is also somewhat of a proxy for ATT because of a strong inverse relationship (Burley et al., 2021), and also demonstrates mixed

associations with cardiorespiratory fitness (Smith et al., 2021). Originally, a positive association between cerebral blood velocity and cardiorespiratory fitness was shown in males (Ainslie et al., 2008) whereas more recent work indicates that this association is not present (Zeller et al., 2022) or only present in females (Lefferts et al., 2022). Given that ATT has been related to brain health outcomes (M. Sun et al., 2022; Takata et al., 2023; Yu et al., 2022), further research is required to understand its determinants.

This cross-sectional study aimed to investigate determinants of global and regional resting CBF and ATT in healthy older adults, with a particular focus on cardiorespiratory fitness, and whether CBF or ATT are associated with cognitive function. Resting grey matter CBF and ATT were estimated using pseudo-continuous ASL with multiple post-labelling delays. It was hypothesised that markers of superior general health (i.e., higher cardiorespiratory fitness/handgrip strength/grey matter volume or lower age/BMI/blood pressure) and cognitive function would be associated with greater CBF and a shorter ATT.

2.3 Materials and methods

2.3.1 Study design

The data for this publication were collected as part of a larger study, The FAB Project (preregistration: https://osf.io/6fqg7, materials and data: https://osf.io/d7aw2/). The study was approved by the STEM Ethical Review Committee at the University of Birmingham (ERN_20-1107). Before taking part in this study, participants were provided with a participant information sheet and provided their informed consent (see Appendices for participant information sheet and consent form).

Participants were screened for eligibility before completing three experimental sessions on different days. Figure 2.1 shows the key outcome measures and desired session order (achieved

for 87% of participants, with all experimental sessions completed within 5.2±3.2 weeks, and with 13±15 days between MRI and exercise sessions (<30 days for 91%). Participants refrained from vigorous physical activity, which acutely alters CBF (Clement et al., 2018), for 24 hours prior to the MRI session.

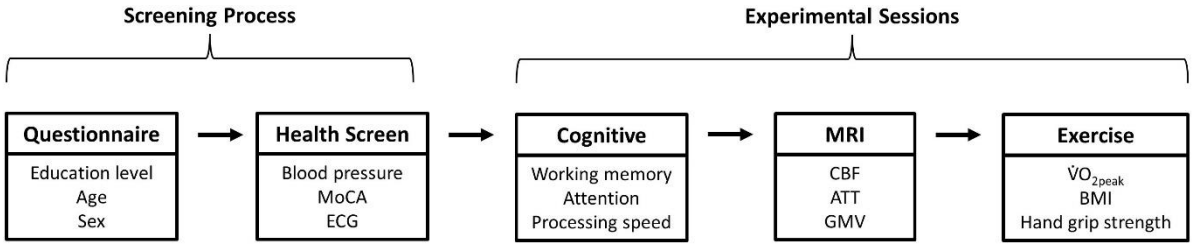

Figure 2.1: Flow chart of the screening and experimental sessions. MoCA: Montreal Cognitive Assessment, ECG: electrocardiogram MRI: magnetic resonance imaging, CBF: cerebral blood flow, ATT: arterial transit time, GMV: grey matter volume, $\dot{V}O_{2peak}$: peak oxygen consumption, BMI: body mass index.

2.3.2 Participants

Ninety-four healthy older adults (aged 60–81 years) were enrolled. Participants were cognitively normal, without historic or current diagnosis of serious health conditions, non-smokers, and self-reported to not meet recommended global activity guidelines (Bull et al., 2020). Section 1 of the Supplemental Material contains detailed inclusion criteria (2.6.1; Supplemental Material found at conclusion of this chapter). MRI data were missing or unusable for n=16, leaving n=78 for analyses presented in this study. Participant characteristics are shown in Table 2.1.

Table 2.1: Participant characteristics.

	Total	Male	Female
n	78	39	39
Age (years)	65±5	65±5	66±5
Education (%)			
Compulsory	28	31	26
Further	33	33	33
Undergraduate	19	18	21
Postgraduate	19	18	21
SBP (mmHg)	140±13	140±12	139±15
DBP (mmHg)	82±8	83±7	82±8
BMI (kg/m^2)	27±4	28±3	26±4
$\dot{V}O_{2peak}$ (mL/kg/min)	28±4	30±4	25±3

Values represent means ± standard deviation. SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption.

2.3.3 Health screening: electrocardiogram (ECG), blood pressure, and cognitive impairment

Participants completed a resting 12-lead ECG (Cardiosoft, Vyaire, USA), three resting blood measurements (705IT, Omron, Japan), and the Montreal Cognitive Assessment (MoCA). Participants were excluded for severe ECG abnormalities, MoCA scores <23 (Carson et al., 2018), and systolic/diastolic blood pressure of >160/>90 mmHg. Excluded participants were referred to their GP.

2.3.4 Outcome Measures

Cardiorespiratory fitness

Participants completed an incremental exercise test on a treadmill (Pulsar 3p, H/P/Cosmos, Germany). Respiratory gases ($\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide production) were recorded continuously using a facemask (7450 V2, Hans Rudolph, USA) and metabolic cart (JAEGER Vyntus CPX, Vyaire, USA), as was heart rate and rhythm using a 12-lead ECG (Cardiosoft, Vyaire, USA). Rating of perceived exertion (RPE) (Borg, 1982) and finger-prick blood [lactate]

(Biosen C-Line, EKF Diagnostics, United Kingdom) were measured between stages. Stages were 4 min with a 1 min rest period between each stage. Treadmill speed started and remained at 3.8 km/h until either all possible elevation stages were completed (4, 7, 10, 13, 16, 19, and 20% gradient) or individual lactate threshold was reached (2.1 mmol/L increase over the mean of the two lowest values (Mamen et al., 2011)). If all elevation stages were completed, 4 min stages continued with speed increasing 0.5 km/h per stage until lactate threshold. After reaching lactate threshold, 1 min stages were completed where speed increased 0.5 km/h per stage (rest periods were removed). Figure 2.S1 shows a treadmill test format example.

Participants were asked to exercise to volitional exhaustion unless halted by the researcher due to ECG abnormalities or injury. Cardiorespiratory fitness was determined using peak oxygen consumption ($\dot{V}O_{2peak}$) (i.e., mean of the two highest 30 s intervals). Nine participants completed a sub-maximal test, $\dot{V}O_{2peak}$ was thus predicted using individual sub-maximal $\dot{V}O_2$ and heart rate data acquired from three of the first possible six stages using a linear regression. Full details and example (Figure 2.S2) of the prediction method can be found in Section 2 of the Supplemental Material (2.6.2).

Hand grip strength

A hand grip dynamometer (5001, Takei, Japan) was adjusted for grip size in participant's dominant hand. Participants stood upright, arms by their sides, and squeezed the dynamometer as hard as possible, maintaining elbow extension and limiting shoulder movement. The highest score from three attempts was taken.

Cognitive function

Working memory: In a 2-back task, participants were presented a 3x3 grid. The stimulus was a single white square that continuously appears, disappears, and then reappears in one of the grid squares at random (n=60 trials, 1 s each). Participants identified when the white square appeared

in the same location as it did two trials prior. Trials were excluded from analysis if incorrect or if response time <200 ms or greater than two standard deviations above/below the mean per participant. The primary outcome measure was *d* prime (*d*'), a measure of discriminability, a greater *d*' indicates superior performance.

Attentional Network Task (ANT): The computerised ANT assessed orienting, alerting, and executive control. The stimulus is a row of five arrows, each pointing left or right. As fast and as accurately as possible, participants reported the direction of the centre arrow using the left and right arrow keys. A central fixation cross is displayed for 400 ms, then a fixation cross (500 ms) and cue (100 ms) are presented simultaneously, and then only the fixation cross is displayed for a further 400 ms. A stimulus is then shown for a maximum of 1700 ms. The centre arrow can be congruent or incongruent (i.e., pointing in the same or opposite direction as the flankers, respectively; n=96 each), or neutral (i.e., central arrow flanked by target-irrelevant black blocks, n=96). The stimulus can appear above or below the fixation cross, cued by a black square (n=216) or not cued (n=72). There are three cue conditions: a spatial cue, a centre cue, or a double cue (n=72 each). The spatial cue indicates if the stimulus appears above or below the fixation cross, whereas the stimulus location remains ambiguous for the centre and the double cue. Twelve practice trials are followed by three blocks of 96 trials.

Trials were excluded from analysis if incorrect or if response time <200 ms or greater than two standard deviations above/below the mean per participant. Alerting scores were calculated as the no cue minus the double cue; Orienting scores by centre cue minus the spatial cue; Executive control scores were calculated by the incongruent target minus the congruent target (all for correct responses). High condition difference scores for alerting and orienting, and low condition difference scores for executive control, indicate better performance.

Processing speed: In a letter comparison task, participants were simultaneously presented with two strings of letters at the top and bottom of the screen, for a maximum of 2500 ms after presentation of a fixation cross (1000 ms). Strings were three or six characters long (n=48 trials, 24 each). As fast and as accurately as possible, participants identified whether the strings were the same or different. Mean response time and accuracy were calculated for each participant using data from trials involving only six-character strings. Trials were excluded from analysis if incorrect or if response time <200 ms or greater than two standard deviations above/below the mean per participant.

MRI acquisition and analysis

An MRI scan session included structural, functional, and arterial spin labelling (ASL) scans, using a 3-T system (MAGNETOM Prisma, Siemens, Germany) with 32-channel receiver head coil. Here, the focus is the ASL data and related scans, analysis of other data acquired can be found elsewhere (Rahman et al., 2023). CBF and ATT data were collected using pseudo-continuous ASL scan with 3D GRASE readout (17:22 mins) (Kilroy et al., 2014; D. J. J. Wang et al., 2013), see also Acknowledgements (2.4.7).

ASL imaging parameters were: repetition time (TR)=4100 ms, echo time (TE)=30.56 ms, in-plane resolution=3.5 mm^2, slice thickness=3.5 mm, transversal slices=32, field of view (FOV)=224x224 mm, labelling duration=1508.8 ms, background suppression=yes, and post-labelling delays (PLD)=200, 975, 1425, 1850, 2025, 2150, 2250, and 2300 ms. Four and twelve volumes of data were acquired for PLD of 200–2250 ms and 2300 ms, respectively. PLD times and number of volumes acquired were optimised according to recommendations (Woods et al., 2019). Slices were positioned axially from the motor cortex and angled anterior-posterior in line with the participant's anterior-posterior commissure (ACPC). A calibration M0 scan was acquired using these same parameters with the PLD set to 2000 ms with PCASL and background suppression

disabled. The T1-weighted structural scan (4:54 mins) was acquired to facilitate data analysis including, normalisation to a standard template brain and differentiation of grey and white matter. Structural T1-weighted (MPRAGE) imaging parameters were: TI=880 ms, TE=2.03 ms, TR=2000 ms, voxel size=1 mm^3, sagittal slices=208, FOV=256×256×208 mm, and flip angle=8°.

ASL data were processed using the Oxford ASL toolbox (https://oxasl.readthedocs.io/en/latest/), which uses the FSL FABBER ASL package and Bayesian Inference to invert the kinetic model for ASL MRI (BASIL) to compute CBF and ATT maps (Chappell et al., 2009; Groves et al., 2009; Woolrich et al., 2006). Parameters input to the kinetic models to estimate CBF and ATT were: bolus duration=1.5088 s, tissue T1=1.3 s, arterial blood T1=1.65 s, labelling efficiency=0.85. All other input parameters were kept with default settings appropriate to PCASL acquisition. Partial volume error correction and adaptive spatial smoothing of the perfusion maps was performed using default settings in oxford_asl (Chappell et al., 2011; Groves et al., 2009).

Global and regional analysis was performed, assessed in native (individual participant) and MNI space, respectively. All CBF and ATT values refer to grey matter only. Regions of interest (ROI) were the cingulate gyrus and frontal, parietal, temporal, occipital, and motor cortices (Figure 2.S3). The chosen ROIs have been used previously (Burley et al., 2021), and were broad because there were no specific *a priori* hypotheses of regions that would be affected by determinants or associated with cognitive function. MNI registration was poor for n=1, leaving n=77 for regional analysis. Difference maps at each PLD for each participant were visually inspected to ensure data quality. Particular attention was paid to ensure there were no: 1) excessive motion resulting in spurious edge effects in difference maps; 2) brain territories which did not appear to be perfused, due to suboptimal label positioning or unaccounted for vasculature; and 3) focal areas of high intensity in final CBF maps which would have suggested that the PLDs were insufficient. Exemplar participants which were excluded after visual inspection are shown in Figure 2.S4. Section 3 of

the Supplemental Material contains additional information regarding grey matter mask configuration (2.6.3).

Grey matter volume was estimated from structural T1 anatomical scans. Brain extraction tool (BET) removed non-brain tissue (Smith, 2002) before segmentation of tissue types using FMRIB's Automated Segmentation Tool (FAST) (Zhang et al., 2001).

2.3.5 Statistical analyses

All statistics used multiple linear regressions (SPSS Statistics v.29, IBM, USA). To identify determinants, global CBF or ATT were the dependent variable with age, sex, blood pressure (systolic and diastolic), BMI, $\dot{V}O_{2peak}$, hand grip strength, and grey matter volume as independent variables. To assess regional associations with cardiorespiratory fitness, mean CBF or ATT of each of the six ROIs were the dependent variable with age, sex, $\dot{V}O_{2peak}$, and any other significant determinants of global CBF or ATT identified from the above analysis as independent variables. To identify associations with cognitive function, global CBF or ATT were the dependent variable with age, sex, education, and scores for processing speed (accuracy and response time), working memory (*d'* prime), and the three attentional domains (alerting, orienting, and executive control scores) as independent variables. Cognitive data were missing for n=2, leaving n=76 for global analysis. The same analyses were performed using regional data, regional analysis details and results are shown in Section 5 of the Supplemental Material (2.6.5).

2.4 Results

Mean global and regional values for CBF and ATT can be found in Table 2.S1 and Figure 2.S5.

2.4.1 Determinants of global CBF

Overall, the eight independent variables did not significantly explain the variance in global CBF (gCBF) ($F(8,69)=1.38$, $P=0.22$, $R^2_{adjusted}=0.38$). BMI was the only significant determinant of gCBF

(β=-0.35, *P*=0.008; Figure 2.2), whereby gCBF decreased with increasing BMI. Data shown in Table 2.2.

2.4.2 Determinants of global ATT

Overall, the eight independent variables did significantly explain the variance in global ATT (gATT) ($F(8,69)=2.66$, $P=0.013$, $R^2_{adjusted}=0.15$). Only age (β=0.43, *P*=0.004; Figure 2.3) and BMI (β=0.30, *P*=0.017; Figure 2.2) were significant determinants of gATT, whereby gATT lengthened with both BMI and age. Data shown in Table 2.2.

Table 2.2: Determinants of global resting CBF and ATT in grey matter.

	gCBF (mL/100 g/min)		**gATT (s)**	
n=78	β	*P*	β	*P*
Age (years)	-0.16	0.298	0.43	**0.004**
Sex (1: male, 2: female)	0.03	0.895	-0.19	0.406
BMI (kg/m^2)	-0.35	**0.008**	0.30	**0.017**
SBP (mmHg)	-0.02	0.877	-0.11	0.371
DBP (mmHg)	-0.11	0.429	0.06	0.651
$\dot{V}O_{2peak}$ (mL/kg/min)	-0.18	0.304	0.24	0.149
Hand grip strength (kgf)	0.06	0.775	-0.33	0.115
Grey matter volume (mm^3)	0.06	0.687	-0.01	0.943

Results from two multiple linear regression analyses. Bold indicates P<0.05. β*: standardised beta coefficient, gCBF: global cerebral blood flow, gATT: global arterial transit time, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure,* $\dot{V}O_{2peak}$*: peak oxygen consumption.*

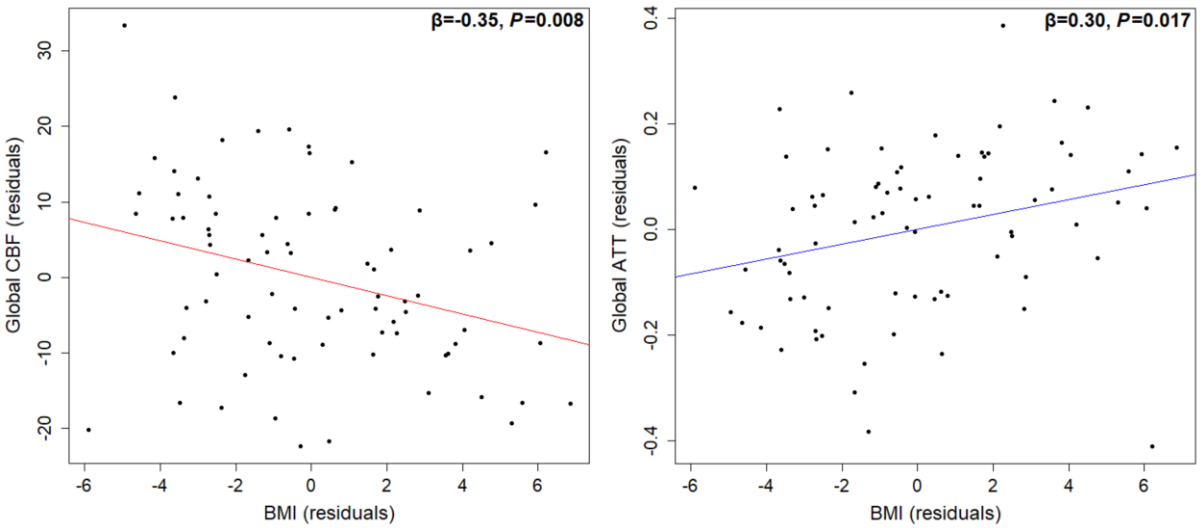

Figure 2.2: Partial regression association between BMI and global CBF (left) or global ATT (right), adjusted for age, sex, blood pressure, cardiorespiratory fitness, hand grip strength, and grey matter volume. Higher BMI was associated with lower CBF and a longer ATT (n=78). CBF: cerebral blood flow, ATT: arterial transit time, BMI: body mass index.

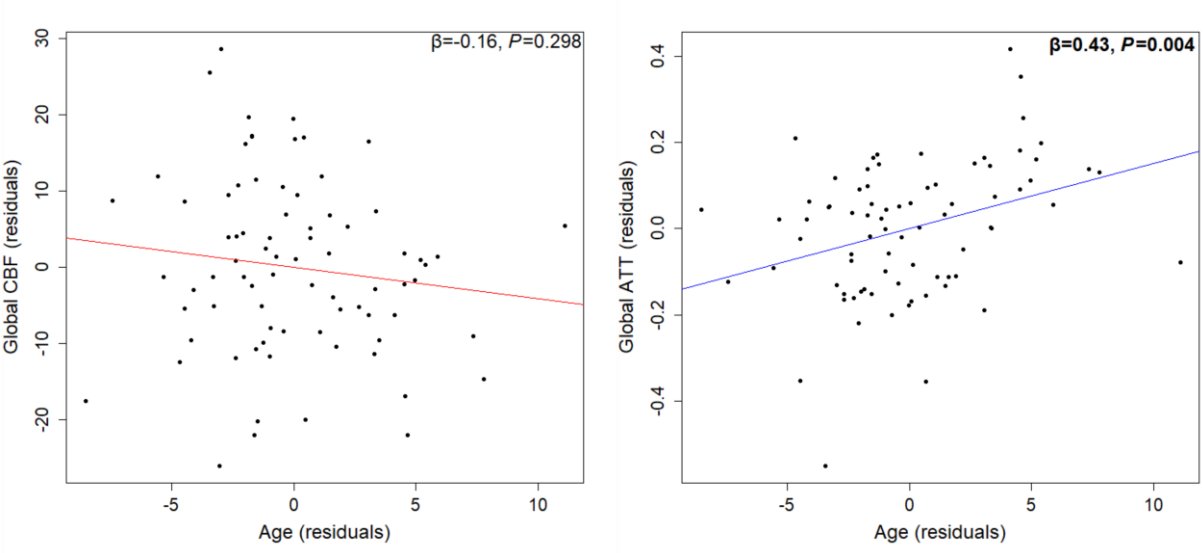

Figure 2.3: Partial regression association between age and global CBF (left) or global ATT (right), adjusted for sex, BMI, blood pressure, cardiorespiratory fitness, hand grip strength, and grey matter volume. ATT may be more sensitive to age-related changes than CBF (n=78). CBF; cerebral blood flow, ATT: arterial transit time, BMI: body mass index.

2.4.3 Associations between regional CBF and ATT with age, BMI, and cardiorespiratory fitness

Regional CBF

Age and cardiorespiratory fitness were not significantly associated with CBF of any region, before or after adjustment for multiple comparisons. Negative associations between BMI and CBF were present in all regions after adjustment for multiple comparisons, with the largest associations in the temporal (β=-0.44, *P*<0.001), occipital (β=-0.43, *P*<0.001), and parietal (β=-0.41, *P*=0.002) regions. Data shown in Table 2.S3.

Regional ATT

Full regional ATT results are shown in Table 2.3. Age was positively associated with ATT in all regions (strongest in occipital), only the cingulate region did not survive adjustment for multiple comparisons. Significant positive associations between BMI and ATT were present in frontal, parietal, temporal, and motor regions, but these did not survive adjustment for multiple comparisons. Cardiorespiratory fitness was positively associated with ATT in frontal, parietal, occipital and motor regions, associations in parietal (β=0.44, *P*=0.004) and occipital (β=0.45, *P*=0.003) regions survived adjustment for multiple comparisons (Figure 2.4).

Table 2.3: Associations between age, BMI, and cardiorespiratory fitness with regional ATT.

	Age		BMI		$\dot{V}O_{2peak}$	
ATT	β	*P*	β	*P*	β	*P*
Frontal	0.47	**<0.001**	0.24	0.046	0.33	0.042
Parietal	0.57	**<0.001**	0.23	0.040	0.44	**0.004**
Temporal	0.46	**<0.001**	0.25	0.043	0.20	0.225
Occipital	0.62	**<0.001**	0.19	0.083	0.45	**0.003**
Motor	0.49	**<0.001**	0.26	0.034	0.40	0.012
Cingulate	0.32	0.017	0.24	0.063	0.12	0.495

Separate multiple linear regressions were performed for each region (n=77), independent variables: age, sex, BMI, and $\dot{V}O_{2peak}$. Bold indicates significant P values survived adjustment for multiple comparisons. β: standardised beta coefficient, ATT: arterial transit time, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption.

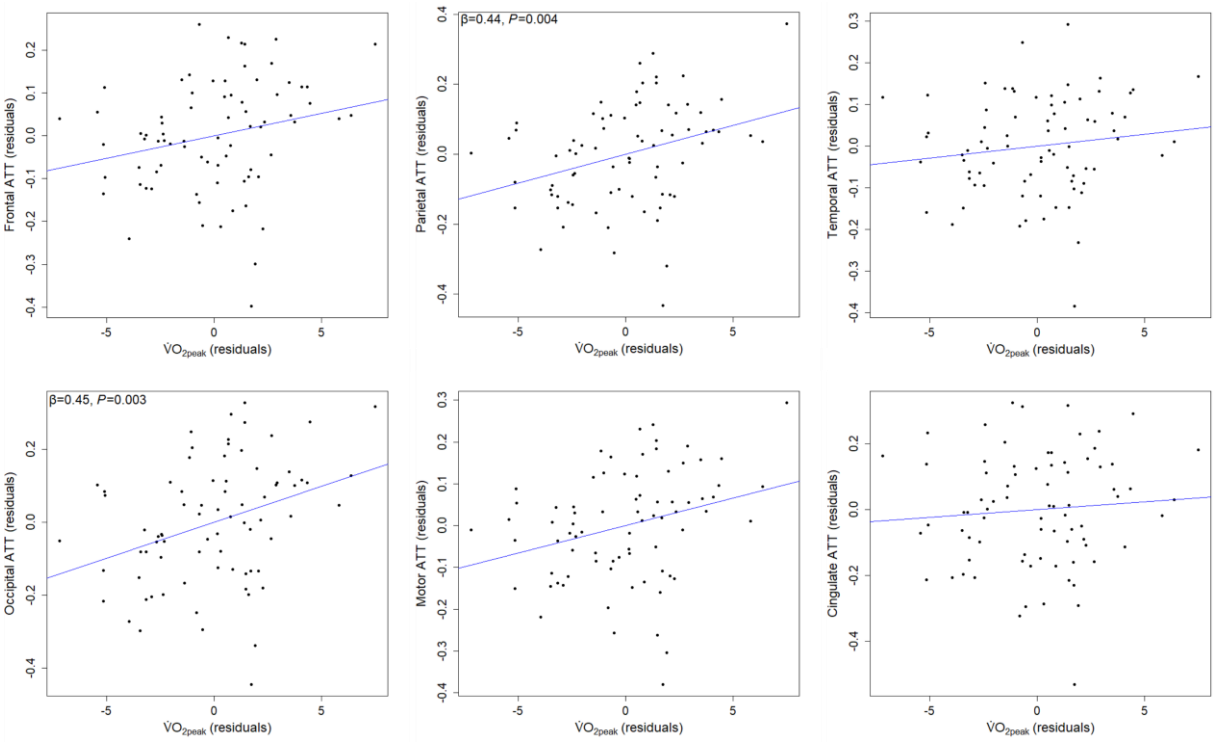

Figure 2.4: Partial regression associations between regional ATT and cardiorespiratory fitness, adjusted for age, sex, and BMI. Older adults with higher cardiorespiratory fitness experience longer ATT in parietal and occipital regions (n=77). ATT: arterial transit time, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption.

2.4.4 Associations between cognitive function and global CBF or ATT

Overall, the models including all independent variables did not significantly explain variance in gCBF ($R^2_{adjusted}$=-0.017, F(9,66)=0.86, *P*=0.56) or gATT ($R^2_{adjusted}$=0.031, F(9,66)=1.27, *P*=0.27). Measures of processing speed, working memory, or attention were not significantly associated with gCBF or gATT. The only noteworthy result between both models was that processing speed accuracy approached significance for predicting gCBF (β=0.25, *P*=0.053). Global regression data and summarised results from regional analyses can be found in Section 5 of the Supplemental Material (2.6.5).

2.5 Discussion

The present study aimed to identify modifiable determinants of CBF and ATT in healthy older adults and assess whether CBF and ATT are associated with cognitive function. The use of multiple post-labelling delay ASL improved CBF estimation accuracy by adjusting for differences in ATT, which is especially important in older populations (Dai et al., 2017). The present data show older adults with a higher BMI had lower global CBF and a longer global ATT, and that global ATT lengthened with age. Sex, blood pressure, cardiorespiratory fitness, hand grip strength, and grey matter volume were not significant determinants of global CBF or ATT. Regional analysis confirmed a lack of association between cardiorespiratory fitness and CBF but indicated that older adults with a higher cardiorespiratory fitness experience longer ATT in parietal and occipital regions. Cognitive function was not associated with CBF or ATT, globally or regionally.

2.5.1 Higher BMI is associated with lower CBF and prolonged ATT in healthy older adults

BMI was the only significant determinant of global CBF, whereby higher BMI was associated with lower CBF (1.2 mL/100 g/min decrease per 1 kg/m^2 increase). This finding agrees with previous population-based research (Knight et al., 2021; Vernooij et al., 2008). A novel finding from the present study was that higher BMI was also associated with prolonged global ATT. This opposes previous research reporting a negative association in male coronary artery disease patients (limited to two clusters within the occipital lobe) (MacIntosh et al., 2015). Making a direct comparison with the present data is problematic due to differences in the populations studied. The present findings reinforce the known damaging effects of excessive weight gain on brain vascular health.

Mechanisms mediating the relationship between BMI and poor cerebrovascular health may relate to the prevalence of metabolic and vascular risk factors that increase with BMI (e.g., hypertension, arterial stiffness, or hyperlipidaemia) (Botvin Moshe et al., 2020; Li et al., 2017; N.

Tang et al., 2022). These risk factors worsen cerebrovascular health (Pires et al., 2013; Suri et al., 2016) and are associated with lower CBF (Jefferson et al., 2018; Jennings et al., 2013; Sabayan et al., 2014). Given that only 17% of the present sample were taking lipid-lowering medication and that blood pressure or grey matter volume were not significant determinants of global CBF or ATT, it is unlikely that higher blood pressure, hyperlipidaemia, or cerebral atrophy typically associated with higher BMI (Hamer and Batty, 2019; N. Tang et al., 2022) explain the observed results. Therefore, adverse structural and functional changes to peripheral and cerebral vessels associated with higher BMI (Botvin Moshe et al., 2020; Li et al., 2017) are potentially the mediators of these associations.

Taken together, poor metabolic health and excessive weight gain have deleterious effects on brain vascular health. Fortunately, research indicates that modifying dietary and physical activity habits to induce ~10 kg weight loss increases CBF in overweight and obese middle-aged adults (Stillman et al., 2021). Evidence also indicates that engagement with high, but not low or moderate, levels of physical activity ameliorates the CBF reductions observed with higher BMI (Knight et al., 2021). The impact of weight loss interventions on ATT warrants further investigation.

2.5.2 Cardiorespiratory fitness lacks association with CBF, whereas associations with ATT may be region-specific

There are conflicting findings regarding the association between cardiorespiratory fitness and CBF in older adults with only one previous ASL study using multiple post-labelling delays (Burley et al., 2021). The present data found no association between cardiorespiratory fitness and global or regional CBF. Other cross-sectional ASL research also reports no global effect (Burley et al., 2021; Krishnamurthy et al., 2022; Thomas et al., 2013), but positive regional associations are generally observed (Dougherty et al., 2020; Johnson et al., 2016; Tarumi et al., 2013; Thomas et al., 2013; Zimmerman et al., 2014). However, these associations relate to smaller regions than

those investigated in the present study, and previous studies used single-delay ASL to estimate CBF. Furthermore, cardiorespiratory fitness has a large genetic component (Bouchard et al., 1999) and refers primarily to the efficiency of oxygen delivery/utilisation at skeletal muscle, not the brain, potentially explaining the present findings.

Cardiorespiratory fitness was not a significant determinant of global ATT, but higher cardiorespiratory fitness was associated with longer ATT in parietal and occipital regions, opposing our hypothesis. The only other study investigating this association in older adults (n=14) reported no global association but regional analysis, investigating the same regions as the present study, did show non-significant prolongation of ATT in high-fitness older adults, likely due to sample size (Burley et al., 2021). Collectively, the present data indicate that, in healthy older adults, cardiorespiratory fitness does not alter the delivery rate of perfusion of blood to cerebral tissue, but instead lengthens the time taken for blood to arrive at parietal and occipital regions from larger cerebral arteries in the neck.

The cardiorespiratory fitness-related prolongation of regional ATT could be related to blood velocity, vascular path length, or both. Faster cerebral artery blood velocity is associated with shorter ATT (Burley et al., 2021); however, its relationship with cardiorespiratory fitness is unclear (Smith et al., 2021). A positive association between blood velocity and fitness was first documented in males (Ainslie et al., 2008) but more recent studies have found no association in males or females (Zeller et al., 2022) or potentially an association in females only (Lefferts et al., 2022). This ambiguity, along with evidence that masters athletes experience less age-related increases in cerebral vessel tortuosity (Bullitt et al., 2009), suggests neither increased large artery blood velocity or vascular path length explains the present findings. However, masters athletes also have more small cerebral vessels (Bullitt et al., 2009). Therefore, given that total vessel cross-sectional area is inversely proportional to blood velocity (assuming constant blood flow),

cardiorespiratory fitness-induced small vessel cerebral angiogenesis may be slowing blood velocity and thus prolonging regional ATT. Interestingly, a longer ATT or larger sCoV (its proxy) has been associated with greater oxygen extraction fraction in patients with cerebrovascular disease (Hara et al., 2022; Takeuchi et al., 2022), potentially indicating that overall slower cerebral blood velocities may translate into longer capillary transit times, thus improving oxygen extraction. Therefore, fitter older adults may have superior cerebral oxygen extraction, which is true in skeletal muscle (Kalliokoski et al., 2001), and could help explain the preservation of cerebral tissue integrity and cognition that is associated with regular exercise training.

2.5.3 Age was not a significant determinant of CBF, but was associated with prolonged ATT

Age-related CBF decline over the lifespan is well documented (Damestani et al., 2023; Leidhin et al., 2021; Yetim et al., 2023), even after partial volume effects due to age-related grey matter atrophy are accounted for (Asllani et al., 2009). However, such findings were not replicated in the present data (with partial volume correction), even when using simple correlations (Table 2.S2), probably due to the limited age range. Previous research using a similar age range also reported no age/CBF relationship (Juttukonda et al., 2022). Yet, age was associated with prolonged global and regional ATT in the present study, conforming with previous findings (Damestani et al., 2023). The fact that both age and cardiorespiratory fitness are associated with a longer ATT in older adults is somewhat contradictive; however, the cause likely differs. Age-related ATT prolongation could be caused by adverse structural cerebrovasculature changes, such as increased cerebral vessel tortuosity (Bullitt et al., 2010; Chen et al., 2019) and prevalence of cerebral stenosis (de Weerd et al., 2010), or by reductions in cerebral artery blood velocity (Ainslie et al., 2008; Lefferts et al., 2022). Despite this, age-related ATT prolongation may consequently serve to improve oxygen extraction fraction (similarly to cardiorespiratory fitness) and help explain why the cerebral metabolic rate of remaining cerebral tissue increases with age (Lu et al., 2011). Given

that age was a significant determinant of ATT but not CBF, ATT may be more sensitive to age-related decline and could therefore be used to identify the onset of cerebrovascular impairment in older adults with low cardiorespiratory fitness.

2.5.4 Blood pressure was not a determinant of CBF or ATT

The present study found no association between global CBF or ATT and blood pressure in healthy older adults (systolic blood pressure=140–160 mmHg in 49%). Alternative multiple linear regressions were performed with either mean arterial pressure or pulse pressure, but this did not affect results (Table 2.S5). Previous single-delay research using ASL found higher systolic and diastolic blood pressure was associated with lower global CBF in older adults (34% hypertensive) (Leidhin et al., 2021). Furthermore, multiple-delay research reports higher mean arterial pressure is associated with lower global CBF in all age groups and longer ATT only in a limited number of regions (Yetim et al., 2023). However, longitudinal changes in blood pressure and global CBF were not associated in hypertensive older adults (van Dalen et al., 2021). Interestingly, hypertension is actually suggested to be a protective response to cerebral hypoperfusion in attempt to maintain CBF (Warnert et al., 2016). The relationship between blood pressure and cerebral haemodynamics is clearly complex and thus between-study differences may be explained by variance in the severity or duration of blood pressure changes experienced. However, given that higher blood pressure increases the risk of cerebrovascular dysfunction (Santisteban et al., 2023) and dementia (Walker et al., 2019), it can be assumed that maintaining normal blood pressure is beneficial for brain health. Further multiple-delay longitudinal research is required to make robust conclusions regarding the short- and long-term effects of blood pressure on CBF and ATT.

2.5.5 No association between CBF or ATT and cognitive function

Chronic cerebral hypoperfusion is thought to contribute to cognitive decline in older adults (Rajeev et al., 2023). However, the present data found no association between CBF and

processing speed, working memory, or attention in healthy older adults. This agrees with previous research showing global and regional CBF were not associated with cognitive function in older adults (Leeuwis et al., 2020). Interestingly, however, a subset of this previous sample were followed-up two years later which found lower baseline global CBF predicted greater decline in global cognition and attention/psychomotor speed whereas specifically frontal and temporal CBF predicted memory decline (van Dinther et al., 2023). Collectively, these data do suggest an importance of CBF for cognitive function, but this is both domain- and region-specific, and only apparent when changes over time are considered. Regarding ATT, the present study is believed to be the first to investigate relationships with cognitive function, reporting no associations. Previous research has investigated relationships with clinical cognitive impairment, reporting that prolonged ATT or its proxy, sCoV, is present in participants with vascular cognitive impairment, vascular dementia, or Alzheimer's disease (Gyanwali et al., 2022; M. Sun et al., 2022).

Taken together, it appears that CBF and ATT lack association with contemporaneous cognitive function in healthy older adults, but changes in these measures may still predict changes in cognitive function over time, as seen with CBF (De Vis et al., 2018; Ebenau et al., 2023; van Dinther et al., 2023). Given that the present data indicates greater age-related sensitivity of ATT than CBF, the predictive capacity of ATT should be investigated as it could indicate cerebrovascular-related cognitive decline in healthy populations. Alternatively, rather than resting state cerebral haemodynamics (i.e., oxygen delivery), oxygen utilisation of the cerebral tissue may be more important for cognitive function.

2.5.6 Future Directions

Cross-sectional analysis does not account for variations in genetics or baseline vascular health, longitudinal/intervention research is needed to make robust conclusions. There are likely other

influential determinants of cerebral haemodynamics not investigated in the present study, such as physical activity, arterial stiffness, cerebrovascular reactivity, blood lipids, or social activities that deserve future investigation. Regarding cognition, future research should investigate the predictive capacity of CBF and ATT. General brain health may be more strongly dictated by the ability of the cerebral tissue to extract and use essential nutrients delivered in the blood (i.e., oxygen extraction fraction and cerebral metabolic rate). Future research should investigate associations between these variables with cardiorespiratory fitness, CBF, ATT, and cognitive function. We used a range of PLDs based on the optimal for ATT <2000 ms (Woods et al., 2019). This appears sufficient for our relatively healthy older population as shown by the observed ATTs (Figure 2.S5). However, the longest PLD used (2300 ms) may not be sufficient to capture more prolonged ATT in older adult samples that are older or less healthy and should be considered in future studies. The present study lacked acute dietary controls prior to MRI acquisition (e.g., caffeine, polyphenols, nitrate, or sugars), which could have impacted results (Clement et al., 2018; Lamport et al., 2015; Vidyasagar et al., 2013). Future studies should measure and correct for arterial partial pressure of CO_2 ($PaCO_2$), or its proxy, partial pressure of end-tidal CO_2 ($PetCO_2$) (Barton and Wang, 1994). These were not measured during CBF and ATT measurements but could be manipulated by anxiety-induced ventilatory changes during an MRI scan. Accelerometer-derived activity measurements were conducted as part of the larger project, revealing 85% of participants met the activity guidelines they self-reported not to, based on moderate-to-vigorous intensity activity (Troiano et al., 2008), although participants possibly modified their normal behaviours (Clemes et al., 2008). The generalisability of findings may therefore be limited, and future research should assess if differences in CBF and ATT exist between habitual high and low activity groups. Power analysis in the present study indicated power=0.61 (pwr, RStudio). Given that previously documented associations were observed (e.g., age/ATT or BMI/CBF), power was sufficient to detect medium-to-strong effects. Larger sample sizes may be required to detect

smaller effects and may explain why cardiorespiratory fitness/ATT associations were not present globally.

2.5.6 Conclusion

This study aimed to identify modifiable determinants of CBF and ATT in healthy older adults and assess whether CBF and ATT were associated with cognitive function. Higher BMI was associated with lower global CBF and longer global ATT. Cardiorespiratory fitness was not associated with CBF, but fitter older adults unexpectedly had prolonged ATT in parietal and occipital regions. Blood pressure and grip strength were not associated with CBF or ATT. Interestingly, data indicates greater age-related sensitivity of ATT than CBF. Regarding cognitive function, neither CBF nor ATT were associated with contemporaneous processing speed, working memory, or attention. Future research should investigate responses of CBF and ATT to exercise training and whether CBF or ATT predict changes in cognitive function over time.

2.5.7 Acknowledgements

This work was funded by the Norwegian Research Council (FRIPRO 300030). We thank Bethany Skinner, Consuelo Vidal Gran, Nicolas Hayston, Rupali Limachya, Amelie Grandjean, Aoife Marley, Shi Miao, and Samuel Thomas for data collection support, and Roksana Markiewicz for cognitive data analysis. We thank Danny Wang and the University of Southern California's Steven Neuroimaging and Informatics Institute for the provision of the PCASL sequence used in this work, which was provided through a C2P agreement with The Regents of the University of California.

2.6 Supplemental Material

2.6.1. Participant inclusion criteria

Inclusion criteria were: 1) aged 60-85 years. 2) do <150 mins of moderate physical activity per week. 3) monolingual. 4) right-handed. 5) no current/historic diagnosis of cardiovascular,

metabolic, respiratory, neurological, kidney, liver, or cancerous disease. 6) resting electrocardiogram (ECG) and blood pressure screened by clinician (i.e., no severe ECG abnormalities (e.g., ST depression, long QT, heart block, wide QRS) and systolic/diastolic blood pressure of <160/<90 mmHg, respectively). 7) Montreal Cognitive Assessment (MoCA) score ≥23. 8) not taking neurotransmitter-altering medication. 9) vaccinated against COVID-19. 9) deemed safe to enter MRI scanner by a qualified MRI-operator (i.e., absence/very small amount of ferrous metal in the body). 10) no language impairments. 11) no post-traumatic stress disorder (PTSD). 12) non-smoker of at least 5 years.

2.6.2. Cardiorespiratory fitness test

Treadmill test format example

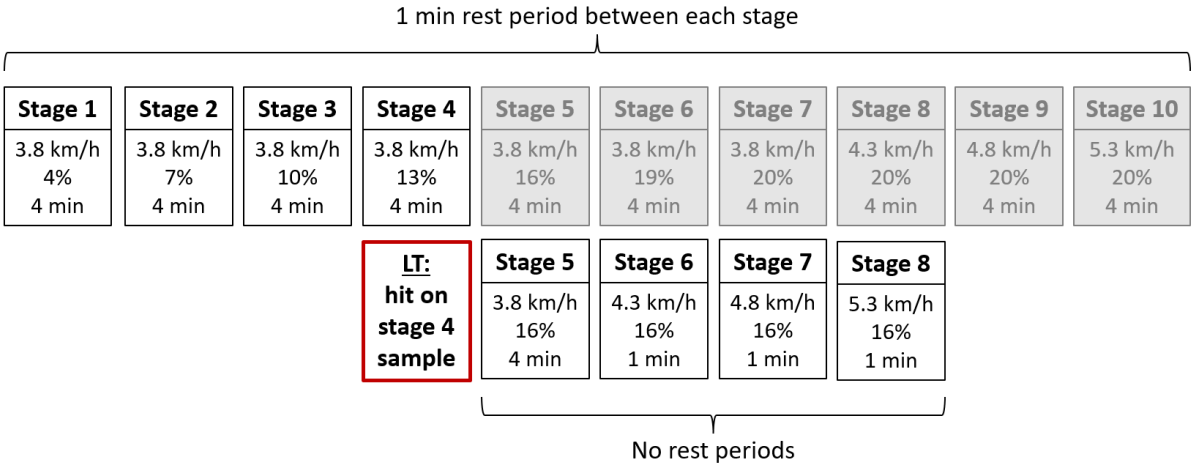

Figure 2.S1: Incremental treadmill test format example where lactate threshold (LT) is reached after stage 4. Stages in grey are possible stages had lactate threshold not been reached after stage 4.

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) measured continuously. Lactate and RPE measured during each rest period and 1 min post-exercise. Heart rate recorded at the end of each stage. Participants began the next stage whilst waiting for lactate analysis from the previous rest period.

Peak oxygen consumption prediction method

Peak oxygen consumption ($\dot{V}O_{2peak}$) was predicted using the equation: x = (y-c)/m. Gradient (m) and intercept (c) were calculated from the line of best fit between three sub-maximal heart rate

and $\dot{V}O_2$ data points from an individual's treadmill test, and y = age-predicted maximal heart rate ($HR_{age\text{-}pred}$; 220-age). This method assumes a largely linear relationship between heart rate and $\dot{V}O_2$ and that $HR_{age\text{-}pred}$ is relatively accurate (generally ±8–12 bpm) (Achten and Jeukendrup, 2003). This method was tested on 13 participants that completed high quality treadmill tests (peak values: $\%HR_{age\text{-}pred}$=103±5, respiratory exchange ratio (RER)= 1.14±0.03). The mean difference between actual ($\dot{V}O_{2peak}$) and predicted ($\dot{V}O_{2peak\text{-}pred}$) peak oxygen consumption was 8.6±2.5% (range=-11–12.3%). For n=8, the difference was <10%.

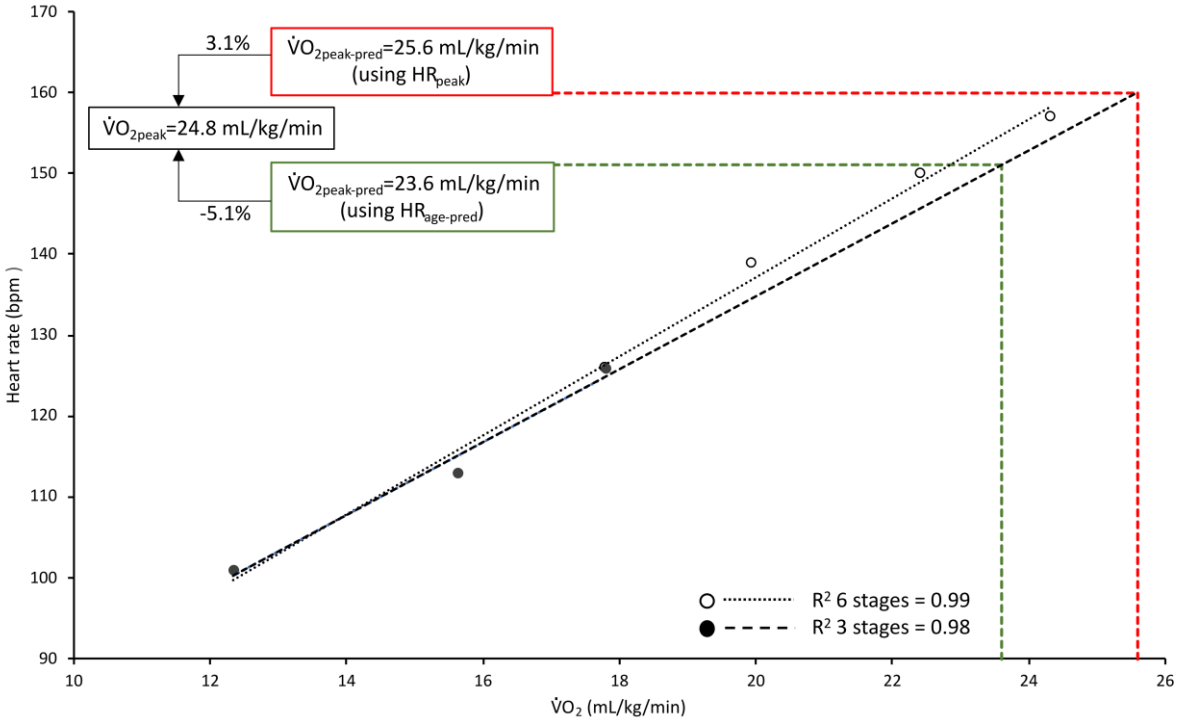

Figure 2.S2: Example of peak oxygen consumption ($\dot{V}O_{2peak}$) prediction from sub-maximal heart rate and $\dot{V}O_2$ data (n=1).

The difference in the slope of the regression lines when using three and six sub-maximal stages is minimal. For this participant, predicted peak oxygen consumption ($\dot{V}O_{2peak\text{-}pred}$) was underestimated (-5.1%) when using $HR_{age\text{-}pred}$ (151 bpm; green line). For this participant, accuracy of this method was improved by 2% when using the peak heart rate (HR_{peak}) recorded in the treadmill test (160 bpm; red line). This method was validated in a sub-sample of participants who completed high quality treadmill test (n=13), mean change in predictive accuracy when using $HR_{age\text{-}pred}$ to HR_{peak}=2.1±3.4%).

2.6.3. MRI acquisition and analysis

As described in the main manuscript, for each participant, ASL data at each PLD (difference of tag and control averaged over repeats of each PLD) and grey matter masks in native space were visually inspected. Participants with abnormal ASL data were further investigated and n=6 data were excluded due to excessive motion, Figure 2.S4 shows some examples of ASL data which were excluded after this visual data quality inspection step. Native space grey matter masks were thresholded at 0.5 probability to ensure only voxels containing primarily grey matter were included in calculations of CBF and ATT. Areas within masks containing incorrect assignment to grey matter, primarily around the eyes and nasal cavity, were manually removed (n=7).

Structural MRI data were aligned to the MNI brain using fsl_anat (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fsl_anat). Registrations to MNI space were visually inspected. For participants with poor registration, the nonlinear registration was disabled and processing re-run. For participants where registration was deemed poor despite best efforts (due to brain atrophy with age), data were excluded from regional analysis requiring data in MNI space (n=1). Regional grey matter masks were made in MNI space and defined using the MNI structural atlas (temporal lobe only) or from the conjunction of the relevant regions from the Harvard atlas (in FSL). For n=9, MNI registration specifically of the inferior frontal lobe was poor (due to significant brain atrophy) and thus frontal lobe CBF and ATT maps were edited to exclude this region from analysis using fslroi.

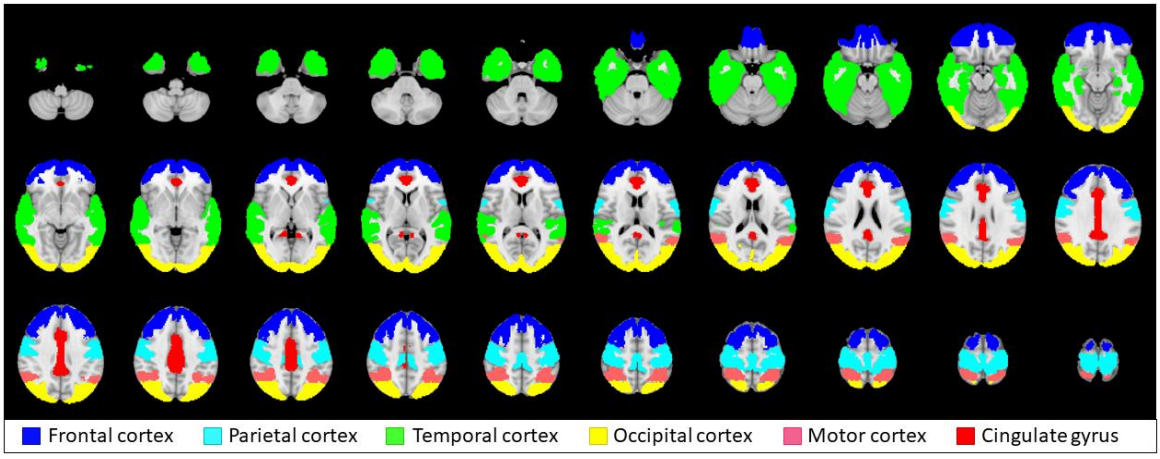

Figure 2.S3: Grey matter masks used for region of interest analysis in MNI space.

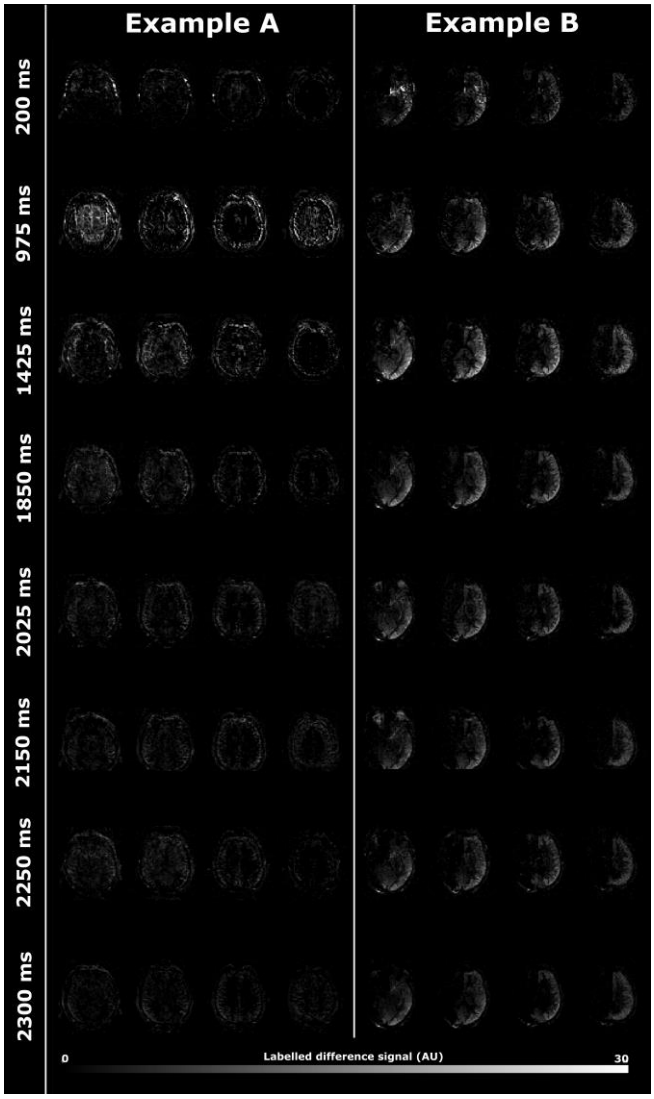

Figure 2.S4: Examples from two excluded participants of arterial spin labelling difference maps at each post-labelling delay. AU: arbitrary units.

2.6.4. Global and regional data for CBF and ATT

Table 2.S1: Global and regional values for grey matter CBF and ATT.

	Total	Male	Female
CBF (mL/100 g/min)			
Global	63±12	62±12	65±12
Frontal cortex	78±17	78±21	79±18
Parietal cortex	85±19	85±23	86±19
Temporal cortex	56±12	55±15	58±11
Occipital cortex	78±18	75±21	81±18
Motor cortex	93±20	92±25	95±20
Cingulate gyrus	90±20	87±23	92±20
ATT (s)			
Global	1.42±0.17	1.43±0.17	1.40±0.17
Frontal cortex	1.37±0.14	1.39±0.26	1.35±0.14
Parietal cortex	1.50±0.16	1.54±0.30	1.46±0.15
Temporal cortex	1.26±0.13	1.27±0.24	1.26±0.13
Occipital cortex	1.59±0.19	1.63±0.32	1.55±0.19
Motor cortex	1.43±0.14	1.45±0.27	1.40±0.14
Cingulate gyrus	1.25±0.18	1.25±0.26	1.25±0.19

Values represent means ± standard deviation. Global (n=78) and regional (n=77) values are from native and MNI space, respectively. CBF: cerebral blood flow, ATT: arterial transit time.

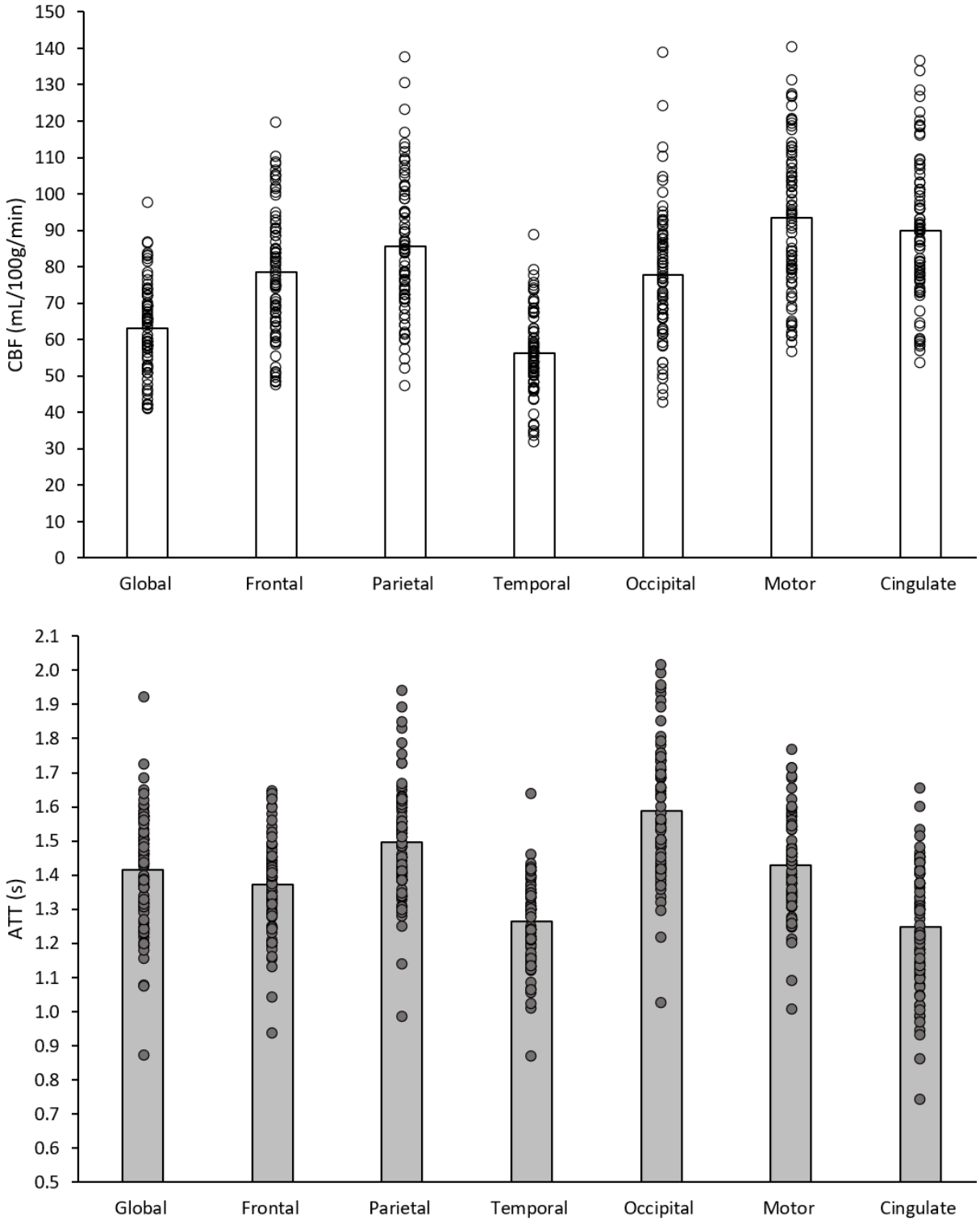

Figure 2.S5: Means and individual data for global and regional cerebral blood flow (CBF; top) and arterial transit time (ATT; bottom) in healthy older adults. Global (n=78) and regional (n=77) analyses were performed in native and MNI space, respectively.

Table 2.S2: Correlations between age and global cerebral blood flow (CBF).

Variables	Co-variates	Pearson correlation	*P*
Age/Global CBF		r(76)=-0.26	0.82
Age/Global CBF	Sex	r(76)=-0.32	0.78

Table 2.S3: Associations between age, BMI, and cardiorespiratory fitness with regional CBF.

	Age		BMI		$\dot{V}O_{2peak}$	
CBF	β	*P*	β	*P*	β	*P*
Frontal	-0.07	0.613	-0.34	**0.008**	-0.16	0.346
Parietal	-0.05	0.722	-0.41	**0.002**	-0.13	0.446
Temporal	-0.12	0.361	-0.44	**<0.001**	-0.20	0.213
Occipital	-0.06	0.639	-0.43	**<0.001**	-0.08	0.597
Motor	-0.03	0.805	-0.35	**0.007**	-0.19	0.257
Cingulate	0.00	0.987	-0.39	**0.002**	-0.11	0.515

Separate multiple linear regressions were performed for each region (n=77), independent variables: age, sex, BMI, and $\dot{V}O_{2peak}$. Bold indicates significant P values survived adjustment for multiple comparisons. β: standardised beta coefficient, CBF: cerebral blood flow, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption.

2.6.5 Associations between cognitive function and CBF or ATT

Global analysis

Table 2.S4: Associations between global CBF or ATT with cognitive function.

	gCBF (mL/100 g/min)		**gATT (s)**	
n=76	β	*P*	β	*P*
Age (years)	0.113	0.400	0.325	0.015
Sex	0.096	0.435	-0.107	0.372
Education	-0.079	0.522	0.075	0.532
Processing speed				
Response time	-0.049	0.696	-0.001	0.995
Accuracy	0.247	0.056	-0.082	0.514
Working memory				
2-back *d'* prime	0.170	0.190	0.016	0.900
Attention (response time)				
Alerting	-0.122	0.344	0.041	0.746
Orienting	0.073	0.580	-0.017	0.896
Executive control	0.111	0.405	-0.034	0.795

Results from two multiple linear regression analyses. β: standardised beta coefficient, gCBF: global cerebral blood flow, gATT: global arterial transit time.

Regional analysis

To analyse regional differences, multiple linear regressions were performed with regional CBF or ATT as dependent variables, and age, sex, education (1; compulsory, 2; further, 3; undergraduate, 4; post-graduate) and scores for processing speed (accuracy and response time), working memory (*d* prime), and the three attentional domains (alerting, orienting, and executive control response times) as independent variables. MNI registration was poor for n=1 and cognitive data was missing for n=2, leaving n=75 for regional analysis.

All associations between CBF or ATT of any region and any cognitive measures were non-significant. Only processing speed accuracy showed any indication of a trend with non-significant positive associations with CBF in all regions (β=0.23–0.25, *P*=0.052–0.080).

Table 2.S5: Alternative multiple linear regression models for global CBF and ATT replacing systolic and diastolic blood pressure with either pulse pressure or mean arterial pressure.

	gCBF (mL/100 g/min)		gATT (s)	
	β	***P***	**β**	***P***
Pulse pressure model				
Age (years)	-0.12	0.425	0.43	**0.003**
Sex	0.05	0.855	-0.19	0.404
BMI (kg/m^2)	-0.37	**0.006**	0.30	**0.016**
Pulse pressure (mmHg)	-0.01	0.921	-0.10	0.386
VO_{2peak} (mL/kg/min)	-0.17	0.328	0.24	0.145
Hand grip strength (kgf)	0.06	0.795	-0.33	0.112
Grey matter volume (mm^3)	0.07	0.618	-0.01	0.943
Mean arterial pressure model				
Age (years)	-0.15	0.330	0.40	**0.006**
Sex	0.03	0.900	-0.18	0.427
BMI (kg/m^2)	-0.35	**0.008**	0.30	**0.017**
Mean arterial pressure (mmHg)	-0.10	0.395	-0.05	0.657
VO_{2peak} (mL/kg/min)	-0.18	0.301	0.24	0.143
Hand grip strength (kgf)	0.06	0.796	-0.31	0.130
Grey matter volume (mm^3)	0.06	0.666	-0.02	0.897

Bold indicates P<0.05. β: *standardised beta coefficient, gCBF: global cerebral blood flow, gATT: global arterial transit time, BMI: body mass index,* $\dot{V}O_{2peak}$*: peak oxygen consumption.*

CHAPTER THREE

CEREBRAL BLOOD FLOW AND ARTERIAL TRANSIT TIME RESPONSES TO HOME-BASED EXERCISE TRAINING IN HEALTHY OLDER ADULTS

The work within this chapter has been submitted for publication at *NeuroImage* and is currently under review. The chapter contains the most recent manuscript and is followed by the accompanying Supplemental Material.

The candidate was the sole recruiter of participants included within all data chapters, was heavily involved with the health screening process to determine participant eligibility, and was primarily responsible for all participant communications and administration. The candidate completed all of the data collection relating to cardiorespiratory fitness testing and physical function testing, and was also heavily involved with the data collection relating to the cognitive function tests and the MRI scan. The candidate was solely responsible for educating participants about the six-month exercise programme and associated equipment, as well as conducting regular follow-ups with participants over the intervention period. The candidate also organised the distribution of accelerometers to participants throughout the intervention period and analysed these data. The candidate completed synthesis and analysis of the presented participants adherence data. Under guidance from KJM, the candidate completed the MRI arterial spin labelling data analysis. The candidate completed the statistical analyses and data visualisation presented in all data chapters and was responsible for leading the writing of the presented manuscripts. KS, SJEL, HS, and SB set-up the larger project. All co-authors read and suggested edits to the manuscript. FR, KEJ, and AG also assisted data collection.

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3.1 Abstract

Brain vascular health worsens with age, as is made evident by grey matter cerebral blood flow (CBF_{GM}) reductions and lengthening arterial transit time (ATT_{GM}). Exercise training can improve aspects of brain health in older adults, yet its effects on CBF_{GM} and ATT_{GM} remain unclear. This randomised controlled trial assessed responses of CBF_{GM} and ATT_{GM} to a 26-week home-based exercise intervention in 65 healthy older adults (control; n=33, exercise; n=32, aged 60–81 years), including whether changes in CBF_{GM} or ATT_{GM} were associated with cognitive function changes. Multi-delay pseudo-continuous arterial spin labelling data were used to estimate resting global and regional CBF_{GM} and ATT_{GM}. Results showed no between-group differences in CBF_{GM} or ATT_{GM} following the intervention. However, exercise participants with the greatest cardiorespiratory gains (n=17; $\Delta\dot{V}O_{2peak}$>2 mL/kg/min) experienced global CBF_{GM} reductions (-4.0 [-7.3, -0.8] mL/100 g/min). Furthermore, within the whole exercise group, cardiorespiratory fitness gains were associated with global CBF_{GM} declines (β=-0.33 [-0.71, 0.06]), but no association was present with ATT_{GM}. No changes in cognitive function or associations with CBF_{GM} or ATT_{GM} changes were observed. Our findings indicate exercise training in older adults may induce global CBF_{GM} reductions, which are associated with the magnitude of cardiorespiratory fitness gains, but do not appear to affect cognitive function.

3.2 Introduction

The global population is ageing rapidly (United Nations Department of Economic and Social Affairs, Population Division, 2022). With age, inevitably, comes cognitive decline, hindering the ability to live independently and maintain quality of life (Salthouse, 2012; Stites et al., 2018). Adverse age-related changes to cerebral haemodynamics, notably cerebral hypoperfusion (Damestani et al., 2023), may contribute to cognitive decline in older adults (De Vis et al., 2018; Ebenau et al., 2023; van Dinther et al., 2023). Regular exercise is considered an essential strategy

to maintain brain health in later life because it benefits cognitive function (Barha et al., 2017; Northey et al., 2018) and dementia risk (Tari et al., 2019). Exercise-induced brain health benefits have been attributed to a multitude of factors, including neurogenesis, angiogenesis, neuroinflammation, and neural plasticity (Chen and Nakagawa, 2023; Lu et al., 2023). However, despite the observed associations between cerebral haemodynamics and cognitive function, the effects of exercise training on cerebral haemodynamics in older adults remain poorly understood (Kleinloog et al., 2023).

Alongside grey matter cerebral blood flow (CBF_{GM}) reductions, arterial transit time (ATT_{GM}) also typically worsens (i.e., lengthens) with age (Damestani et al., 2023), though ATT_{GM} prolongation has been associated with greater cardiorespiratory fitness (Chapter 2) (Feron et al., 2024b). ATT_{GM} is the time taken for blood to travel from large arteries in the neck to the cerebral tissue. Prolonged ATT is associated with impaired cerebrovascular reactivity (Takata et al., 2023) and atherosclerotic risk (Hafdi et al., 2022), and is present in patients with Alzheimer's disease (Gyanwali et al., 2022; M. Sun et al., 2022) or cerebral artery stenosis (Yu et al., 2022). The MRI sequence, arterial spin labelling (ASL) can estimate both CBF_{GM} and ATT_{GM} if data are acquired at multiple post-labelling delays. Using multiple post-labelling delays also improves CBF_{GM} estimation accuracy by enabling adjustment for regional and individual differences in ATT_{GM} (Dai et al., 2017). Despite this, compared with single-delay ASL, only a minority of ASL studies have utilised this technique due to increased data collection time requirements, although shorter multi-delay sequences are now available (Woods et al., 2024).

Exercise training induces favourable changes in arterial stiffness, endothelial function, blood pressure, body composition, and grey matter volume (Erickson et al., 2014; Hellsten and Nyberg, 2015; Slentz et al., 2005). Despite these variables being associated with higher resting volumetric CBF (Poels et al., 2008; Sabayan et al., 2014) or CBF_{GM} (Jefferson et al., 2018; Leidhin et al., 2021),

and evidence that long-term exercise training reduces age-related declines in regional (Tarumi et al., 2013; Thomas et al., 2013) and global (Sugawara et al., 2020) CBF_{GM}, there is limited evidence for training-induced CBF_{GM} increases in healthy older adults. For example, exercise training for eight or twelve weeks increased CBF_{GM} in small regions (Alfini et al., 2019; Chapman et al., 2013; Kleinloog et al., 2019; Maass et al., 2015); however, regional CBF_{GM} decreases were also present (Kleinloog et al., 2019; Maass et al., 2015). Furthermore, global effects in whole-brain CBF (Chapman et al., 2013) or CBF_{GM} (Flodin et al., 2017; Kleinloog et al., 2019; Maass et al., 2015) have not been observed. Interestingly, evidence that a one-year exercise intervention increased global volumetric CBF in older adults (Tomoto et al., 2023b) and a one-year weight loss intervention (diet and exercise) induced widespread CBF_{GM} increases in overweight/obese middle-aged adults (Stillman et al., 2021) indicates that longer interventions may be required to induce global effects. Differences in the exercise intervention, CBF measurement technique, or regions investigated may explain discrepancies in results. All, except two (Maass et al., 2015; Tomoto et al., 2023b), aforementioned studies measured CBF with ASL, but none used multiple post-labelling delays.

Regarding ATT_{GM}, to our knowledge, no studies have investigated responses to exercise training. Two cross-sectional studies assessed the relationship between cardiorespiratory fitness and ATT_{GM} in older adults, with one reporting no correlation (n=14) (Burley et al., 2021) and the other unexpectedly reporting higher cardiorespiratory fitness was associated with longer ATT_{GM} in parietal and occipital regions (n=77) (Chapter 2) (Feron et al., 2024b). Furthermore, training-induced changes to cerebral blood velocity, which is inversely related to ATT_{GM} (Burley et al., 2021), are also unclear (Kleinloog et al., 2023; Smith et al., 2021). Exercise training studies are required to improve understanding of ATT_{GM} responses.

The present randomised controlled trial used multiple post-labelling delay ASL to investigate responses of resting CBF_{GM} and ATT_{GM} to home-based exercise training in older adults, and to assess if any changes were associated with training-induced cognitive improvements. High-intensity interval training was performed, hypothesised to induce superior cerebrovascular and cognitive benefits due to repeated lactate exposure (El Hayek et al., 2019; Morland et al., 2017). Exercise training was hypothesised to increase cardiorespiratory fitness and CBF_{GM}, lengthen ATT_{GM}, and improve cognitive function. Furthermore, anticipated changes in CBF_{GM} and ATT_{GM} within the exercise group were hypothesised to be associated with improvements in cardiorespiratory fitness and cognitive function.

3.3 Materials and Methods

The data for the present study were collected as part of a larger study, The FAB Project (preregistration: https://osf.io/6fqg7, materials and data: https://osf.io/d7aw2/). The data in the present study are from a sub-group of a larger shared participant cohort, had unique outcome measures to other publications related to the project (Feron et al., 2024b; Fosstveit et al., 2024a; Rahman et al., 2023) and addressed *a priori* questions (as per preregistration). The study was approved by the STEM Ethical Review Committee at the University of Birmingham (ERN_20-1107). Before taking part in this study, participants were provided with a participant information sheet and provided their informed consent (see Appendices for participant information sheet and consent form).

3.3.1 Study design

Participants were screened for eligibility before completing three experimental sessions (cognitive, MRI, and exercise sessions). Participants were randomised to control or exercise groups after the cognitive session. Participants then entered a 26-week intervention period, before returning to complete the same three experimental sessions, but in the reverse order.

Post-intervention exercise and MRI sessions were conducted within a two-week period, starting on the first day of the final intervention week, achieved for 53% and 88% of the control and exercise group, respectively. Pre-intervention sessions were completed within 5.4±3.2 and 4.5±2.5 weeks and post-intervention sessions were completed within 2.8±2.3 and 1.8±1.3 weeks for control and exercise groups, respectively. Mean duration between the start of the intervention period and the start of post-intervention experimental sessions were 26.3±1.9 and 27.8±2.9 weeks for the control and exercise groups, respectively.

3.3.2 Participants

Ninety-four healthy older adults entered the study, participant flow is shown in Figure 3.1. Participants were aged 60–81 years cognitively normal, without historic or current diagnosis of serious health conditions, non-smokers, and self-reported to not meet recommended global activity guidelines (Bull et al., 2020). Of these, 77 had pre-intervention ASL data, nine dropped out of the intervention, and post-intervention ASL data was unusable for a further three, leaving a sample of 65 older adults (characteristics in Table 3.1). Full inclusion criteria and health screening procedure (resting electrocardiogram, blood pressure, and cognitive assessment) can be found in Section 1 of the Supplemental Material (3.6.1; Supplemental Material found at conclusion of this chapter).

Table 3.1: Participant characteristics.

	Control	Exercise
n (male:female)	33 (16:17)	32 (17:15)
Age (years)	65±5	66±5
Highest education level reached (%)		
Compulsory	27	25
Further	27	38
Undergraduate	27	9
Postgraduate	18	28
Resting SBP (mmHg)	141±14	139±12
Resting DBP (mmHg)	82±8	83±7
BMI (kg/m^2)	27.9±4.1	26.2±2.3
$\dot{V}O_{2peak}$ (mL/kg/min)	27.6±3.5	27.8±4.3

Values represent means ± standard deviation. SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption.

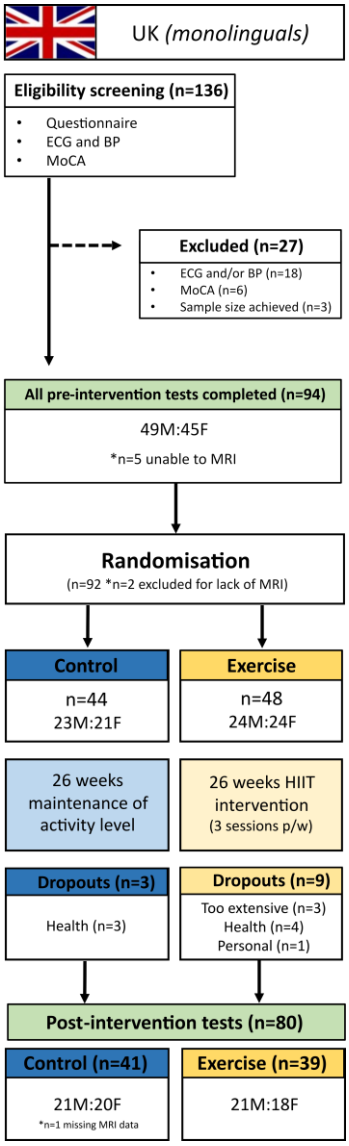

Figure 3.1: CONSORT diagram of participant flow. ECG: electrocardiogram, BP: blood pressure, MoCA: Montreal Cognitive Assessment, MRI: magnetic resonance imaging, HIIT: high-intensity interval training.

3.3.3 26-week intervention

Control participants were asked to continue living their normal life (i.e., avoid making significant changes to their physical activity and dietary habits).

Exercise participants completed a 26-week unsupervised home-based high-intensity interval training programme involving two interval and one circuit training sessions each week (Fosstveit et al., 2024a). Participants had regular contact and feedback from the researcher (every 1-2 weeks), but more so during the familiarisation period (weeks 1-4). Participants completed a logbook and were given a fitness watch (Polar Unite, Finland) and chest heart rate monitor (Polar H9, Finland) to monitor real-time heart rate and record each session. Exercise intensity was guided using the percentage of peak heart rate (%HR_{peak}) achieved during the pre-intervention cardiorespiratory fitness test.

Interval sessions involved alternating between two minutes of high-intensity exercise and active recovery. Participants were attempting to reach >80%HR_{peak} by the end of each high-intensity interval. The majority opted to walk uphill, jog, or run. Participants started with five intervals per session, the workload progressively increased and finished with ten intervals. Circuit sessions involved six body-weight exercises (squats, high-knees, step-ups, press-ups, reverse lunges, and mountain climbers). Participants progressed from completing one to three sets of each exercise per session during the familiarisation and then completed 3 x 45 second sets in weeks 5-26, aiming to perform as many repetitions as possible and reach >80%HR_{peak} by the end of each set. Further exercise intervention details, including progressive overload (Tables 3.S1 and 3.S2) and example heart rate graphs (Figure 3.S1), can be found in Section 2 of the Supplemental Material (3.6.2).

3.3.4 Intervention adherence

Detailed adherence methodology can be found in Section 2 of the Supplemental Material (3.6.2).

Physical activity levels in the control group were objectively measured during the first, middle, and final week of the intervention period using a waist-worn accelerometer (GT3X+, ActiGraph Inc., USA), categorised as, minutes per day, sedentary behaviour, light physical activity (LPA), and moderate-to-vigorous intensity physical activity (MVPA) (Troiano et al., 2008).

In the exercise group, the logbook and heart rate recordings for each session enabled accurate determination of the number of sessions completed, session duration, and heart rate (mean, peak, and zones) for each participant. Adherence was calculated for a variety of metrics as the percentage completion relative to what was planned. The primary metric was adherence to cumulative metabolic equivalents minutes (MET-mins) during the intervention period (Nilsen et al., 2018). MET-mins for each session were calculated by multiplying session duration by the MET value corresponding to the mean $\%HR_{peak}$ of each session (Garber et al., 2011). Heart rate-specific MET values (Table 3.S3) were calculated based on American College of Sports Medicine (ACSM) guidelines (Garber et al., 2011).

3.3.5 Outcome Measures

Cardiorespiratory fitness

Participants completed an incremental exercise test on a treadmill (Pulsar 3p, H/P/Cosmos, Germany). Respiratory gases ($\dot{V}O_2$: oxygen consumption, $\dot{V}CO_2$: carbon dioxide production) were recorded continuously using a facemask (7450 V2, Hans Rudolph, USA) and metabolic cart (JAEGER Vyntus CPX, Vyaire, USA), as was heart rate and rhythm using a 12-lead ECG (Cardiosoft, Vyaire, USA). Rating of perceived exertion (RPE) (Borg, 1982) and finger-prick blood [lactate] (Biosen C-Line, EKF Diagnostics, United Kingdom) were measured between stages. Stages were

4 min with a 1 min rest period between each stage. Treadmill speed started and remained at 3.8 km/h until either all possible elevation stages were completed (4, 7, 10, 13, 16, 19, and 20% gradient) or individual lactate threshold was reached (2.1 mmol/L increase over the mean of the two lowest values (Mamen et al., 2011)). If all elevation stages were completed, 4 min stages continued with speed increasing 0.5 km/h per stage until lactate threshold. After reaching lactate threshold, 1 min stages were completed where speed increased 0.5 km/h per stage (rest periods were removed). Figure 3.S2 shows a treadmill test format example.

Participants were asked to exercise to volitional exhaustion unless halted by the researcher due to ECG abnormalities. Cardiorespiratory fitness was determined using peak oxygen consumption ($\dot{V}O_{2peak}$) (i.e., mean of the two highest 30 s intervals). Five control and ten exercise participants completed a sub-maximal test at pre-intervention and/or post-intervention. For these participants, predicted $\dot{V}O_{2peak}$ values were used at both timepoints even if only one test was sub-maximal (for consistency). Predictions used individual sub-maximal $\dot{V}O_2$ and heart rate data acquired from three of the first possible six stages using a linear regression. See Section 3 of the Supplemental Material (3.6.3) for full details and example (Figure 3.S3) of the prediction method.

MRI acquisition and analysis

An MRI scan session included structural, functional, and arterial spin labelling (ASL) scans, using a 3-T system (MAGNETOM Prisma, Siemens, Germany) with 32-channel receiver head coil. Here, the focus is the ASL data and related scans, analysis of other data acquired can be found elsewhere (Rahman et al., 2023). CBF and ATT data were collected using pseudo-continuous ASL sequence with 3D GRASE readout (17:22 mins) (Kilroy et al., 2014; D. J. J. Wang et al., 2013), see also Acknowledgements (3.5.7).

ASL imaging parameters were: repetition time (TR)=4100 ms, echo time (TE)=30.56 ms, in-plane resolution=3.5 mm^2, slice thickness=3.5 mm, transversal slices=32, field of view (FOV)=224x224

mm, background suppression=yes, and post-labelling delays (PLD)=200, 975, 1425, 1850, 2025, 2150, 2250, and 2300 ms. Four and twelve volumes of data were acquired for PLD of 200–2250 ms and 2300 ms, respectively. PLD times and number of volumes acquired were optimised according to recommendations (Woods et al., 2019). Slices were positioned axially from the motor cortex and angled anterior-posterior in line with the participant's anterior-posterior commissure (ACPC). A calibration M0 scan was acquired using these same parameters with the PLD set to 2000 ms with PCASL and background suppression disabled. The T1-weighted structural scan (4:54 mins) was acquired to facilitate data analysis including, normalisation to a standard template brain and differentiation of grey and white matter. Structural T1-weighted (MPRAGE) imaging parameters were: TI=880 ms, TE=2.03 ms, TR=2000 ms, voxel size=1 mm^3, sagittal slices=208, FOV=256×256×208 mm, and flip angle=8°.

ASL data were processed using the Oxford ASL toolbox (https://oxasl.readthedocs.io/en/latest/), which uses the FSL FABBER ASL package and Bayesian Inference to invert the kinetic model for ASL MRI (BASIL) to compute CBF and ATT maps (Chappell et al., 2009; Groves et al., 2009; Woolrich et al., 2006). Parameters input to the kinetic models to estimate CBF and ATT were: bolus duration=1.5088 s, tissue T1=1.3 s, arterial blood T1=1.65 s, labelling efficiency=0.85. All other input parameters were kept with default settings appropriate to PCASL acquisition. Partial volume error correction and adaptive spatial smoothing of the perfusion maps was performed using default settings in oxford_asl (Chappell et al., 2011; Groves et al., 2009).

Global and regional analysis was performed, assessed in native (individual participant) and MNI space, respectively. All CBF and ATT values refer to grey matter only. Regions of interest were the cingulate gyrus and frontal, parietal, temporal, occipital, and motor cortices (Figure 3.S4). Only participants with useable ASL data in both native and MNI space were included in analyses (n=65). Native space difference maps at each PLD for each participant were visually inspected to ensure

data quality. Particular attention was paid to ensure there were no: 1) excessive motion resulting in spurious edge effects in difference maps; 2) brain territories which did not appear to be perfused, due to suboptimal label positioning or unaccounted for vasculature; and 3) focal areas of high intensity in final CBF maps which would have suggested that the PLDs were insufficient. Seven participants were excluded for poor ASL data after visual inspection, difference maps from two exemplar excluded participants are shown in Figure 3.S5. One further participant was excluded for poor MNI registration due to brain atrophy. Section 4 of the Supplemental Material (3.6.4) contains additional information regarding data quality assessment and grey matter mask configuration.

Cognitive function

Working memory: In a 2-back task, participants were presented a 3x3 grid. The stimulus was a single white square that continuously appears, disappears, and then reappears in one of the grid squares at random (n=60 trials, 1 s each). Participants identified when the white square appeared in the same location as it did two trials prior. Trials were excluded from analysis if incorrect or if response time <200 ms or greater than two standard deviations above/below the mean per participant. The primary outcome measure was *d* prime (*d*'), a measure of discriminability, a greater *d*' indicates superior performance.

Attentional Network Task (ANT): The computerised ANT assessed orienting, alerting, and executive control. The stimulus is a row of five arrows, each pointing left or right. As fast and as accurately as possible, participants reported the direction of the centre arrow using the left and right arrow keys. A central fixation cross is displayed for 400 ms, then a fixation cross (500 ms) and cue (100 ms) are presented simultaneously, and then only the fixation cross is displayed for a further 400 ms. A stimulus is then shown for a maximum of 1700 ms. The centre arrow can be congruent or incongruent (i.e., pointing in the same or opposite direction as the flankers,

respectively; n=96 each), or neutral (i.e., central arrow flanked by target-irrelevant black blocks, n=96). The stimulus can appear above or below the fixation cross, cued by a black square (n=216) or not cued (n=72). There are three cue conditions: a spatial cue, a centre cue, or a double cue (n=72 each). The spatial cue indicates if the stimulus appears above or below the fixation cross, whereas the stimulus location remains ambiguous for the centre and the double cue. Twelve practice trials are followed by three blocks of 96 trials.

Trials were excluded from analysis if incorrect or if response time <200 ms or greater than two standard deviations above/below the mean per participant. Alerting scores were calculated as the no cue minus the double cue; Orienting scores by centre cue minus the spatial cue; Executive control scores were calculated by the incongruent target minus the congruent target (all for correct responses). High condition difference scores for alerting and orienting, and low condition difference scores for executive control, indicate better performance.

Processing speed: In a letter comparison task, participants were simultaneously presented with two strings of letters at the top and bottom of the screen, for a maximum of 2500 ms after presentation of a fixation cross (1000 ms). Strings were three or six characters long (n=48 trials, 24 each). As fast and as accurately as possible, participants identified whether the strings were the same or different. Mean response time and accuracy were calculated for each participant using data from trials involving only six-character strings. Trials were excluded from analysis if incorrect or if response time <200 ms or greater than two standard deviations above/below the mean per participant.

3.3.6 Statistical analyses

The intention-to-treat principle was used: analysis was performed with all data, excluding dropouts, according to the original group allocation, despite adherence variations. All regressions

used absolute changes, were completed separately for the control and exercise groups, and regional *P*-values were adjusted for multiple comparisons using the Bonferroni correction.

A mixed-design ANOVA compared within- and between-group differences in BMI and cardiorespiratory fitness ($\dot{V}O_{2peak}$), including age and sex as covariates (SPSS Statistics v.29, IBM, USA). A separate mixed-design ANOVA compared differences in global and regional CBF_{GM} and ATT_{GM}, including age, sex, and pre-intervention BMI as covariates (SPSS Statistics v.29, IBM, USA). Variation in training-induced $\dot{V}O_{2peak}$ response was anticipated (Bouchard et al., 1999) and therefore sub-group analysis was planned to split the exercise group into high and low $\dot{V}O_{2peak}$ response groups, stratified according to one standard deviation greater than the mean $\dot{V}O_{2peak}$ change of the control group.

Multiple linear regressions using RStudio (RStudio Team, 2021) assessed associations between changes in CBF_{GM} or ATT_{GM} and cardiorespiratory fitness. Dependant variables were ΔCBF_{GM} or ΔATT_{GM} with age, sex, ΔBMI, and $\Delta\dot{V}O_{2peak}$ as independent variables. BMI has strong associations with both CBF_{GM} and ATT_{GM} (Chapter 2) (Feron et al., 2024b) and thus was included as a covariate in these analyses because, despite randomisation, the exercise group had a significantly lower BMI at pre-intervention (~-1.7 kg/m^2) and experienced significant BMI reductions at post-intervention (-0.4±0.8 kg/m^2).

Two control and six exercise participants had incomplete cognitive data sets leaving 57 participants for analysis. A mixed-design ANOVA compared differences in processing speed (accuracy and response time), working memory (*d'*), and the three attentional domains (alerting, orienting, and executive control scores), including age, sex, and education as covariates (SPSS Statistics v.29, IBM, USA). Multiple linear regressions using RStudio (RStudio Team, 2021) assessed associations between changes in cognitive function and $\dot{V}O_{2peak}$, CBF_{GM} or ATT_{GM}. The

dependent variable was either $\Delta\dot{V}O_{2peak}$, ΔCBF_{GM}, or ΔATT_{GM}, while independent variables were age, sex, education, and the change of the aforementioned six cognitive measures.

3.4 Results

3.4.1 Intervention adverse events, dropouts, and adherence

Overall, there were no serious adverse events resulting from the study and there were nine dropouts (one control and eight exercise participants), leaving n=65 for primary analyses. Adherence to the exercise programme was high. Percentage adherence of the exercise group for cumulative MET-mins, sessions per week, and minutes >80%HR_{peak} per session were 90±20%, 87±15%, and 92±56%. In the control group, participants did not substantially change their normal physical activity levels. Further details can be found in the Section 5 of the Supplemental Material (3.6.5).

3.4.2 Changes in cardiorespiratory fitness and BMI

There was a significant group × time interaction for $\dot{V}O_{2peak}$ ($F_{1,61}$=17.4, P<0.001) and BMI ($F_{1,61}$=4.4, P=0.041). Specifically, $\dot{V}O_{2peak}$ increased from pre-to-post in the exercise group (descriptive; 2.2±2.3 mL/kg/min or 8.3±9.1%, estimated; 2.2 [1.4, 3.0] mL/100 g/min), whereas the control group did not change (Table 3.2). However, Figure 3.2 highlights the inter-individual variability in $\dot{V}O_{2peak}$ responses to the exercise intervention. Regarding BMI, compared to the control group, BMI of the exercise group was significantly lower at both pre- and post-intervention, and the exercise group exhibited a significant reduction in BMI from pre-to-post (descriptive; -0.4±0.8 kg/m^2, estimated; -0.4 [-0.6, -0.1] kg/m^2) whereas the control group did not change (Table 3.2).

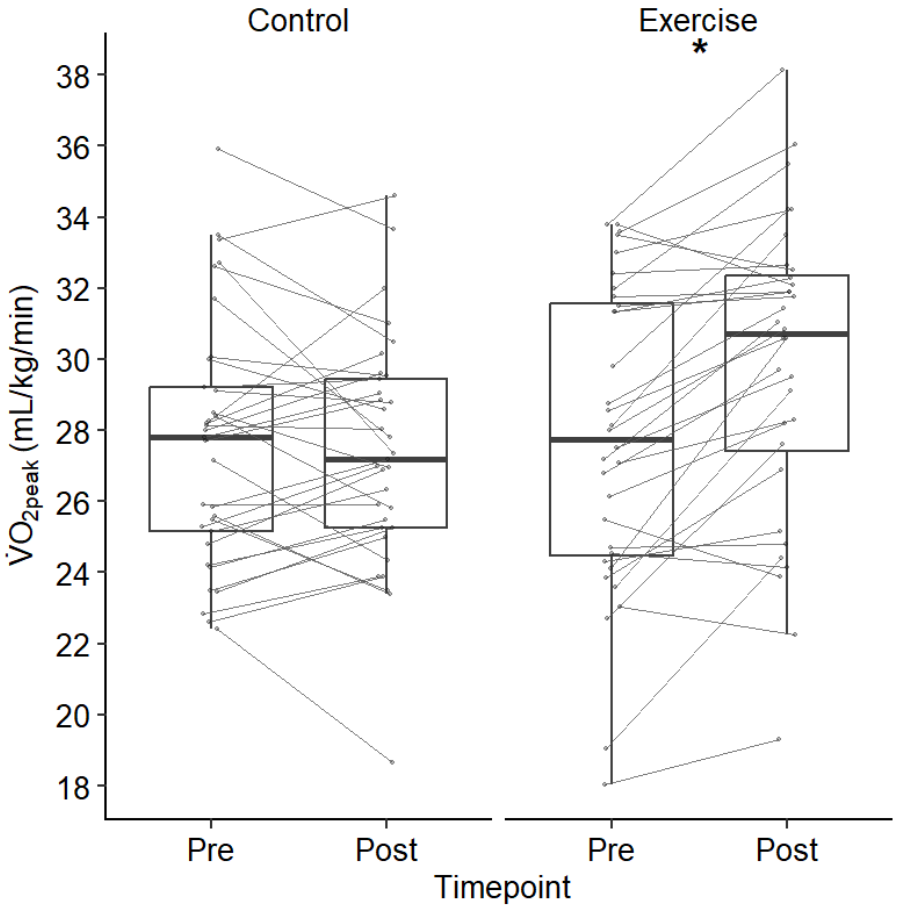

Figure 3.2: Descriptive changes in cardiorespiratory fitness from pre-to-post intervention. Values are median (bold line) and interquartile range, with individual participant changes from pre-to-post intervention (grey lines). $\dot{V}O_{2peak}$: peak oxygen consumption, *: significant difference from pre-to-post.

3.4.3 Pre-to-post intervention changes in CBF_{GM} and ATT_{GM}

There were no significant group × time interactions for global or regional CBF_{GM} or ATT_{GM} (Table 3.2). Figure 3.3 highlights the inter-individual variability in global CBF_{GM} and ATT_{GM} responses for both groups. Repeating analyses after excluding exercise participants with <80% cumulative MET-mins adherence (n=7) did not affect results. Descriptive means at pre-intervention, post-intervention, and for pre-to-post intervention change are shown in Table 3.2. See Figure 3.S6 for pre- and post-intervention CBF maps. Comparisons of individual changes in $\dot{V}O_{2peak}$ (Figure 3.2) and CBF_{GM} or ATT_{GM} (Figure 3.3) were also explored and are shown in Figure 3.S7.

Table 3.2: Mean changes in cardiorespiratory fitness, CBF_{GM}, and ATT_{GM} to the control and exercise intervention.

	Control (n=33)			Exercise (n=32)		
	Pre	Post	Δ	Pre	Post	Δ
BMI (kg/m^2)	27.9±4.1	27.9±4.3	0.0±0.7	26.2±2.3*	25.8±2.2$^{*\#}$	-0.4±0.8
$\dot{V}O_{2peak}$ (mL/kg/min)	27.6±3.5	27.4±3.2	-0.2±2.2	27.8±4.3	30.0±4.3$^{*\#}$	2.2±2.3
CBF_{GM} (mL/100 g/min)						
Global	62.7±13.2	63.6±12.0	1.0±8.6	62.2±11.2	60.8±9.8	-1.3±9.0
Frontal	77.8±17.6	79.3±16.4	1.5±12.4	78.5±18.1	76.4±13.6	-2.1±15.9
Parietal	85.5±19.3	86.1±17.6	0.6±13.6	84.3±18.1	82.2±14.0	-2.2±13.1
Temporal	56.2±13.3	57.4±11.9	1.2±7.4	55.5±10.2	53.5±9.3	-2.0±7.9
Occipital	79.1±20.3	80.1±19.2	0.9±15.1	76.1±14.8	74.5±13.9	-1.5±10.2
Motor	95.0±21.2	96.1±19.5	1.1±15.5	90.3±19.0	88.8±15.4	-1.5±15.3
Cingulate	90.1±21.1	90.4±18.0	0.3±12.8	87.9±18.9	85.3±15.0	-2.6±14.8
ATT_{GM} (s)						
Global	1.41±0.19	1.40±0.17	-0.01±0.14	1.41±0.14	1.41±0.15	0.00±0.15
Frontal	1.38±0.15	1.38±0.15	0.00±0.13	1.37±0.13	1.38±0.15	0.02±0.13
Parietal	1.50±0.18	1.52±0.18	0.02±0.10	1.49±0.14	1.51±0.16	0.02±0.11
Temporal	1.26±0.14	1.26±0.13	0.00±0.11	1.26±0.11	1.26±0.12	0.00±0.12
Occipital	1.59±0.21	1.59±0.20	0.01±0.11	1.58±0.17	1.60±0.18	0.02±0.12
Motor	1.44±0.16	1.44±0.14	0.01±0.10	1.42±0.13	1.44±0.14	0.02±0.10
Cingulate	1.25±0.20	1.23±0.17	-0.03±0.18	1.24±0.16	1.24±0.15	-0.01±0.18

*Values are descriptive means ± standard deviations. BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption, CBF_{GM}: grey matter cerebral blood flow, ATT_{GM}: grey matter arterial transit time, *: significant between-group difference at that timepoint (P<0.05), #: significant within-group difference from pre-to-post (P<0.05).*

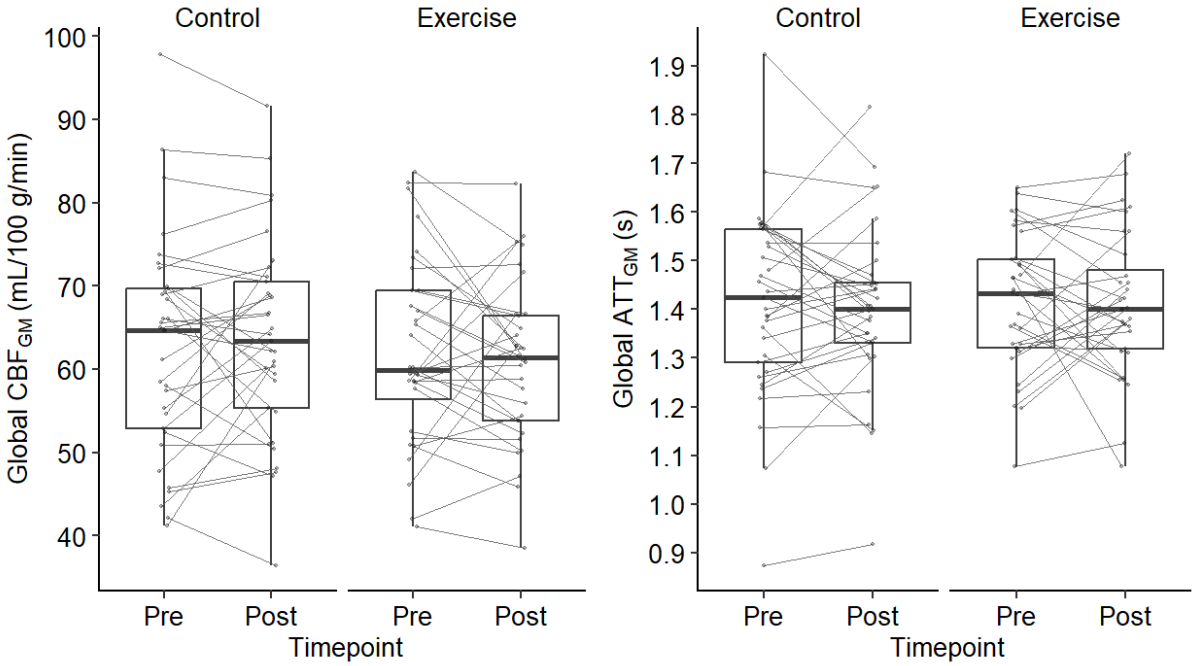

Figure 3.3: Descriptive changes in global CBF_{GM} (left) and ATT_{GM} (right) from pre-to-post intervention. Values are median (bold line) and interquartile range, with individual participant changes from pre-to-post intervention (grey lines). CBF_{GM}: grey matter cerebral blood flow, ATT_{GM}: grey matter arterial transit time.

The exercise group was stratified into high and low $\dot{V}O_{2peak}$ response groups (<2 or >2 mL/kg/min, respectively), based upon control group $\dot{V}O_{2peak}$ change (-0.2±2.2 mL/kg/min). A two-tailed one-sample T-test showed the mean global CBF_{GM} change of the high $\dot{V}O_{2peak}$ response group (n=17, 9M:8F, $\Delta\dot{V}O_{2peak}$=4.0±1.4 mL/kg/min) was significantly different from zero (ΔCBF_{GM}=-4.0±6.3 [-7.3, -0.8] mL/100 g/min; t_{16}=2.6, P=0.018), whereas the low group (n=15, 8M:7F, $\Delta\dot{V}O_{2peak}$=0.2±1.0 mL/kg/min) was not (ΔCBF_{GM}=1.7±10.7 [-4.2, 7.7] mL/ 100 g/min; t_{15}=0.6, P=0.545) (Figure 3.4A). Changes in ATT_{GM} were not significantly different from zero in either group (Figure 3.4B). Figure 3.S8 provides maps of pre-to-post intervention changes in CBF_{GM} for control and exercise groups that show widespread CBF_{GM} reductions for the exercise group, which are more pronounced in the high $\dot{V}O_{2peak}$ response group.

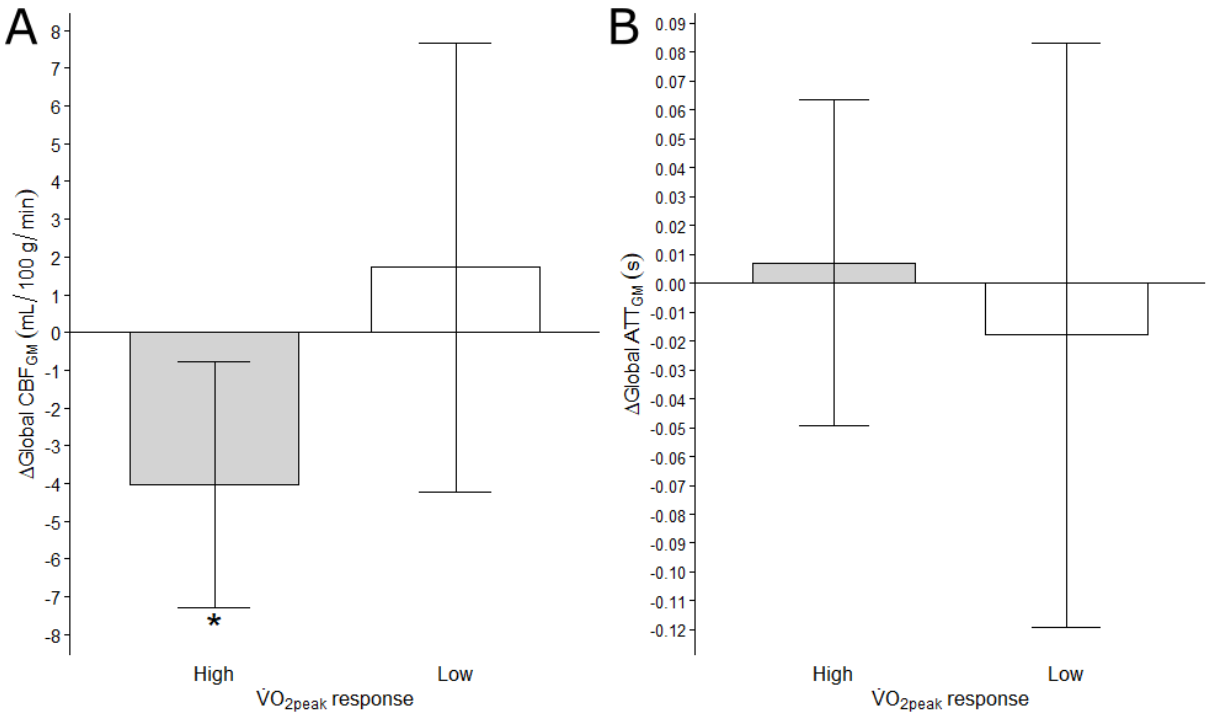

Figure 3.4: Pre-to-post intervention change in global CBF_{GM} (panel A) and global ATT_{GM} (panel B) with 95% confidence intervals from exercise group participants with either a high or low $\dot{V}O_{2peak}$ response to exercise training (i.e., >2 or <2 mL/kg/min increase, respectively). In the high group, but not the low, CBF_{GM} was significantly different from zero (-4.0 [-7.3, -0.8] mL/100 g/min). CBF_{GM}: cerebral blood flow, $\dot{V}O_{2peak}$: peak oxygen consumption, *: significant difference from zero (*P*=0.018).

3.4.4 Associations between changes in CBF_{GM} or ATT_{GM} and cardiorespiratory fitness

Regression analysis over the whole exercise group (n=32) revealed a non-significant negative association between the absolute changes in global CBF_{GM} and cardiorespiratory fitness (β=-0.33 [-0.71, 0.06], *P*=0.093) (Figure 3.S9 and 3.S10). A post-hoc analysis to identify if specific regions of the brain were driving this association identified that the association was strongest in parietal, motor, and, particularly, occipital regions (Figure 3.5 and Table 3.S5). In the control group, absolute changes in global CBF_{GM} and cardiorespiratory fitness were not associated (β=0.28 [-0.14, 0.71], *P*=0.187; Figure 3.S9). There were no significant associations between the absolute change in ATT_{GM} and cardiorespiratory fitness in the control (for all regions, *P*>0.12) or exercise (for all regions, *P*>0.19) group.

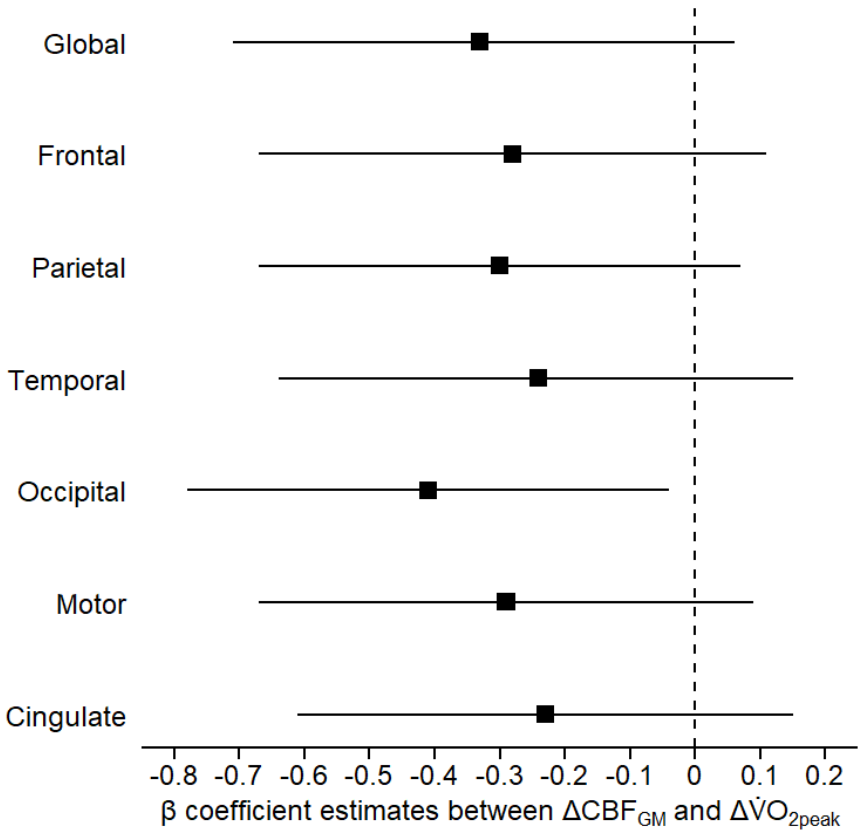

Figure 3.5: Partial regression associations with 95% confidence intervals between pre-to-post intervention changes in global or regional CBF_{GM} and cardiorespiratory fitness within the exercise group (n=32), adjusted for age, sex, and ΔBMI. β: standardised beta coefficient, CBF_{GM}: grey matter cerebral blood flow, $\dot{V}O_{2peak}$: peak oxygen consumption, BMI: body mass index.

3.4.5 Changes in cognitive performance and associations with CBF_{GM} or ATT_{GM}

There were no significant group × time interactions for any of the cognitive measures, nor were changes in these measures associated with changes in cardiorespiratory fitness in either group. Similarly, there were no associations between changes in global CBF_{GM} or ATT_{GM} and any cognitive function measure in either group.

3.5 Discussion

The present 26-week home-based exercise intervention increased cardiorespiratory fitness and reduced BMI in healthy older adults. There were no between-group differences in pre-to-post intervention changes in CBF_{GM} or ATT_{GM}; however, exercise participants with the greatest gains in

cardiorespiratory fitness experienced reductions in global CBF_{GM}. Neither group experienced changes in cognitive function, nor were changes in cognitive function associated with changes in $\dot{V}O_{2peak}$, CBF_{GM}, or ATT_{GM} in either group. Collectively, these data indicate that exercise training induces widespread CBF_{GM} reductions in older adults when cardiorespiratory fitness gains are greater than 2 mL/kg/min, but these changes in cardiorespiratory fitness or CBF_{GM} were not associated with changes in cognitive function.

3.5.1 Global CBF_{GM} was reduced in participants with a high cardiorespiratory fitness response

The cardiorespiratory fitness-dependant reductions in global CBF_{GM} observed in the present study indicate that for each 1 mL/kg/min increase in $\dot{V}O_{2peak}$, global CBF_{GM} fell by -1.3 [-2.8, 0.23] mL/100 g/min (P =0.093). Of note, given that global CBF_{GM} is reported to decrease by 3.1±1.1 mL/100 g/min per decade of life (Damestani et al., 2023), the reductions observed in the high $\dot{V}O_{2peak}$ response group would equate to ~13 years of age-related changes. Thus, these shorter-term changes seem likely to reflect changes in brain vascular physiology that are different to those mediated via ageing.

All previous research has reported no changes to global CBF_{GM} following an 8-week (Kleinloog et al., 2019), 12-week (Alfini et al., 2019; Chapman et al., 2013; Maass et al., 2015), or 6-month (Flodin et al., 2017) exercise intervention in healthy older adults. Notably, these studies generally used shorter intervention periods and had smaller sample sizes than the present study, potentially limiting the time for CBF_{GM} changes to occur and the ability to analyse cardiorespiratory fitness-based sub-groups. Differences in CBF_{GM} of focal regions have been observed previously, with both increases (Alfini et al., 2019; Chapman et al., 2013; Kleinloog et al., 2019) and decreases (Kleinloog et al., 2019; Maass et al., 2015) reported. Only two previous studies have investigated relationships between changes in cardiorespiratory fitness and global

CBF_{GM}, reporting either no association in control or exercise groups (Flodin et al., 2017), or a positive association when combining groups (Maass et al., 2015). Interestingly, in opposition to present findings, a one-year exercise intervention increased global volumetric CBF (i.e., blood flow through large cerebral arteries normalised for total brain volume), and these increases were positively associated with cardiorespiratory fitness changes within the exercise group (Tomoto et al., 2023b). The present data also oppose single-delay ASL findings that masters athletes experience reduced age-related global CBF_{GM} declines (Sugawara et al., 2020) or have elevated regional CBF_{GM} compared to sedentary peers (Tarumi et al., 2013). However, it is possible that reduced CBF_{GM} is a short-term response which changes when training is maintained for longer periods (i.e. several years).

Regarding adherence, compared to the low response group, the high $\dot{V}O_{2peak}$ response group had higher percentage adherence for cumulative MET-mins, total session number, and minutes >80%HR_{peak} per session by ~9%, ~7%, and ~30%, respectively, though these differences were not statistically significant (T-tests, see Table 3.S6). Notably, the largest adherence difference was to exercise intensity, rather than volume, whereby the high response group spent an additional 3.0 [-1.1, 7.2] mins/session >80%HR_{peak}. Furthermore, across the whole exercise group, changes in global CBF_{GM} were significantly negatively correlated with adherence to exercise intensity (r_{30}=-0.37 [-0.63, -0.02]), but not to volume (r_{30}=-0.06 [-0.40, 0.29]) (Figure 3.S11), indicating that specifically high-intensity exercise may be important for cerebrovascular adaptations in older adults. Indeed, the present study is the first to investigate CBF_{GM} responses to high-intensity interval training in healthy older adults, rather than a more traditional aerobic exercise intervention. High-intensity exercise training may induce more substantial cerebral adaptations because of repeated lactate exposure, shown to mediate training-induced cerebral angiogenesis in rodents (Morland et al., 2017).

3.5.2 Mechanisms for training-induced CBF_{GM} reductions

Whilst our findings regarding CBF_{GM} oppose previous studies, they do align with literature investigating training-induced peripheral blood flow changes. Training-induced resting CBF_{GM} reductions could be indicative of lowered blood flow requirements of the cerebral tissue resulting from adaptations that improve oxygen extraction and/or utilisation. This concept is true for skeletal muscle, where exercise training reduces blood flow during sub-maximal exercise (Proctor et al., 2001; Varnauskas et al., 1970) through improvements in oxygen extraction and metabolic efficiency (Bransford and Howley, 1977; Skattebo et al., 2020). Interestingly, a pilot study in young adult athletes reports a positive correlation between cardiorespiratory fitness and resting cerebral oxygen extraction of the right striatum (Bao et al., 2019) and 3-months exercise training reduced global cerebral metabolic rate of oxygen during sub-maximal exercise in overweight adults (Seifert et al., 2009).

Several exercise-induced adaptations could be contributing to CBF_{GM} reductions. For example, exercise training increases red blood cell count and [haemoglobin] in middle-aged adults (Sellami et al., 2021), subsequently increasing the oxygen carrying capacity of the blood (Mairbäurl, 2013). Furthermore, structural cerebrovascular changes are also evident, including a larger number of small cerebral vessels in masters athletes (Bullitt et al., 2009) and cerebral capillarisation in exercising rodents (Morland et al., 2017; Stevenson et al., 2020). Increased capillary density reduces diffusion distance, a key determinant of oxygen extraction (Dunn et al., 2016). Additionally, exercise training improves cerebral artery elasticity in middle-aged and older adults (Tarumi et al., 2013; Tomoto et al., 2023b). Changes in cerebral arterial vessel diameters are essential for CBF regulation (Claassen et al., 2021) and thus increased vessel elasticity may improve CBF regulation efficiency. Regarding cerebral oxygen utilisation, exercise training in rodents increases cerebral mitochondrial number and function (Braga et al., 2021; Dominiak et

al., 2022; Steiner et al., 2011). Similarly, in older adults, cardiorespiratory fitness is positively associated with *N*-acetyl-aspartate (NAA), a marker of neuronal integrity and metabolism (Erickson et al., 2012; Gonzales et al., 2013). Training-induced improvements to cerebral metabolic efficiency could also reduce blood flow requirements. Research is required to determine whether cerebral oxygen extraction and/or utilisation changes in response to exercise training. Such changes could help explain the poorly understood mechanisms regarding exercise training-induced cognitive benefits.

3.5.3 Exercise training did not induce changes in ATT_{GM}

The present study is the first to examine changes in ATT_{GM} following exercise training, reporting no changes in healthy older adults, globally or regionally, irrespective of $\dot{V}O_{2peak}$ response. Training-induced lengthening of ATT_{GM} was hypothesised based on cross-sectional data from the present cohort showing cardiorespiratory fitness was associated with longer ATT in parietal and occipital regions (Chapter 2) (Feron et al., 2024b). However, cerebral blood velocity, which is inversely correlated with ATT_{GM} (Burley et al., 2021), was unaffected by a one-year exercise intervention in older adults (Tomoto et al., 2023b), supporting the present findings.

Given that ATT_{GM} reflects the speed at which blood travels from large cerebral arteries to the grey matter tissue, the primary determinants are blood velocity and the vascular path length. Masters athletes have more small cerebral vessels and reduced cerebral vessel tortuosity (Bullitt et al., 2009). Theoretically, long-term training-induced small vessel angiogenesis could reduce overall blood velocity by increasing total vessel cross-sectional area, lengthening ATT_{GM}. Conversely, tortuosity reductions could shorten vascular path length, shortening ATT_{GM}. Regarding large artery cerebral blood velocity, both non-significant elevations (Bliss et al., 2023; Sugawara et al., 2020) and reductions (Zhu et al., 2013) in masters athletes have been reported. The present intervention

may not have been intense or long enough to induce these types of structural adaptations that could affect blood velocity, vessel diameter, and vascular path length.

Another factor to consider is BMI, which has strong and widespread associations with both CBF_{GM} and ATT_{GM} (Chapter 2) (Feron et al., 2024b). Although the present study reports CBF_{GM} changes in the absence of substantial changes in BMI or body mass (-1.1±2.4 kg), this could be more relevant for ATT_{GM}. A one-year diet or diet and exercise intervention inducing ~10 kg losses lead to widespread CBF_{GM} increases in overweight or obese middle-aged adults (Stillman et al., 2021). Longer-term exercise interventions or interventions specifically targeting body mass in overweight older adults may have more pronounced effects on ATT_{GM}.

3.5.4 Changes to CBF or ATT were not associated with changes to cognitive function

The present study reports no changes to processing speed, working memory, or attention following a six-month exercise programme. Findings regarding training-induced cognitive improvements in older adults are mixed, with meta-analyses reporting a lack of (Kelly et al., 2014; Young et al., 2015) or beneficial effects (Barha et al., 2017; Colcombe and Kramer, 2003; Northey et al., 2018). Studies specifically involving six-month interventions have reported cognitive improvements (Jonasson et al., 2017; Vidoni et al., 2015), although not by all (Brown et al., 2021). Interestingly, these reported cognitive benefits were seen in participants with lower baseline cardiorespiratory fitness than the present sample and thus experienced greater training-induced improvements (~9–28% vs. 8.3±9.1%). Indeed, cardiorespiratory fitness gains, rather than the volume or intensity of exercise, is suggested to be most predictive of cognitive benefits (Voss and Jain, 2022). This has been shown in studies both with (Kovacevic et al., 2020; Vidoni et al., 2015) and without (Brown et al., 2021; Maass et al., 2015; Voss et al., 2012) group-level cognitive differences. However, associations with cardiorespiratory fitness changes were also absent in the present study. The present findings may be explained by the sample having not experienced

significant age-related cognitive decline due to their health and education status (28% of the exercise group achieved post-graduate education). Additionally, higher intensity exercise interventions (i.e., sprint intervals) may elicit stronger cognitive benefits because of greater exercise-induced lactate exposure, linked with learning and memory in rodents (El Hayek et al., 2019).

Given the lack of group-level changes in CBF_{GM}, ATT_{GM}, and cognitive function, it is unsurprising that there were also no significant associations observed between changes in global CBF_{GM} or ATT_{GM} and any of the cognitive measures in either group. Previous research indicates that lower CBF_{GM} predicts cognitive decline over the longer term (i.e., years) in older adults (De Vis et al., 2018; Ebenau et al., 2023; van Dinther et al., 2023). As discussed, the training-induced CBF_{GM} reductions observed may indicate improved cerebral oxygen extraction and/or utilisation. If so, these changes may not have been present long enough to induce detectable cognitive improvements, explaining the lack of associations. Alternatively, CBF_{GM} reductions are potentially only a short-term training response that changes over time, and these longer-term, training-induced changes to cerebral haemodynamics may be more predictive of cognitive function. Additionally, other exercise-induced cerebral improvements may play a larger role in cognitive health, such as grey matter volume (Erickson et al., 2014), cerebral angiogenesis (Bullitt et al., 2009; Morland et al., 2017), or neuronal viability (Erickson et al., 2012).

3.5.5 Future directions and technical considerations

The present study's unexpected findings that exercise training induces CBF_{GM} reductions unveil new research questions. Primarily, 1) whether reductions are associated with changes in cerebral oxygen extraction and/or utilisation, and 2) whether reductions are a short-term response or persistent over longer-term exercise training (i.e., years). Furthermore, future research is

warranted investigating whether ATT_{GM} changes in response to longer-term exercise training or weight-loss interventions.

Generally, greater CBF_{GM} is assumed to indicate better brain health, but the present findings and evidence of compensatory hyperperfusion in cognitive impairment (Swinford et al., 2023) challenge this assumption. This should be considered when planning future research to further improve understanding. Given that the present study is the first to report training-induced global CBF_{GM} changes in healthy older adults (with a high $\dot{V}O_{2peak}$ response) and the only to use a high-intensity interval training programme, future research investigating similar brain health measures should prescribe, at least some, high-intensity exercise because of the potential substantial impacts of lactate exposure on brain health (El Hayek et al., 2019; Kujach et al., 2020; Morland et al., 2017).

Comparisons between the present study and previous findings, using single-delay ASL (Alfini et al., 2019; Chapman et al., 2013; Flodin et al., 2017; Kleinloog et al., 2019), are limited by measurement technique. Unlike the present study, CBF_{GM} was not adjusted for regional and individual ATT_{GM} differences, compromising estimation accuracy. Assuming ATT_{GM} is homogenous across the group and unchanged by exercise training, single-delay measurements would cause a systematic reduction in CBF_{GM} estimation. However, our previous work in the present sample found higher baseline cardiorespiratory fitness was associated with longer regional ATT_{GM} (Chapter 2) (Feron et al., 2024b). Therefore, we believe that multi-delay ASL is vital for this type of investigation to ensure accurate characterisation of CBF_{GM}. We used a range of PLDs based on the optimal for ATT <2000 ms (Woods et al., 2019). This appears sufficient for our relatively healthy older population as shown by the observed ATTs (Figure 3.3). However, the longest PLD used (2300 ms) may not be sufficient to capture more prolonged ATT in adult samples that are older or less healthy than our cohort.

Future research assessing cerebral haemodynamic changes should consider controlling for diet and partial pressure of end-tidal CO_2 ($PetCO_2$), which was not done in the present study. For example, caffeine and polyphenol intake can acutely alter global CBF_{GM} by 20-60% (Francis et al., 2006; Lamport et al., 2015; Vidyasagar et al., 2013). Moreover, $PetCO_2$ is a proxy for arterial partial pressure of CO_2 ($PaCO_2$), the most powerful regulator of CBF (Willie et al., 2014), which could be manipulated by anxiety-induced ventilatory changes during an MRI scan.

Finally, whole-group baseline accelerometery data indicated 86% of participants completing this study actually met the activity guidelines they self-reported not to, although participants possibly modified their normal behaviours (Clemes et al., 2008). Therefore, the generalisability of findings may be limited, and future research should assess if training-induced CBF_{GM} or ATT_{GM} changes differ between habitual high and low activity groups.

3.5.6 Conclusion

In summary, a 26-week home-based high-intensity interval exercise intervention was well adhered to and improved cardiorespiratory fitness in healthy older adults. Furthermore, global CBF_{GM} reductions were present in exercise participants with the greatest gains in cardiorespiratory fitness, but there were no changes in ATT_{GM}. A lower resting CBF_{GM} could indicate lower cerebral tissue blood flow requirements, resulting from exercise-induced adaptions that enhance oxygen extraction and/or utilisation. Regarding cognitive function, the exercise intervention had no group-level effects on processing speed, working memory, or attention, nor were changes in cognitive function associated with changes in cardiorespiratory fitness, CBF_{GM}, or ATT_{GM} in either group. Future research should investigate how cerebral oxygen extraction responds to exercise training and whether longer-term exercise training (i.e., years) changes the CBF_{GM} response and whether potential accompanying longer-term structural cerebral adaptations influence ATT_{GM}.

3.5.7 Acknowledgements

This work was funded by the Norwegian Research Council (FRIPRO 300030). We thank Bethany Skinner, Consuelo Vidal Gran, Nicolas Hayston, Rupali Limachya, Amelie Grandjean, Aoife Marley, Shi Miao, and Samuel Thomas for data collection support, and Roksana Markiewicz for cognitive data analysis. We thank Danny Wang and the University of Southern California's Steven Neuroimaging and Informatics Institute for the provision of the PCASL sequence used in this work, which was provided through a C2P agreement with The Regents of the University of California.

3.6 Supplemental Material

3.6.1 Participant enrolment

Inclusion criteria

Inclusion criteria were: 1) aged 60-85 years. 2) do <150 mins of moderate physical activity per week. 3) monolingual. 4) right-handed. 5) no current/historic diagnosis of cardiovascular, metabolic, respiratory, neurological, kidney, liver, or cancerous disease. 6) resting electrocardiogram (ECG) and blood pressure screened by clinician (i.e., no severe ECG abnormalities (e.g., ST depression, long QT, heart block, wide QRS) and systolic/diastolic blood pressure of <160/<90 mmHg, respectively). 7) Montreal Cognitive Assessment (MoCA) score ≥23. 8) not taking neurotransmitter-altering medication. 9) vaccinated against COVID-19. 9) deemed safe to enter MRI scanner by a qualified MRI-operator. 10) no language impairments. 11) no post-traumatic stress disorder (PTSD). 12) non-smoker of at least 5 years.

Health screening: electrocardiogram (ECG), blood pressure, and cognitive impairment

Participants completed a resting 12-lead ECG (Cardiosoft, Vyaire, USA), three resting blood measurements (705IT, Omron, Japan), and the Montreal Cognitive Assessment (MoCA). Participants were excluded for severe ECG abnormalities, MoCA scores <23 (Carson et al., 2018),

and resting systolic/diastolic blood pressure of >160/>90 mmHg, respectively. Excluded participants were referred to their GP.

3.6.2 Intervention periods

Exercise intervention

The exercise intervention was home-based, but participants received regular contact from the researcher, predominantly via email and phone. Twenty-six consecutive weeks were desired but not always possible (i.e., due to illness/injury/travel/personal reasons). In this case, intervention periods were extended to allow completion of 26-weeks with exercise sessions. Intervention periods were also extended for four participants who were due to finish in December so that gains were maintained before post-intervention testing was possible in January. Heart rate data recorded by participants using the fitness watch and heart rate monitor were automatically uploaded to a connected Polar Flow account to be accessed immediately by both the participant and researcher. The fitness watch and heart rate monitor allowed real-time intensity monitoring for participants and the option for them to look at their sessions retrospectively to gauge effort levels, as well the ability for the researcher to give detailed and regular feedback on their sessions.

Table 3.S1: Progressive overload of the interval training sessions.

Week	Session description	Target interval intensity	High-intensity duration	Total session duration
1	20 min continuous walk	n/a	n/a	20 mins
2	20 min continuous walk	n/a	n/a	20 mins
3	5 x 2 min intervals	>75%HR_{peak}	10 mins	20 mins
4-7	5 x 2 min intervals	>80%HR_{peak}	10 mins	20 mins
8-11	6 x 2 min intervals	>80%HR_{peak}	12 mins	24 mins
12-15	7 x 2 min intervals	>80%HR_{peak}	14 mins	28 mins
16-19	8 x 2 min intervals	>80%HR_{peak}	16 mins	32 mins
20-23	9 x 2 min intervals	>80%HR_{peak}	18 mins	36 mins
24-26	10 x 2 min intervals	>80%HR_{peak}	20 mins	40 mins

Two interval sessions per week with all 2 min intervals were separated by 2 min active recovery. HR_{peak}: peak heart rate.

Table 3.S2: Progressive overload of the circuit training sessions.

Week	Sets	Set format	Rest periods	Target intensity	High-intensity duration	Session duration
1	1	12 reps	n/a	n/a	n/a	n/a
2	2	12 reps	Between-sets: 60 s Between-exercises: 90 s	n/a	n/a	n/a
3	3	12 reps	Between-sets: 60 s Between-exercise: 90 s	n/a	n/a	n/a
4	2	45 s	Between-sets: 60 s Between-exercises: 90 s	>80%HR_{peak}	6 mins	24 mins
5-26	3	45 s	Between-sets: 30 s Between-exercises: 90 s	>80%HR_{peak}	9 mins	28.5 mins

One circuit session per week. The number of sets is completed for each of the six exercises. HR_{peak}: peak heart rate, reps: repetitions, s: seconds.

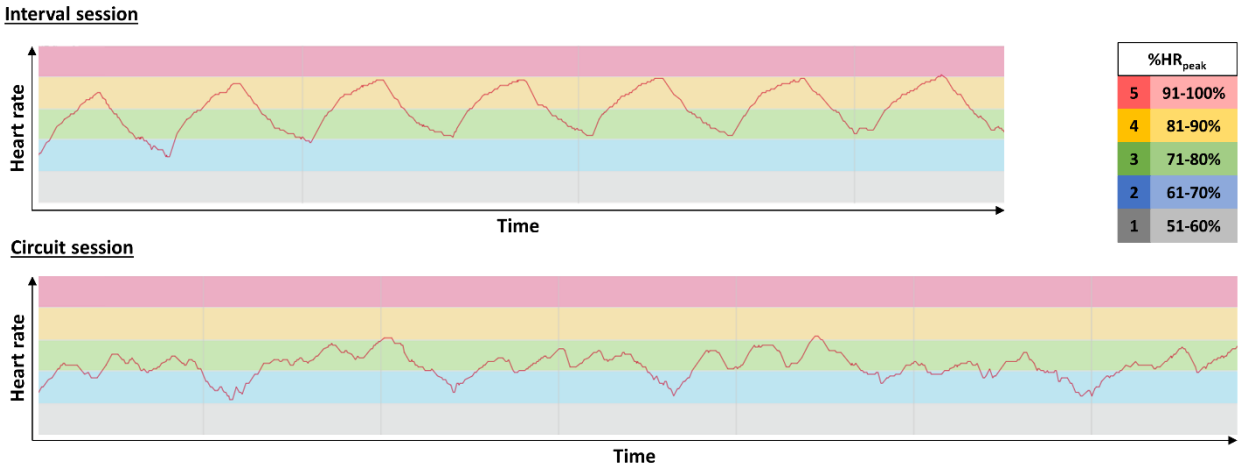

Figure 3.S1: Example heart rate graphs from an interval (top) and circuit (bottom) training session (n=1). Colours indicate heart rate zones, defined by percentage of peak heart rate (HR_{peak}).

Control intervention adherence

The waist-worn accelerometer (GT3X+, ActiGraph Inc., USA) was worn during all waking hours for seven consecutive days at each timepoint (excluding showering and water-based activities). Accelerometers were initialised and downloaded using ActiLife software (ActiGraph Inc., USA). Data were collected in 10 s epochs and only included for analysis if there were ≥10 hours of recordings on at least 4 out of 7 days. Non-wear time was characterised as intervals of ≥60 consecutive minutes with no activity counts, with allowance for 1 min with counts greater than zero. Activity levels were categorised based on uniaxial counts per minute (cpm) and defined as mean minutes per day of sedentary behaviour (0-99 cpm), light physical activity (100-2019 cpm), and moderate-to-vigorous physical activity (>2019 cpm) (Troiano et al., 2008).

Exercise intervention adherence

The written logbook and heart rate recordings for each session allowed accurate determination of the number of sessions completed, session duration, the number of intervals/sets completed each session, and heart rate (mean, peak, and zones). All heart rate data were visually inspected to determine inclusion. Adherence was calculated using only post-familiarisation data (i.e.,

weeks 5-26; 44 interval sessions, 22 circuit sessions). Exercise sessions were classed as interval, circuit, or other – 'other' refers to a non-prescribed session involving continuous exercise of any intensity. A novel method of reporting adherence to exercise interventions using metabolic equivalents (METs) has been proposed where the planned exercise dose is compared with what participants actually completed (Nilsen et al., 2018). Adherence to cumulative MET-mins was the primary adherence measure. Planned cumulative MET-mins for the intervention period were calculated by summating the planned MET-mins from each planned session. Planned MET-mins of each session were estimated by multiplying the planned session duration by the MET value corresponding to 70-76%$HR_{age\text{-}pred}$. Heart rate-specific MET values (Table 3.S3) were calculated based on American College of Sports Medicine (ACSM) guidelines (Garber et al., 2011). Mean heart rate for each session was estimated to fall within this range. This process was then completed for all participant exercise sessions (using actual session duration and mean %HR_{peak} of each session). Not all sessions had useable heart rate data meaning individual cumulative intervention MET-mins were calculated by multiplying mean METs/session by the number of completed sessions. Mean percentage of sessions with useable heart rate data was 96±6% across all exercise participants. Cumulative MET-mins was normalised for total intervention weeks (post-familiarisation), which differed between participants (range=18-31 weeks). This includes weeks where no exercise sessions were completed (e.g., due to illness or travel). For participants completing more than the planned post-familiarisation weeks (i.e., >22), MET-mins completion was relative to the possible achievable MET-mins in those weeks, not only to the MET-mins achievable in the planned weeks (e.g., planned MET-mins for 22 and 24 weeks are 8567 and 9346, respectively). Similarly, adherence to planned weekly session number was relative to total intervention weeks. For each metric, adherence was calculated as the percentage completion relative to what was planned.

Table 3.S3: Exercise intensity classification based on ACSM guidelines.

Intensity	%HR_{peak}	%$\dot{V}O_{2peak}$	RPE	METs calculation	METs
Low	<63	<45	<11		**Range: 1.6-3.1**
	≤56	≤36	≤8	≤1.5	**1.5**
	57-60	37-40	9	(1.6+2.4)/2	**2.0**
	61-63	41-45	10-11	(2.5+3.1)/2	**2.8**
Moderate	64-76	46-63	12-13		**Range: 3.2-4.7**
	64-69	46-54	12	(3.2+3.9)/2	**3.6**
	70-76	55-63	13	(4.0+4.7)/2	**4.4**
High	>77	>64	>14		**Range: 4.8-6.7 & ≥6.8**
	77-86	64-76	14-15	(4.8+5.8)/2	**5.3**
	87-95	77-90	16-17	(5.9+6.7)/2	**6.3**
	≥96	≥91	≥18	≥6.8	**6.8**

HR_{peak}: peak heart rate, $\dot{V}O_{2peak}$: peak oxygen uptake, RPE: rating of perceived exertion, METs: metabolic equivalents. Example calculation: Intensity of 67%HR_{peak} = 3.6 METs. Moderate-intensity METs range = 3.2 – 4.7 (4.7-3.2=1.5). Sub-group 1 = 3.2+(3.2+(1.5/2))/2 = 3.6.

3.6.3 Cardiorespiratory fitness test

Treadmill test format example

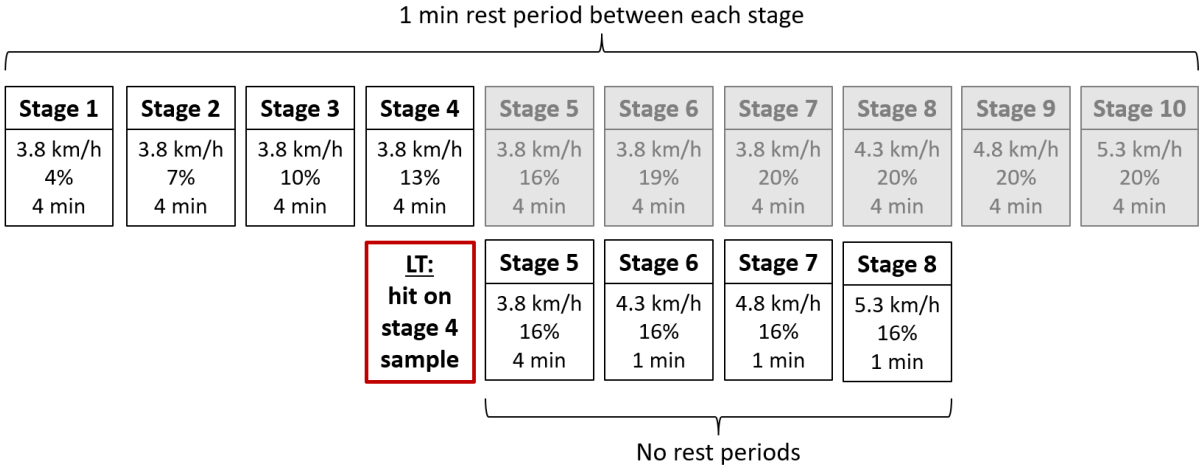

Figure 3.S2: Incremental treadmill test format example where lactate threshold (LT) is reached after stage 4. Stages in grey are possible stages had lactate threshold not been reached after stage 4.

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) measured continuously. Lactate and RPE measured during each rest period and 1 min post-exercise. Heart rate recorded at the end of each stage. Participants began the next stage whilst waiting for lactate analysis from the previous rest period.

Peak oxygen consumption prediction method

Peak oxygen consumption ($\dot{V}O_{2peak}$) was predicted using the equation: x = (y-c)/m. Gradient (m) and intercept (c) were calculated from the line of best fit between three sub-maximal heart rate and $\dot{V}O_2$ data points from an individual's treadmill test, and y = age-predicted maximal heart rate ($HR_{age\text{-}pred}$; 220-age). This method assumes a largely linear relationship between heart rate and $\dot{V}O_2$ and that $HR_{age\text{-}pred}$ is relatively accurate (generally ±8–12 bpm) (Achten and Jeukendrup, 2003). This method was tested on 13 participants that completed high quality treadmill tests (peak values: $\%HR_{age\text{-}pred}$=103±5, respiratory exchange ratio (RER)= 1.14±0.03). The mean difference between actual ($\dot{V}O_{2peak}$) and predicted ($\dot{V}O_{2peak\text{-}pred}$) peak oxygen consumption was 8.6±2.5% (range=-11–12.3%). For n=8, the difference was <10%.

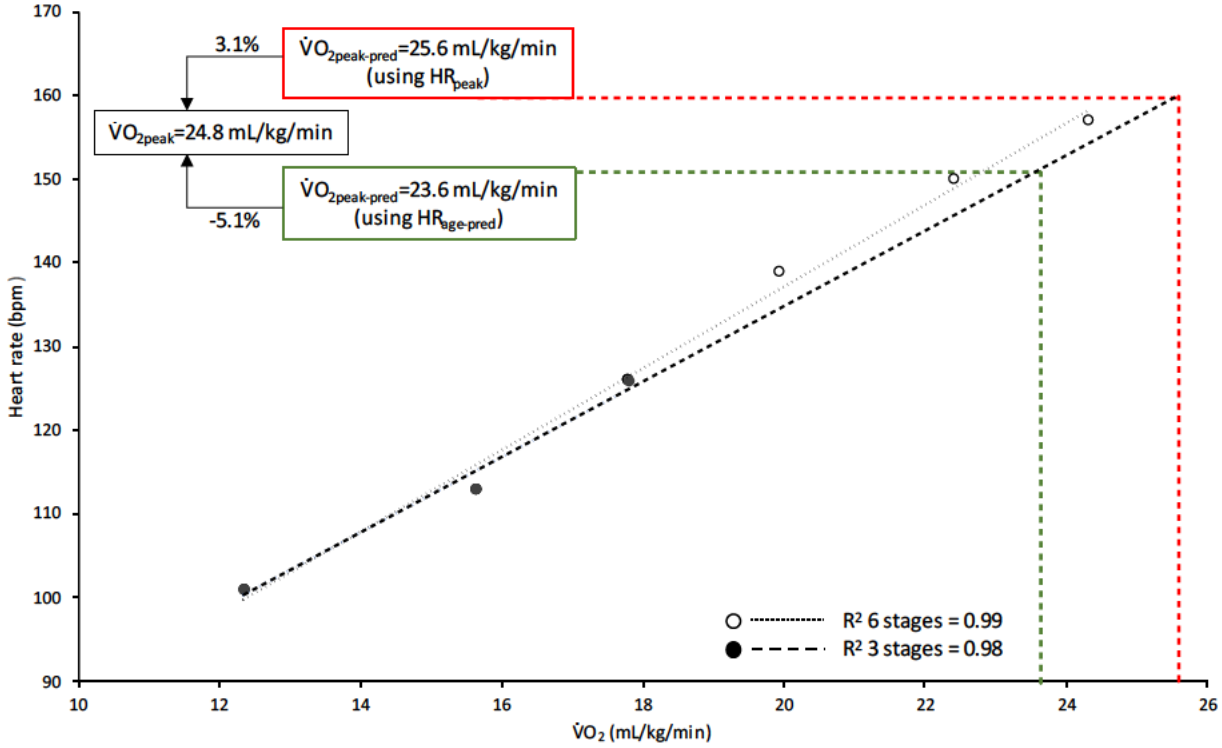

Figure 3.S3: Example of peak oxygen consumption ($\dot{V}O_{2peak}$) prediction from sub-maximal heart rate and $\dot{V}O_2$ data (n=1).

The difference in the slope of the regression lines when using three and six sub-maximal stages is minimal. For this participant, predicted peak oxygen consumption ($\dot{V}O_{2peak\text{-}pred}$) was underestimated (-5.1%) when using $HR_{age\text{-}pred}$ (151 bpm; green line). For this participant, accuracy of this method was improved by 2% when using the peak heart rate (HR_{peak}) recorded in the treadmill test (160 bpm; red line). This method was validated in a sub-sample of participants who completed high quality treadmill test (n=13), mean change in predictive accuracy when using $HR_{age\text{-}pred}$ to HR_{peak}=2.1±3.4%).

3.6.4 MRI acquisition and analysis

As mentioned in the methods above, for each participant, ASL data at each PLD (difference of tag and control averaged over repeats) and grey matter masks in native space were visually inspected. Seven participants were excluded due to excessive motion, poor labelling, or unbalanced perfusion maps (suggesting unusual vasculature or poor labelling). Figure 3.S5 shows two examples of ASL data which were excluded after this visual data quality inspection step. Native space grey matter masks were further thresholded at 0.5 probability to ensure only voxels

containing primarily grey matter were included in calculations of CBF_{GM} and ATT_{GM}. Sixteen masks (10 participants) contained areas with incorrect assignment to grey matter, primarily around the eyes and nasal cavity, which were manually removed.

Structural MRI data were aligned to the MNI brain using fsl_anat (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fsl_anat). Registrations to MNI space were visually inspected. For participants with poor registration, the nonlinear registration was disabled and processing re-run. One participant was excluded because registration was deemed poor despite best efforts (due to brain atrophy with age). Regional grey matter masks were made in MNI space and defined using the MNI structural atlas (temporal lobe only) or from the conjunction of the relevant regions from the Harvard atlas (in FSL). For 22 scans (13 participants), MNI registration specifically of the inferior frontal lobe was poor (due to significant brain atrophy) and thus frontal lobe CBF and ATT maps were truncated to exclude this region from analysis using fslroi. The chosen regions of interest (Figure 3.S4) have been used previously (Burley et al., 2021), and were broad because there were no specific *a priori* hypotheses of regions that would be affected by exercise training or associated with cognitive function.

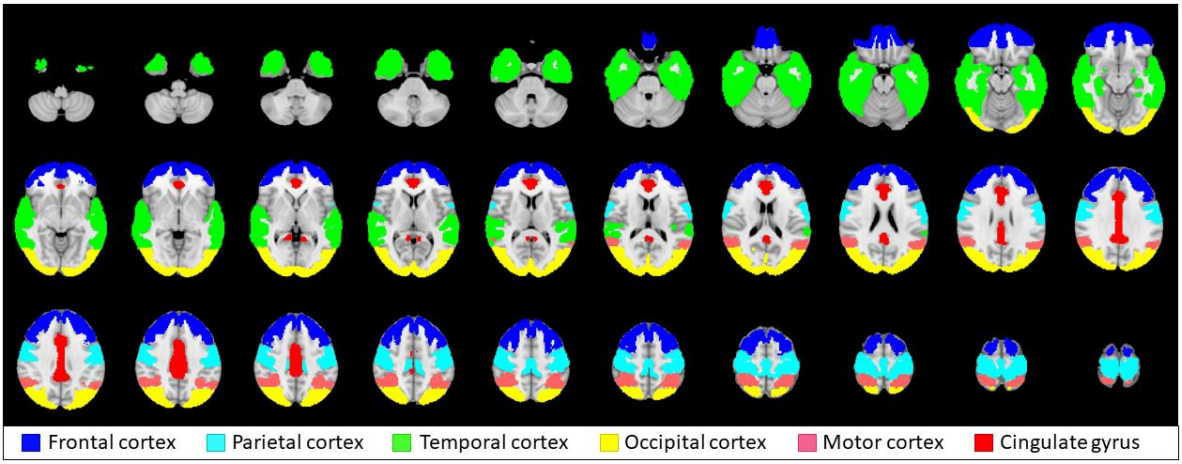

Figure 3.S4: Grey matter masks used for region of interest analysis in MNI space.

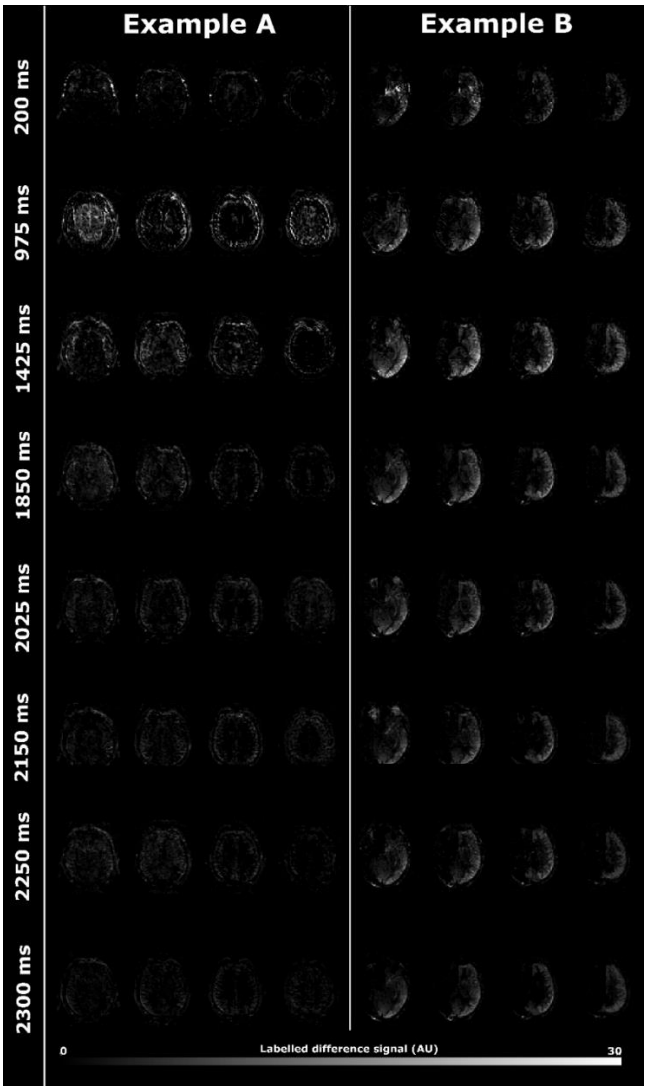

Figure 3.S5: Examples of arterial spin labelling difference maps at each post-labelling delay from two excluded participants. AU: arbitrary units.

3.6.5 Intervention adverse events, dropouts, and adherence

Adverse events

There were no serious adverse events resulting from participation in experimental sessions or the exercise programme. However, for a minority of participants, some exercises aggravated historical injuries/vulnerabilities (e.g., joint pain) or induced minor soft issue injuries (e.g., muscle strain) that were remedied with rest (~1 week) or were overcome by adapting exercises appropriately.

Dropouts

One control (1M:0F, 60 years, $\dot{V}O_{2peak}$=36.3 mL/kg/min) and eight exercise (2M:6F, 65±5 years, $\dot{V}O_{2peak}$=25.2±4.7 mL/kg/min) participants did not return for post-intervention testing sessions, leaving n=65 for primary analyses. Reasons cited for exercise group dropouts: muscle/joint pain (n=3), lack of time (n=1), personal issues (n=1), or unknown (n=3). Notably, 75% of exercise group dropouts were female; mean age and pre-intervention $\dot{V}O_{2peak}$ (sex-specific) of exercise group dropouts were similar to those that returned for post-intervention testing.

Adherence

Percentage adherence of the exercise group for cumulative MET-mins, sessions per week, and minutes >80%HR_{peak} per session were 90±20%, 87±15%, and 92±56%. Full details of exercise programme adherence can be found in Table S4. In the control group, participants did not substantially change their normal physical activity levels. Accelerometery data were incomplete for one control participant, leaving 32 for analysis. A one-way repeated measures ANOVA found a significant main effect of time on sedentary behaviour ($F_{2, 62}$=6.2, *P*=0.004) and LPA ($F_{2, 62}$=3.5, *P*=0.037), but not for MVPA (*P*>0.05). Specifically, sedentary behaviour in the final intervention week was significantly greater than both the first and middle intervention weeks by 26 [5, 47] and

31 [7, 54] mins/day, respectively, and LPA in the final intervention week was significantly lower than the middle intervention week by 14 [1, 28] mins/day.

Table 3.S4: Adherence of the exercise group to the exercise intervention using post-familiarisation data (weeks 5-26).

		Completed		
	Planned	Mean	Range	%
Weeks	22	23.6±3.2	18-31	107±15
Session number				
Overall	66	61±10	29-75	91±15
Interval	44	36±12	0-49	82±26
Circuit	22	19±5	6-29	88±22
Other	—	5±10	0-41	—
Sessions per week	3	2.6±0.5	1.6-3.8	87±15
Session duration (mins)				
Overall	29.5	31.5±4.4	25.2-49.4	107±16
Interval	30	29.9±3.0	22.0-39.1	99±10
Circuit	28.5	31.9±6.1	24.1.-51.3	113±20
Other	—	39.1±21.9	20.1-116.4	—
Intervals/sets per session				
Interval	7	6.7±1.2	3.3-8.1	90±17
Circuit	3	3.0±0.1	2.7-3.0	99±3
Mean heart rate (%HR_{peak})				
Overall	70-76	74±6	61-83	—
Interval	70-76	77±6	58-89	—
Circuit	70-76	68±7	56-79	—
Other	—	69±10	38-83	—
Minutes >80%HR_{peak}/session				
Overall	10.5	9.7±5.8	0-21	92±56
Interval	11.25	13.2±6.2	0.4-23.7	117±55
Circuit	9	4.3±4.9	0.0-16.8	48±54
Other	—	7.0±9.5	0.0-36.5	—
Cumulative MET-mins				
Overall	8567	8214±1875	3743-11791	90±20

Values are means ± standard deviations. HR_{peak}: peak heart rate, MET: metabolic equivalents.

3.6.6 Changes in cardiorespiratory fitness, CBF_{GM}, and ATT_{GM}

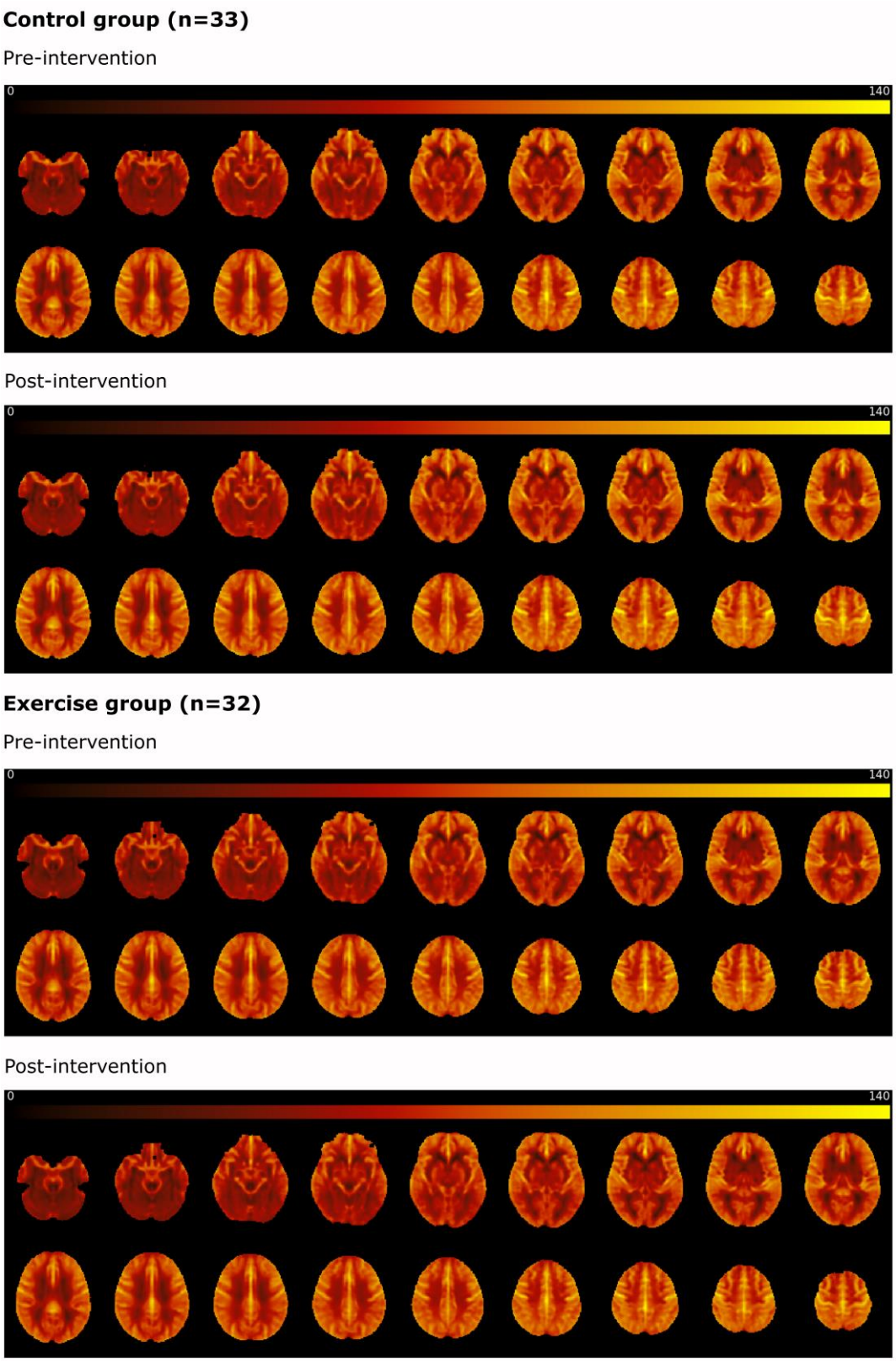

Figure 3.S6: Group average pre- and post-intervention cerebral blood flow (mL/100 g/min) maps for control and exercise groups.

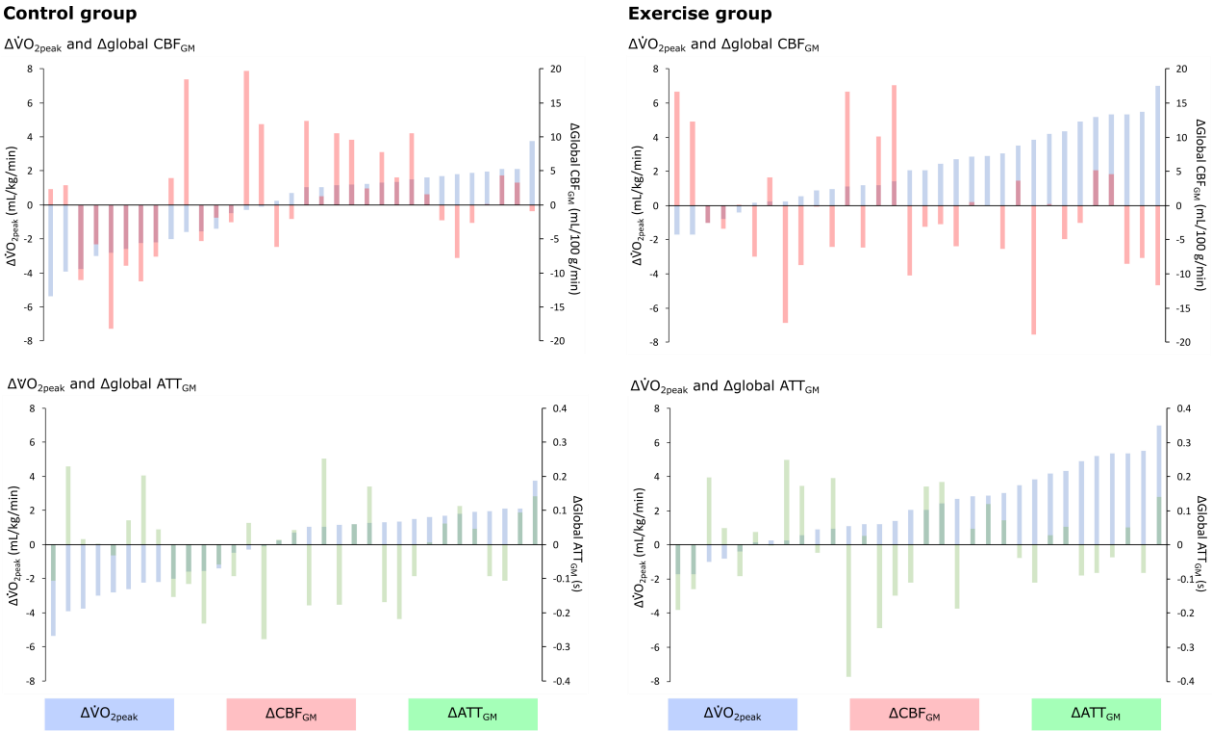

Figure 3.S7: Comparisons between individual participant pre-to-post intervention changes in $\dot{V}O_{2peak}$ and global CBF_{GM} (top panel) or global ATT_{GM} (bottom panel) for control (left) and exercise (right) groups. $\dot{V}O_{2peak}$: cardiorespiratory fitness, CBF_{GM}: grey matter cerebral blood flow, ATT_{GM}: grey matter arterial transit time.

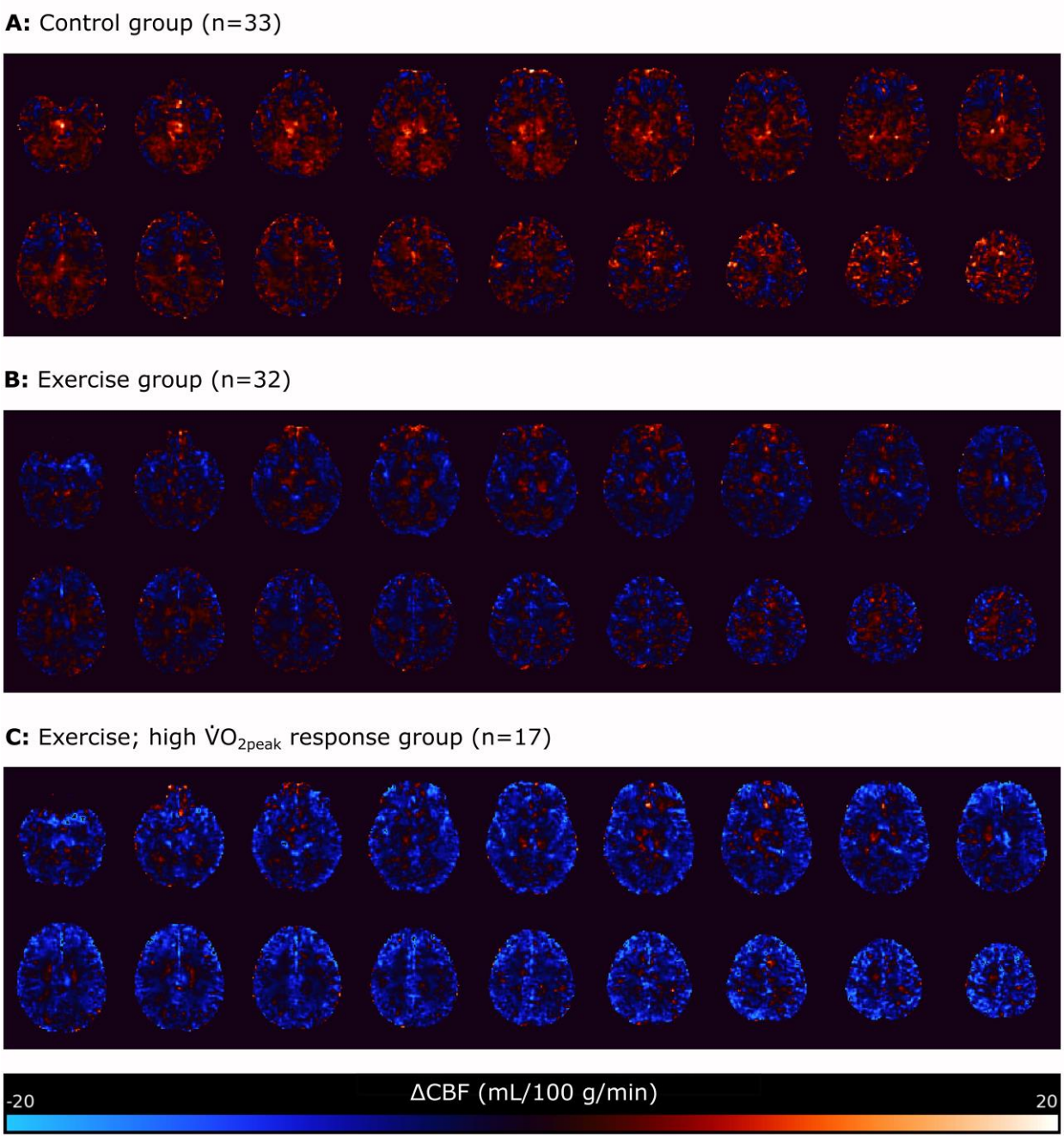

Figure 3.S8: Group difference maps of the pre-to-post intervention changes in cerebral blood flow (CBF: mL/100 g/min) for the control (A), exercise (B), and high $\dot{V}O_{2peak}$ response (C) groups. Blue indicates a reduction in CBF, red indicates an increase in CBF.

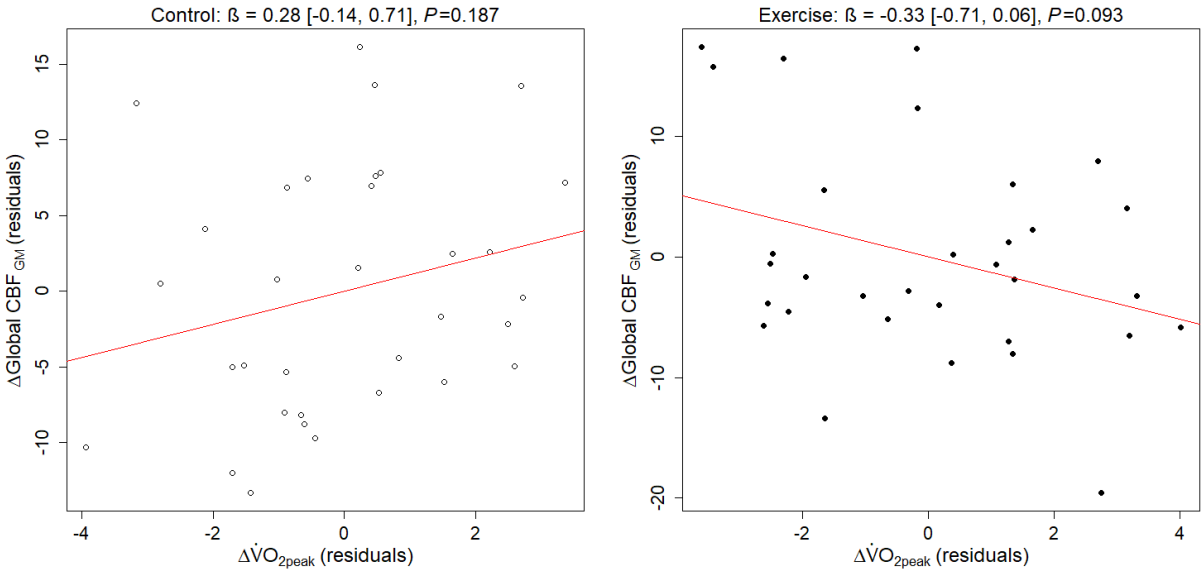

Figure 3.S9: Partial regression association between pre-to-post intervention change in global CBF_{GM} and cardiorespiratory fitness in the control (n=33; left) exercise group (n=32; right). CBF_{GM}: grey matter cerebral blood flow, $\dot{V}O_{2peak}$: peak oxygen consumption.

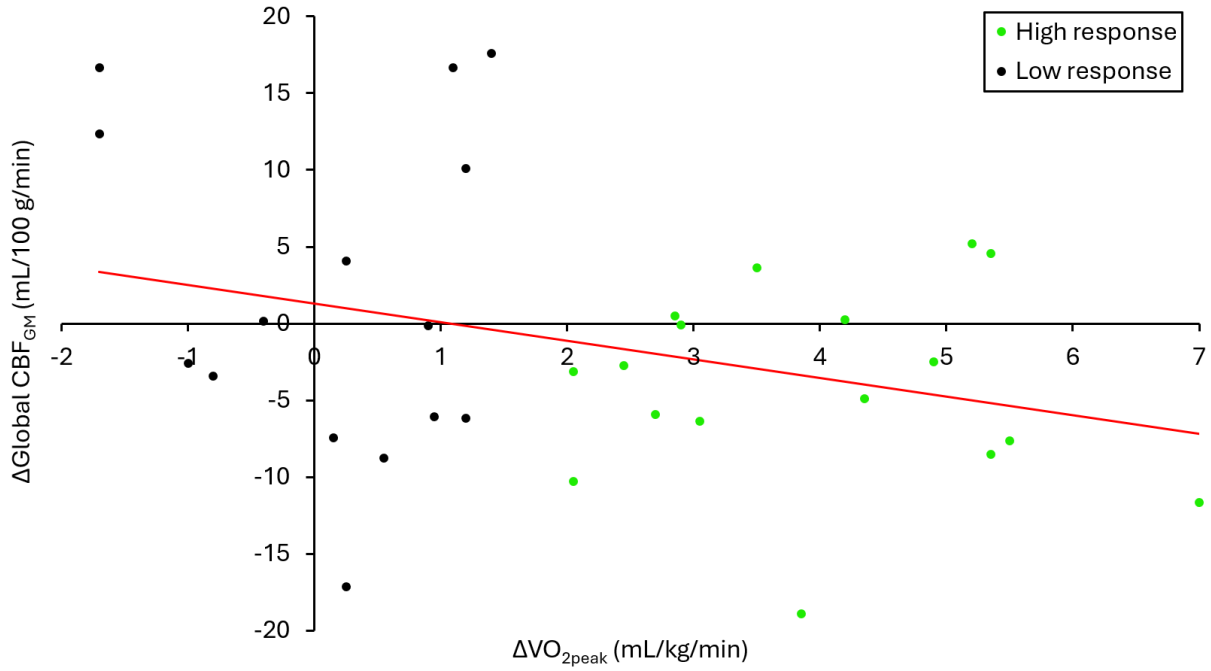

Figure 3.S10: Correlation between pre-to-post intervention changes in global CBF_{GM} and cardiorespiratory fitness in the exercise group (n=32: r_{30}=-0.31 [-0.59, 0.05]), with identification of participants in low and high $\dot{V}O_{2peak}$ response groups (< or > Δ2 mL/kg/min, respectively). CBF_{GM}: grey matter cerebral blood flow, $\dot{V}O_{2peak}$: peak oxygen consumption.

Table 3.S5: Associations between changes in cerebral blood flow and cardiorespiratory fitness in the exercise group from pre-to-post intervention.

	Exercise group (n=32)		
$\Delta\dot{V}O_{2peak}$	β	95%CI	*P*
ΔCBF_{GM}			
Global	-0.33	[-0.71, 0.06]	0.093
Frontal	-0.28	[-0.67, 0.11]	0.152
Parietal	-0.30	[-0.67, 0.07]	0.111
Temporal	-0.24	[-0.64, 0.15]	0.215
Occipital	-0.41	[-0.78, -0.04]	0.028
Motor	-0.29	[-0.67, 0.09]	0.134
Cingulate	-0.23	[-0.61, 0.15]	0.229

Dependant variable: ΔCBF_{GM} of each region, independent variables: age, sex, ΔBMI, and $\Delta\dot{V}O_{2peak}$. The presented regional P-values are unadjusted. CBF_{GM}: grey matter cerebral blood flow, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption, β: standardised beta coefficient, CI: confidence interval.

Table 3.S6: Two-way independent samples T-test comparing adherence metrics between high and low $\dot{V}O_{2peak}$ response groups.

Adherence metric	Mean difference (high-low response)	T-test
Cumulative MET-mins (%)	9.2 [-5.3, 23.7]	t(30)=1.29, *P*=0.21
Session number (%)	7.4 [-3.0, 17.7]	t(30)=1.45, *P*=0.16
Minutes >80%HR_{peak} (%)	30.2 [-9.1, 69.1]	t(30)=1.57, *P*=0.14

Mean difference with 95% confidence intervals. $\dot{V}O_{2peak:}$ peak oxygen consumption, MET-mins: metabolic equivalent minutes, HR_{peak}: peak heart rate.

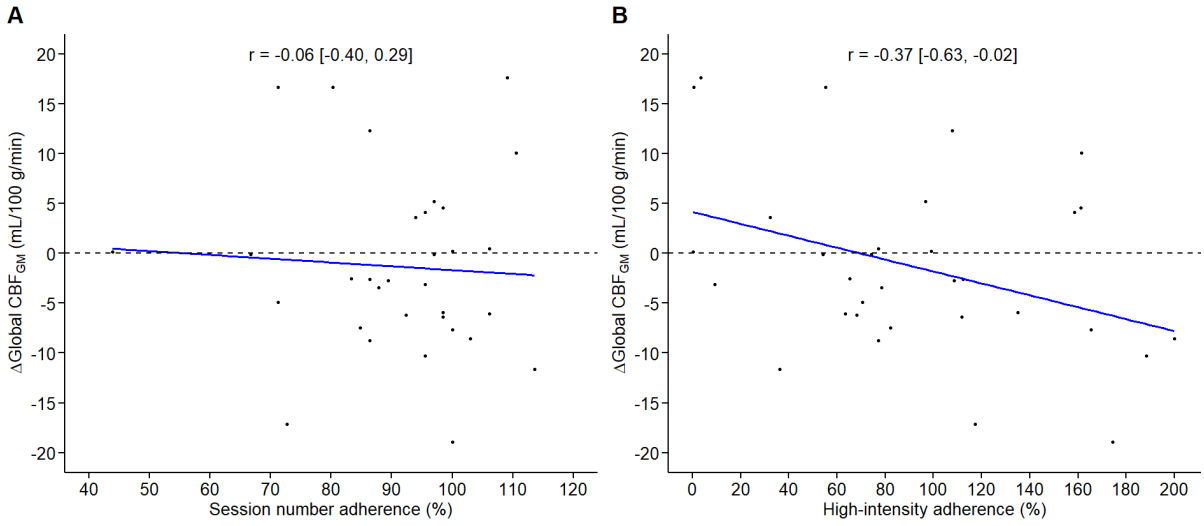

Figure 3.S11: Bivariate Pearson correlations between pre-to-post intervention changes in global grey matter cerebral blood flow (ΔCBF_{GM}) and percentage adherence to exercise volume (A: total exercise session number) or exercise intensity (B: minutes ≥80%HR_{peak}) within the exercise group (n=32).

CHAPTER FOUR

ACCELEROMETER-DERIVED PHYSICAL ACTIVITY BEHAVIOURS AND CEREBRAL HAEMODYNAMICS IN OLDER ADULTS

The work within this chapter is intended to be submitted for publication. The present manuscript, followed by the accompanying Supplemental Material, is currently receiving comments from co-authors before being submitted for publication.

The candidate was the sole recruiter of participants included within all data chapters, was heavily involved with the health screening process to determine participant eligibility, and was primarily responsible for all participant communications and administration. The candidate completed all of the data collection relating to cardiorespiratory fitness testing and physical function testing, and was also heavily involved with the data collection relating to the cognitive function tests and the MRI scan. The candidate organised the distribution of accelerometers to participants over three timepoints throughout the intervention period and analysed these data. Under guidance from KJM, the candidate completed the MRI arterial spin labelling data analysis. The candidate completed the statistical analyses and data visualisation presented in all data chapters and was responsible for leading the writing of the presented manuscripts. KS, SJEL, HS, and SB set-up the larger project. All co-authors read and suggested edits to the manuscript. FR, KEJ, and AG also assisted data collection.

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4.1 Abstract

Sedentary behaviour and physical activity levels worsen with age and may contribute to age-related cognitive decline. Structural and functional cerebral changes are linked with these cognitive effects, with cerebral haemodynamics hypothesised to be an underlying mechanism. However, associations between physical activity behaviours and cerebral haemodynamics are poorly understood. This study assessed cross-sectional and longitudinal (over 6-months) associations between cerebral haemodynamics and accelerometer-derived physical activity behaviours (n=62 and n=39, for cross-sectional and longitudinal cohorts respectively) or overall composite physical function scores (n=61 and n=50, respectively) in healthy older adults (60–81 years). Accelerometer analyses used partial least squares regressions, handling the inherent multicollinearity between variables. Multiple-delay pseudo-continuous arterial spin labelling data were used to estimate resting global and regional grey matter cerebral blood flow (CBF_{GM}) and arterial transit time (ATT_{GM}). Results showed that neither cross-sectional or longitudinal time-point analyses identified any significant associations between CBF_{GM} or ATT_{GM} and sedentary behaviour, light physical activity, moderate-to-vigorous physical activity, or physical function composite scores. Thus, findings indicate that resting cerebral haemodynamics were not associated with physical activity behaviours or physical function in healthy older adults.

4.2 Introduction

Sedentary behaviour and physical activity levels are modifiable lifestyle factors that worsen substantially in older adults, particularly those over 60 years (Doherty et al., 2017; Hallal et al., 2012; Wennman et al., 2019), potentially hindering a healthy ageing process. These unfavourable age-related changes have been linked with adverse structural and functional cerebral changes (Domingos et al., 2021a; Maasakkers et al., 2022), and increased risk of cognitive decline and dementia in older adults (Buchman et al., 2012; Middleton et al., 2011). Cerebral

haemodynamics, which also worsen with age (Damestani et al., 2023), may underpin the protective effects of favourable physical activity behaviours on brain health because the brain lacks intracellular energy stores and thus blood flow dictates energy availability (Öz et al., 2007; Zimmerman et al., 2021). Indeed, lower cerebral blood flow (CBF) is shown to detrimentally affect cognitive function in later life (van Dinther et al., 2023; Wolters et al., 2017). Despite this, the relation between physical activity behaviours and cerebral haemodynamics is poorly understood.

Existing older adult research has focussed on associations between CBF and cardiorespiratory fitness, rather than physical activity behaviours. Findings from cardiorespiratory fitness-based research for both cross-sectional (Feron et al., 2024b; Intzandt et al., 2020; Tarumi et al., 2013) and intervention (Kleinloog et al., 2023; Smith et al., 2021) studies have been inconsistent, possibly attributable to the large genetic component of cardiorespiratory fitness, causing substantial variation in fitness responses to habitual physical activity and/or exercise training (Bouchard et al., 1999). Regarding physical activity behaviours, the limited available objective evidence (i.e., accelerometer-derived) tends to report positive associations with CBF in small regions. For example, light (LPA) and moderate-to-vigorous (MVPA) physical activity were associated with greater CBF in frontal gyri (Zlatar et al., 2019). Although, other research reports only LPA, and not MVPA, was associated with greater CBF in temporal and frontal gyri (Bangen et al., 2023). A larger study found moderate positive associations between regional CBF and LPA (insula and pallidum) or MVPA (accumbens area), but only weak evidence that baseline physical activity levels predicted regional CBF changes over time (Sanders et al., 2023). Even less research has investigated sedentary behaviour. Zlatar et al. (2019) found that sedentary behaviour was associated with lower CBF in clusters within the frontal lobe, while others report that global CBF was not different between high and low sedentary groups, regardless of whether sedentary behaviour was determined using questionnaires (Launer et al., 2015) or accelerometers (Maasakkers et al., 2021).

Nevertheless, conclusions from these accelerometer-based studies are somewhat limited because the strong inverse relationship between sedentary behaviour and physical activity (Mansoubi et al., 2014), particularly LPA, was not fully considered (Collins et al., 2023). For example, Sanders et al. (2023) only measured physical activity levels, while others did not statistically control for this relationship (Bangen et al., 2023; Maasakkers et al., 2021; Zlatar et al., 2019), meaning it is impossible to truly isolate effects. Furthermore, all previous studies investigating the association between CBF and physical activity or sedentary behaviour have used single-delay arterial spin labelling (ASL) to estimate CBF, limiting estimation accuracy because regional and individual differences in arterial transit time (ATT) were not accounted for (Dai et al., 2017; Woods et al., 2024), but this can be achieved with multiple-delay ASL. ATT is the time taken for blood to travel from large arteries in the neck to the cerebral tissue, which lengthens with age and disease (Damestani et al., 2023; M. Sun et al., 2022; Yu et al., 2022) and may experience greater age-related changes than CBF (Damestani et al., 2023; Feron et al., 2024b). Moreover, the impact of physical activity behaviours on ATT, per se, remains unexplored.

Finally, physical activity behaviours have bidirectional relationships with physical function (Yerrakalva et al., 2022), a similar but distinct concept. Physical function is defined as the ability to perform both basic and instrumental activities of daily living (D. X. M. Wang et al., 2020), commonly assessed using grip strength and chair stands tests. Similar to physical activity behaviours, superior physical function has favourable effects on cognitive function, dementia risk, grey matter volume, and white matter hyperintensities (Demnitz et al., 2023; Duchowny et al., 2022; Jiang et al., 2022; Makizako et al., 2022). However, only a limited number of studies have investigated relationships between cerebral haemodynamics and physical function, and those that have report mixed findings. For example, grip strength was not associated with global CBF or ATT in older adults (Chapter 2) (Feron et al., 2024b), but a higher composite physical activity score

(including grip strength) was associated with greater posterior cingulate CBF (Boraxbekk et al., 2016).

Therefore, the primary aim of this study was to assess both cross-sectional and longitudinal (6-month follow-up) associations between accelerometer-derived sedentary behaviour, LPA, or MVPA and resting grey matter CBF or ATT in healthy older adults. Associations between cerebral haemodynamics and physical function were also investigated.

4.3 Material and methods

4.3.1 Study design

The data for this publication were collected as part of a larger study, The FAB Project (preregistration: https://osf.io/6fqg7). The data in the present study are from a sub-group of a larger shared participant cohort, had unique outcome measures to other publications related to the project (Feron et al., 2024b, p. 202, 2024a; Fosstveit et al., 2024a; Rahman et al., 2023), and addressed *a priori* questions (as per preregistration). The study was approved by the STEM Ethical Review Committee at the University of Birmingham (ERN_20-1107). Before taking part in this study, participants were provided with a participant information sheet and provided their informed consent (see Appendices for participant information sheet and consent form).

Participants were screened for eligibility before completing physical function tests, an ASL MRI scan, and a seven-day physical activity assessment using a waist-worn accelerometer. As part of the larger project, participants were then randomised to a control or exercise intervention group (lifestyle maintenance vs. six-month, thrice weekly, home-based high-intensity interval training, respectively), described in detail in (Fosstveit et al., 2024a). The physical function tests and MRI scan were repeated after six-months, whereas accelerometer activity monitoring was repeated at the middle and final intervention weeks (Figure 4.1).

For cross-sectional participants, the physical function tests, MRI scan, and the start of accelerometery monitoring occurred within 12±15 days. For longitudinal participants, these measures were completed at follow-up within 17±18 days.

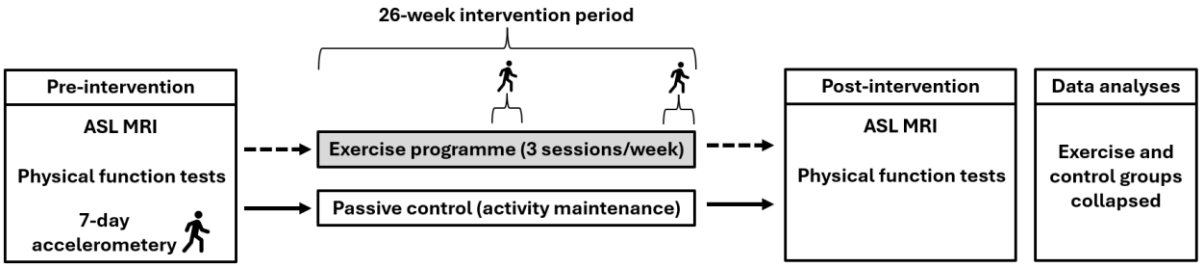

Figure 4.1: Schematic of the study design including pre- and post-intervention outcomes and the timing of the second and third accelerometer assessments (middle and final intervention week, respectively). ASL: arterial spin labelling, MRI: magnetic resonance imaging.

4.3.2 Participants

Ninety-four healthy older adults (aged 60–81 years) were enrolled. Participants were cognitively normal, without historic or current diagnosis of serious health conditions, non-smokers, and self-reported to not meet recommended global activity guidelines (Bull et al., 2020). Section 1 of the Supplemental Material (4.6.1; Supplemental Material found at conclusion of this chapter). contains detailed inclusion criteria. Of these, 78 participants had useable baseline ASL data but accelerometery data was missing for 16, leaving 62 for the cross-sectional analyses presented in this study. Of the 62 participants at baseline, eight dropped out of the intervention (control; n=1, exercise; n=7), a further two had unusable post-intervention ASL data, and a further 13 had incomplete accelerometery data, leaving 39 for longitudinal analyses. Of these 39, 21 and 18 were allocated to the control and exercise groups, respectively. Participant characteristics are shown in Table 4.1. Note that both the cross-sectional and longitudinal cohorts are subsets of those reported in the previous two chapters, which examined the relationship between CBF or ATT and cardiorespiratory fitness (see Chapters 2 and 3).

Table 4.1: Baseline characteristics of participants included in cross-sectional and longitudinal analyses.

	Total	Male	Female
Cross-sectional cohort			
n	62	29	33
Age (years)	66±5	66±6	66±5
Systolic blood pressure (mmHg)	139±13	141±11	137±14
Diastolic blood pressure (mmHg)	82±8	83±8	81±8
BMI (kg/m^2)	26.9±3.6	27.9±3.2	26.1±3.8
Sedentary behaviour (mins/day)	664±58	653±66	673±49
LPA (mins/day)	184±42	197±45	173±37
MVPA (mins/day)	45±21	50±24	40±18
Meeting MVPA guidelines (%)	85	86	85
$\dot{V}O_{2peak}$ (mL/kg/min)	27.7±4.5	30.4±4.1	25.5±3.4
Longitudinal cohort			
n	39	19	20
Age (years)	66±5	66±5	66±4
Systolic blood pressure (mmHg)	139±13	141±11	139±14
Diastolic blood pressure (mmHg)	83±8	82±8	84±8
BMI (kg/m^2)	26.7±3.4	28.2±3.4	25.3±2.9
Sedentary behaviour (mins/day)	672±63	668±71	676±56
LPA (mins/day)	182±41	189±44	175±38
MVPA (mins/day)	44±21	48±25	40±16
Meeting MVPA guidelines (%)	85	84	85
$\dot{V}O_{2peak}$ (mL/kg/min)	27.9±3.6	29.7±3.7	26.1±2.5

Values represent means ± standard deviation. SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption. LPA: light physical activity, MVPA: moderate-to-vigorous physical activity.

4.3.3 Health screening: electrocardiogram (ECG), blood pressure, and cognitive impairment

Participants completed a resting 12-lead ECG (Cardiosoft, Vyaire, USA), three resting blood measurements (705IT, Omron, Japan), and the Montreal Cognitive Assessment (MoCA). Participants were excluded for severe ECG abnormalities, MoCA scores <23 (Carson et al., 2018) and systolic/diastolic blood pressure of >160/>90 mmHg. Excluded participants were referred to their GP.

4.3.4 Outcome Measures

Accelerometer-derived physical activity and sedentary behaviour

A waist-worn accelerometer (GT3X+, ActiGraph Inc., USA) measured physical activity and sedentary behaviour. The device was worn for seven consecutive days during all waking hours (excluding showering and water-based activities) after pre-intervention tests and then during the middle (+3-months), and final (+6-months) intervention week. Accelerometers were initialised and downloaded using ActiLife software (ActiGraph Inc., USA). Data were collected and analysed in 10 s epochs. Wear-time criteria was ≥13 hours (Herrmann et al., 2014) on at least four out of seven days. Non-wear time was characterised as intervals of ≥60 consecutive minutes with no activity counts, with allowance for 1 min with counts >0. Activity levels were categorised based on uniaxial counts per minute (cpm) and defined as mean minutes per day of sedentary behaviour (0-99 cpm), light physical activity (LPA; 100-2019 cpm), and moderate-to-vigorous physical activity (MVPA; >2019 cpm) (Troiano et al., 2008).

Physical function composite score (PF_{comp})

Participants completed five physical function tests: grip strength, chair stands, sit and reach, timed up and go, and balance. Scores for each test were standardised (Z-score) and then summed to give the composite score (PF_{comp}). 8-ft up and go score was reversed so that a higher score represented superior performance. Cross-sectional PF_{comp} data was missing for one participant (i.e., n=61), whilst longitudinal data were available for n=50 of these participants.

Grip strength: A hand grip dynamometer (5001, Takei, Japan) was adjusted for grip size of the dominant hand. Participants stood upright, arms by their sides, and squeezed the dynamometer as hard as possible, maintaining elbow extension and limiting shoulder movement. The highest score from three attempts was taken.

Chair stands: Participants sat upright in a chair with both feet flat on the ground, shoulder width apart, and hands held against their chest. Participants had one attempt to fully stand up (knees fully extended) and fully sit down as many times as possible in 30 s.

Sit and reach: Participants sat on the edge of a chair and straightened one of their legs, placing the heel on the floor with their toes pointing up to the ceiling. Participants interlaced their fingers and reached towards their toes, getting as close to or as far past their toes as they could. A ruler was used to measure the distance from/past the toes. The best score was taken from two attempts.

Timed up and go: Participants sat upright on a chair with both feet on the floor and hands on their knees. Participants were asked to stand up, walk out to and around a cone placed 8-ft away, and return to their seat as fast as possible. Running was not allowed. The fastest time from two attempts was recorded.

Balance: Participants stood on one leg of their choice for as long as possible (maximum 60 s). The heel of the non-standing leg was brought to and kept against the medial knee of the standing leg. Arm position was unrestricted. Participants could briefly practice before having two attempts, the best time was recorded.

MRI data acquisition and analysis

An MRI scan session included structural, functional, and arterial spin labelling (ASL) scans, using a 3-T system (MAGNETOM Prisma, Siemens, Germany) with 32-channel receiver head coil. Here, the focus is the ASL data and related scans, analysis of other data acquired can be found elsewhere (Rahman et al., 2023). CBF and ATT data were collected using pseudo-continuous ASL sequence with 3D GRASE readout (17:22 mins) (Kilroy et al., 2014; D. J. J. Wang et al., 2013), see also Acknowledgements (4.5.6).

ASL imaging parameters were: repetition time (TR)=4100 ms, echo time (TE)=30.56 ms, in-plane resolution=3.5 mm^2, slice thickness=3.5 mm, transversal slices=32, field of view (FOV)=224x224 mm, background suppression=yes, and post-labelling delays (PLD)=200, 975, 1425, 1850, 2025, 2150, 2250, and 2300 ms. Four and twelve volumes of data were acquired for PLD of 200–2250 ms and 2300 ms, respectively. PLD times and number of volumes acquired were optimised according to recommendations (Woods et al., 2019). Slices were positioned axially from the motor cortex and angled anterior-posterior in line with the participant's anterior-posterior commissure (ACPC). A calibration M0 scan was acquired using these same parameters with the PLD set to 2000 ms with PCASL and background suppression disabled. The T1-weighted structural scan (4:54 mins) was acquired to facilitate data analysis including, normalisation to a standard template brain and differentiation of grey and white matter. Structural T1-weighted (MPRAGE) imaging parameters were: TI=880 ms, TE=2.03 ms, TR=2000 ms, voxel size=1 mm^3, sagittal slices=208, FOV=256×256×208 mm, and flip angle=8°.

ASL data were processed using the Oxford ASL toolbox (https://oxasl.readthedocs.io/en/latest/), which uses the FSL FABBER ASL package and Bayesian Inference to invert the kinetic model for ASL MRI (BASIL) to compute CBF and ATT maps (Chappell et al., 2009; Groves et al., 2009; Woolrich et al., 2006). Parameters input to the kinetic models to estimate CBF and ATT were: bolus duration=1.5088 s, tissue T1=1.3 s, arterial blood T1=1.65 s, labelling efficiency=0.85. All other input parameters were kept with default settings appropriate to PCASL acquisition. Partial volume error correction and adaptive spatial smoothing of the perfusion maps was performed using default settings in oxford_asl (Chappell et al., 2011; Groves et al., 2009).

Global and regional analysis was performed, assessed in native (individual participant) and MNI space, respectively. All CBF and ATT values refer to grey matter only. Regions of interest were the cingulate gyrus and frontal, parietal, temporal, occipital, and motor cortices (Figure 4.S1). Only

participants with useable ASL data in both native and MNI space were included in analyses. Native space difference maps at each PLD for each participant were visually inspected to ensure data quality. Particular attention was paid to ensure there were no: 1) excessive motion resulting in spurious edge effects in difference maps; 2) brain territories which did not appear to be perfused, due to suboptimal label positioning or unaccounted for vasculature; and 3) focal areas of high intensity in final CBF maps which would have suggested that the PLDs were insufficient. ASL data difference maps from two exemplar excluded participants are shown in Figure 4.S2. Section 4.6.2 of the Supplemental Material contains additional information regarding data quality assessment and grey matter mask configuration.

4.3.5 Statistical analyses

Age, sex, BMI, and accelerometer wear-time were included as covariates in analyses described below because of their associations with cerebral haemodynamics (Damestani et al., 2023; Feron et al., 2024b) or accelerometer-derived measures (Guo et al., 2019; Herrmann et al., 2014). For longitudinal analyses, changes in accelerometer-derived measures were calculated as the mean of the middle and final intervention weeks minus the pre-intervention week. During the time between baseline and follow-up measures, participants were allocated to a control (n=21) or exercise (n=18) group as part of a larger study (Fosstveit et al., 2024a). A mixed-design ANOVA, controlled for age and sex, found no significant main effect of time or group, or group × time interaction for absolute or relative (i.e., mins/day or percentage of wear-time, respectively) sedentary behaviour, LPA, or MVPA throughout the 26-week follow-up period. Therefore, for longitudinal analyses of change-change associations, participant groups were collapsed. Individual participant changes in physical activity behaviours by group allocation are shown in Figure 4.S3 and 4.S4.

Pearson correlations, considered the second best method for assessing associations with accelerometer-derived measures (Aadland et al., 2019b), were used to assess associations between relative sedentary behaviour, LPA, or MVPA (i.e., percentage of wear-time) and CBF_{GM} or ATT_{GM} (globally and regionally).

Partial least squares regression (PLSR) analysis is recommended to assess associations between accelerometery-derived measures and outcome variables because this analysis can handle the inherent multicollinearity that exists between accelerometery measures (Aadland et al., 2019a; Migueles et al., 2022). PLSR models were conducted using the *mvpa* package (Baumeister et al., 2024) in RStudio (RStudio Team, 2021; https://github.com/liningtonlab/mvpa). Models included CBF_{GM} or ATT_{GM} (global or regional) as the outcome variable and accelerometery measures as explanatory variables (sedentary behaviour, LPA, and MVPA). Prior to PLSR, outcome and explanatory variables were automatically adjusted for relevant covariates (age, sex, and BMI) using the R package. Models used relative accelerometer data to control for accelerometer wear-time (i.e., percentage of wear-time). Prior to PLSR, all variables were centred and standardised to unit variance. Monte Carlo resampling (Kvalheim et al., 2018) with 1000 repetitions was used to cross-validate PLSR models and obtain a single predictive PLS component (50% of participants were used as an external validation set when estimating the models). PLSR models produce selectivity fractions (SF), a measure of explained predictive variance for the explanatory variables varying between −1 and +1 with 95% confidence intervals (CI) (Baumeister et al., 2024).

Multiple linear regressions assessed associations between CBF_{GM} or ATT_{GM} and daily steps or the physical function composite score (PF_{comp}) (SPSS Statistics v.29, IBM, USA). Daily steps were adjusted for wear-time for each participant by first multiplying individual mean steps/min of wear-time by the difference between individual total wear time and the longest recorded wear time from all participants, this value was then summed with the original individual daily steps value. For

cross-sectional analyses: dependant variable = CBF_{GM} or ATT_{GM}, independent variables = age, sex, BMI, and either estimated daily steps (n=62) or PF_{comp} (PF_{comp} data was available for n=61). For longitudinal analyses: dependant variable = ΔCBF_{GM} or ΔATT_{GM}, independent variables = age, sex, ΔBMI, and either Δestimated daily steps (n=39) or ΔPF_{comp} (n=50).

4.4 Results

4.4.1 Cross-sectional

Participants wore the accelerometer for 5.7±1.1 days for 14.9±0.7 hrs/day. Characteristics of the sample's sedentary behaviour and physical activity levels are shown in Table 4.1.

Partial correlations, adjusted for age, sex, and BMI, identified no significant associations between CBF_{GM} or ATT_{GM} and relative sedentary behaviour, LPA, or MVPA.

Partial least squares regression analyses confirmed correlation analyses, finding no associations between CBF_{GM} or ATT_{GM} and sedentary behaviour, LPA, or MVPA.

Multiple linear regression analyses found no significant associations between global or regional CBF_{GM} or ATT_{GM} and PF_{comp} or daily steps, a simple proxy for overall physical activity level.

4.4.2 Longitudinal

Participants wore the accelerometer for 5.9±1.1 days for 15.1±0.8 hrs/day during the middle intervention week (+13.3±2.2 weeks) and for 6.0±1.1 days for 15.0±0.8 hrs/day during the final intervention week (+26.4±3.0 weeks). Individual changes in relative physical activity behaviours between each timepoint are shown in Figure 4.2.

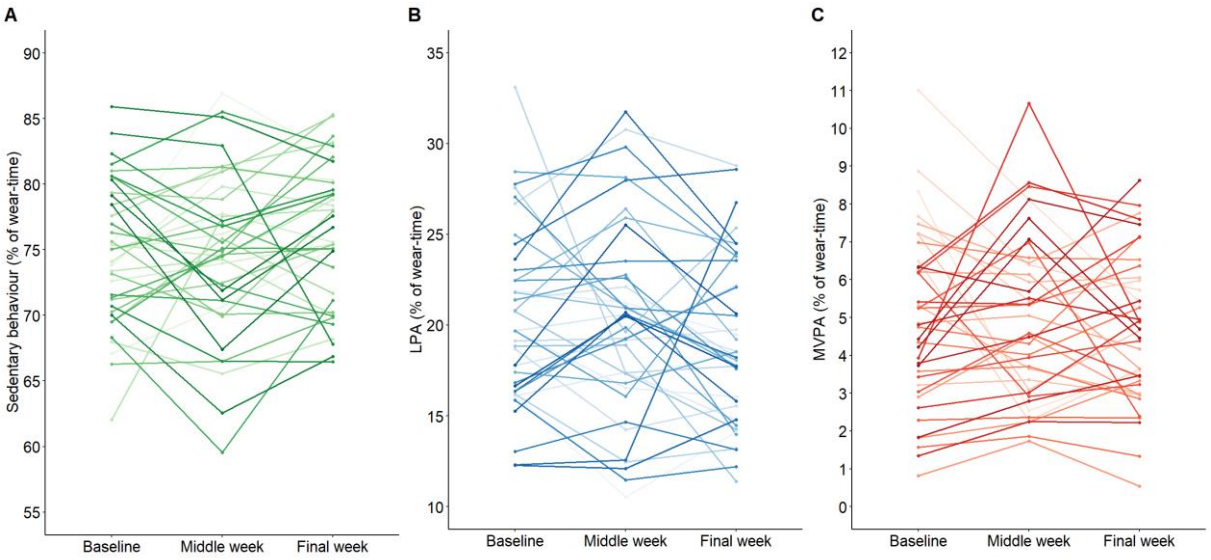

Figure 4.2: Individual participant changes (n=39) in sedentary behaviour (A), light intensity physical activity (B: LPA), and moderate-to-vigorous intensity physical activity (C: MVPA) from the pre-intervention, middle (+3 months), and final (+6 months) follow-up weeks, expressed as percentage of total accelerometer wear-time.

Partial correlations, adjusted for age, sex, and change in BMI, identified no significant associations between changes in CBF_{GM} or ATT_{GM} and changes in relative sedentary behaviour, LPA, or MVPA.

Partial least squares regression analyses confirmed correlation analyses, finding no associations between changes in CBF_{GM} or ATT_{GM} and changes in relative sedentary behaviour, LPA, or MVPA.

Multiple linear regression analyses found no significant associations between changes in global or regional CBF_{GM} or ATT_{GM} and changes in PF_{comp} or daily steps.

4.5 Discussion

The present study investigated associations between accelerometer-derived physical activity behaviours or physical function and resting cerebral haemodynamics in healthy older adults, both cross-sectionally and longitudinally (over six-months). Unlike previous research investigating cerebral haemodynamics, accelerometer analyses used partial least squares regression models

to enable inclusion of all accelerometer variables, despite multicollinearity, and thus accounted for the complex interplay between sedentary behaviour and physical activity levels (Aadland et al., 2019a; Collins et al., 2023). Cross-sectional analyses found no significant associations between resting CBF_{GM} or ATT_{GM} and sedentary behaviour, LPA, MVPA, steps/day or composite physical function scores. Similarly, over the six-month period, changes in physical activity behaviours or physical function scores were not associated with changes in CBF_{GM} or ATT_{GM}.

4.5.1 Cerebral blood flow and physical activity behaviours

The present study reports no cross-sectional or longitudinal associations between global or regional CBF_{GM} and sedentary behaviour, LPA, or MVPA in healthy older adults. Sedentary behaviour has been associated with lower cerebral blood velocity, cerebrovascular conductance, and total brain volume (Hartman et al., 2021; Launer et al., 2015). Despite this, the present findings report no association with resting CBF_{GM}, consistent with previous research comparing global CBF_{GM} between high and low sedentary groups (Launer et al., 2015; Maasakkers et al., 2021). In contrast, other accelerometer research reports that lower sedentary behaviour is associated with greater CBF_{GM} in some small clusters within the middle frontal gyrus (Zlatar et al., 2019), but only MVPA was controlled for in that analysis. Although the present sample was highly sedentary at baseline (87% for >10 hrs/day), they were also (unintentionally) highly active with 85% meeting MVPA guidelines, which is considerably greater than the ~60% reported in English adults aged 55–74 years (NHS England, 2023). Previous research shows how high physical activity levels can offset the detrimental impacts of sedentary behaviour on mortality and cardiovascular disease risk (Ekelund et al., 2016; Lee et al., 2020). Therefore, any effect of sedentary behaviour on CBF_{GM} may have been offset by the high physical activity levels of this cohort.

Regarding MVPA, previous studies report that lobular and (smaller) regional CBF_{GM} generally lacks cross-sectional associations with MVPA (Bangen et al., 2023; Sanders et al., 2023; Zlatar et al.,

2019), apart from within the accumbens area (Sanders et al., 2023) and a frontal gyrus cluster (Zlatar et al., 2019). Although not supported by the present data, previous evidence indicates that only LPA, not MVPA, is associated with greater CBF_{GM} in the inferior temporal gyrus and middle frontal gyrus (Bangen et al., 2023). Similar associations between CBF_{GM} and LPA have been reported in cingulate, insula, and pallidum regions, although effects were not present when looking more broadly across lobes (Sanders et al., 2023). For comparison, the present data were also analysed identically to the statistical methods used by Bangen et al. (2023), which found no association with LPA or MVPA (Table 4.S1). Overall physical activity (i.e., steps/day) also lacked association with CBF_{GM}, differing with previous cross-sectional research showing that total accelerometer activity counts were associated with greater CBF_{GM} in four frontal gyri clusters (Zlatar et al., 2019).

Longitudinal investigations in this area lacking. One study has assessed whether baseline LPA and MVPA predict changes in CBF over time (~500 days), reporting only weak evidence of a positive association between higher baseline LPA and changes in frontal, temporal, and insula CBF (Sanders et al., 2023). The present data found that, over six-months, changes in accelerometer-measures were not associated with changes in cerebral haemodynamics. The shorter follow-up time in the present study (~182 vs. ~500 days) potentially explains why the impact of LPA was not replicated. Even so, ~500 days follow-up is still relatively short and may not capture the full impact of physical activity behaviours on CBF. Longer interventions (e.g., 5–10 years) specifically targeting sedentary behaviour and/or physical activity levels are required to make definitive conclusions.

It should be considered that physical activity behaviours may have larger effects on cerebral haemodynamics in older adults to that of the present study (i.e., >75 years); for whom, generally, sedentary time increases and moderate-to-vigorous activity decreases (Husu et al., 2016). It has been shown that overall physical activity levels are relatively well preserved in adults aged up to

65 years, with more considerable step-wise reductions occurring in adults aged 65–74 and 75+ years (Doherty et al., 2017; Wennman et al., 2019). In the present sample, 53% were ≤65 years and 81% were <70 years, and thus their physical activity behaviours may not have worsened enough to have a meaningful effect on cerebral haemodynamics. Furthermore, previous studies investigating sedentary behaviour and LPA did not account for the interplay between these two variables (Collins et al., 2023), and compared with previous cohorts, the present sample were generally more sedentary but also did more MVPA, potentially offsetting effects (Ekelund et al., 2016; Lee et al., 2020). Moreover, only the present study controlled for BMI, which has strong associations with both CBF_{GM} and physical activity levels (Feron et al., 2024b; Guo et al., 2019), and used multiple-delay ASL to improve the estimation of CBF_{GM} (Dai et al., 2017) in comparison to previous single-delay research (Bangen et al., 2023; Launer et al., 2015; Maasakkers et al., 2021; Sanders et al., 2023; Zlatar et al., 2019).

The present study investigated how changes in objective physical activity measures, rather than cardiorespiratory fitness, affect resting cerebral haemodynamics. As part of a larger study (Fosstveit et al., 2024a), 18 participants in the present longitudinal analyses completed a thrice weekly, high-intensity exercise programme during the six-month follow-up period, increasing cardiorespiratory fitness levels (Δ1.8 [0.7, 2.8] mL/kg/min). Despite this, within this sample, there were no between-group differences in MVPA at any timepoint and no correlation between changes in fitness and MVPA within the exercise group (r_{16}=0.10 [-0.34, 0.52]). A potential explanation for this relates to the considerable genetic component of cardiorespiratory fitness (Bouchard et al., 1999), whereby different individuals completing equivalent MVPA will have a different fitness response. Analysis of a larger participant cohort this subset was part of unexpectedly found that the exercise intervention induced global CBF_{GM} reductions, but this was limited only to those with a high cardiorespiratory fitness response (i.e., >2 mL/kg/min) (Chapter 3) (Feron et al., 2024a), indicating a greater importance of fitness gains rather than physical activity changes.

Taken together, the present and previous data indicate limited cross-sectional or longitudinal associations between physical activity behaviours and global or lobular CBF_{GM} at rest in healthy older adults. There is evidence to suggest increases in cardiorespiratory fitness levels are key to cerebral haemodynamics changes in older adults (Chapter 3) (Feron et al., 2024a), rather than simply changes in physical activity behaviours. However, longer-term investigations (i.e., years) which follow changes in physical activity behaviours, or interventions designed to modify a specific physical activity behaviour, rather than a more general exercise intervention, may reveal more meaningful associations. Furthermore, the impact of physical activity behaviours on cerebral haemodynamics may be more prominent in older, older adults.

4.5.2 Arterial transit time and physical activity behaviours

The present study is the first to investigate associations between ATT_{GM} and sedentary behaviour or physical activity levels, reporting no significant cross-sectional or longitudinal associations in healthy older adults. Previous longitudinal research has investigated changes in cerebral blood velocity, which is inversely associated with ATT_{GM} (Burley et al., 2021), in response to a 16-week reduced sitting intervention in older adults, reporting increases in resting cerebral blood velocity (Hartman et al., 2021). This finding contrasts with the present data which found no association between changes in sedentary behaviour or LPA (which increased following the reduced-sitting intervention) and ATT_{GM} over the six-month follow-up period. However, the reduced-sitting intervention reduced sedentary behaviour by ~1-hr/day and increased daily steps by ~2700/day, whereas only ten participants in the present longitudinal sample experienced changes (±) of this magnitude in either metric.

Taken together, the present data report no obvious associations between ATT_{GM} and physical activity behaviours, although more targeted interventions that induce larger changes in physical activity behaviours could have more pronounced effects on ATT_{GM}. Given that ATT_{GM} changes were

not evident following a six-month exercise intervention (irrespective of cardiorespiratory gains) (Chapter 3) (Feron et al., 2024a), targeting modifications towards sedentary behaviour and LPA may induce more meaningful effects. Compared with CBF_{GM}, ATT_{GM} may be more sensitive to age-related declines (Damestani et al., 2023; Feron et al., 2024b) and thus may show greater responses to changes in physical activity behaviours over the longer-term.

4.5.3 Cerebral haemodynamics and physical function

A positive bidirectional relationship between physical function and physical activity levels has been reported previously (Ramsey et al., 2021; Yerrakalva et al., 2022); however, in the present data, the only notable cross-sectional correlation was between overall composite physical function and sedentary behaviour (r_{60}=-0.25 [-0.47, 0.00]). Similarly to the accelerometer measures, physical function was not cross-sectionally or longitudinally associated with CBF_{GM} or ATT_{GM}, adding to only a limited pool of research. In line with the present data, within a largely shared participant cohort, specifically hand grip strength lacked association with global CBF_{GM} or ATT_{GM} (Chapter 2) (Feron et al., 2024b). Furthermore, no relationship between cerebral blood velocity (inversely related to ATT) and grip strength was observed in healthy adults (Rosonke et al., 2024). However, a higher composite physical activity score (including grip strength, blood pressure, resting heart rate, and BMI) has been associated with greater posterior cingulate cortex CBF across the lifespan (Boraxbekk et al., 2016). Interventions targeting muscular strength, endurance, and flexibility in older adults are required to identify whether specifically improving physical function affects cerebral haemodynamics.

4.5.4 Future directions and technical considerations

Most research investigating the impact of physical activity behaviours on resting cerebral haemodynamics are cross-sectional (with inconsistent findings) and therefore lacks causal inferences. Investigation of how either natural or intervention-induced changes in physical activity

behaviours affect cerebral haemodynamics over the longer-term may reveal more valuable insights. Interventions that specifically target either sedentary behaviour, LPA, or MVPA, could help isolate potential effects of each activity domain, and effects might be most beneficial in older, older adults (i.e., >75 years) who tend to have considerably worse physical activity behaviours. Furthermore, longitudinal or intervention studies could help identify whether changes in cerebral haemodynamics are important for the commonly reported cognitive benefits of physical activity in older adults (Mellow et al., 2022; Rojer et al., 2021).

Regarding sedentary behaviour, it should also be considered that the cognitive demands of specific activities that are engaged with whilst sedentary may have differential effects on brain health and thus cerebral haemodynamics (e.g., reading, watching television, or socialising). For example, reading whilst being sedentary offsets the normally deleterious effects on cognitive function (Mellow et al., 2022). Similarly, activities outside of the "physical" domain may have more prominent effects on cerebral haemodynamics. For example, in older adults, social activities appear to partially mediate the beneficial effects of physical activity on cognition (Cohn-Schwartz and Khalaila, 2022) and are associated with greater temporal and parietal CBF_{GM} (Sanders et al., 2023).

Furthermore, future research should consider controlling for diet and partial pressure of end-tidal CO_2 ($PetCO_2$) during CBF/ATT measurement (not controlled in the present study). For example, caffeine and polyphenol intake can acutely alter global CBF_{GM} by 20–60% (Francis et al., 2006; Lamport et al., 2015; Vidyasagar et al., 2013). Moreover, $PetCO_2$ is a proxy for arterial partial pressure of CO_2 ($PaCO_2$), the most powerful regulator of CBF (Willie et al., 2014), which could be manipulated by anxiety-induced ventilatory changes during an MRI scan. Inclusion criteria for the present study included that participants self-reported not meeting physical activity guidelines of 150 min/week; however, the accelerometery data indicates 84% of participants did meet these

activity guidelines. Therefore, present findings may lack generalisability to the larger population, although participants possibly modified their normal behaviours during assessment periods (Clemes et al., 2008).

4.5.5 Conclusion

To summarise, the present study found no clear cross-sectional associations between cerebral haemodynamics (CBF_{GM} or ATT_{GM}) and sedentary behaviour, LPA, MVPA, or overall physical function in healthy older adults. Similarly, over a six-month follow-up period, longitudinal changes in physical activity behaviours or physical function did not predict changes in cerebral haemodynamics. Longer-term longitudinal studies or interventions specifically designed to modify a specific physical activity behaviour (e.g., sedentary behaviour) are required to make more definitive conclusions. Future studies should target older, older adults who exhibit considerably worse profiles of physical activity behaviours.

4.5.6 Acknowledgements

This work was funded by the Norwegian Research Council (FRIPRO 300030). We thank Bethany Skinner, Consuelo Vidal Gran, Nicolas Hayston, Rupali Limachya, Amelie Grandjean, Aoife Marley, Shi Miao, and Samuel Thomas for data collection support, and Roksana Markiewicz for cognitive data analysis. We thank Danny Wang and the University of Southern California's Steven Neuroimaging and Informatics Institute for the provision of the PCASL sequence used in this work, which was provided through a C2P agreement with The Regents of the University of California.

4.6 Supplemental Material

4.6.1 Participant enrolment

Inclusion criteria

Inclusion criteria were: 1) aged 60-85 years. 2) do <150 mins of moderate physical activity per week. 3) monolingual. 4) right-handed. 5) no current/historic diagnosis of cardiovascular, metabolic, respiratory, neurological, kidney, liver, or cancerous disease. 6) resting electrocardiogram (ECG) and blood pressure screened by clinician (i.e., no severe ECG abnormalities (e.g., ST depression, long QT, heart block, wide QRS) and systolic/diastolic blood pressure of <160/<90 mmHg, respectively). 7) Montreal Cognitive Assessment (MoCA) score ≥23. 8) not taking neurotransmitter-altering medication. 9) vaccinated against COVID-19. 9) deemed safe to enter MRI scanner by a qualified MRI-operator. 10) no language impairments. 11) no post-traumatic stress disorder (PTSD). 12) non-smoker of at least 5 years.

4.6.2 MRI acquisition and analysis

As mentioned in the methods above, for each participant, ASL data at each PLD (difference of tag and control averaged over repeats) and grey matter masks in native space were visually inspected. Participants were excluded due to excessive motion, poor labelling, or unbalanced perfusion maps (suggesting unusual vasculature or poor labelling). Figure 4.S2 shows two examples of ASL data which were excluded after this visual data quality inspection step. Native space grey matter masks were further thresholded at 0.5 probability to ensure only voxels containing primarily grey matter were included in calculations of CBF_{GM} and ATT_{GM}. Areas within masks containing incorrect assignment to grey matter, primarily around the eyes and nasal cavity, were manually removed.

Structural MRI data were aligned to the MNI brain using fsl_anat (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/fsl_anat). Registrations to MNI space were visually

inspected. For participants with poor registration, the nonlinear registration was disabled and processing re-run. One participant was excluded because registration was deemed poor despite best efforts (due to brain atrophy with age). Regional grey matter masks were made in MNI space and defined using the MNI structural atlas (temporal lobe only) or from the conjunction of the relevant regions from the Harvard atlas (in FSL). For 21scans (12 participants), MNI registration specifically of the inferior frontal lobe was poor (due to significant brain atrophy) and thus frontal lobe CBF and ATT maps were truncated to exclude this region from analysis using fslroi. The chosen regions of interest (Figure 4.S1) have been used previously (Burley et al., 2021), and were broad because there were no specific *a priori* hypotheses of regions that would be affected by exercise training or associated with cognitive function.

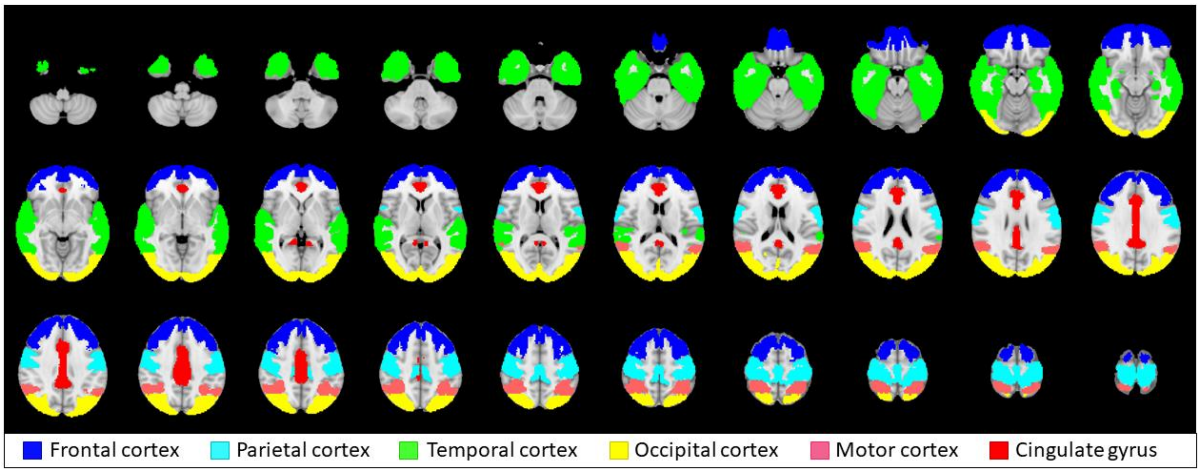

Figure 4.S1: Grey matter masks used for region of interest analysis in MNI space.

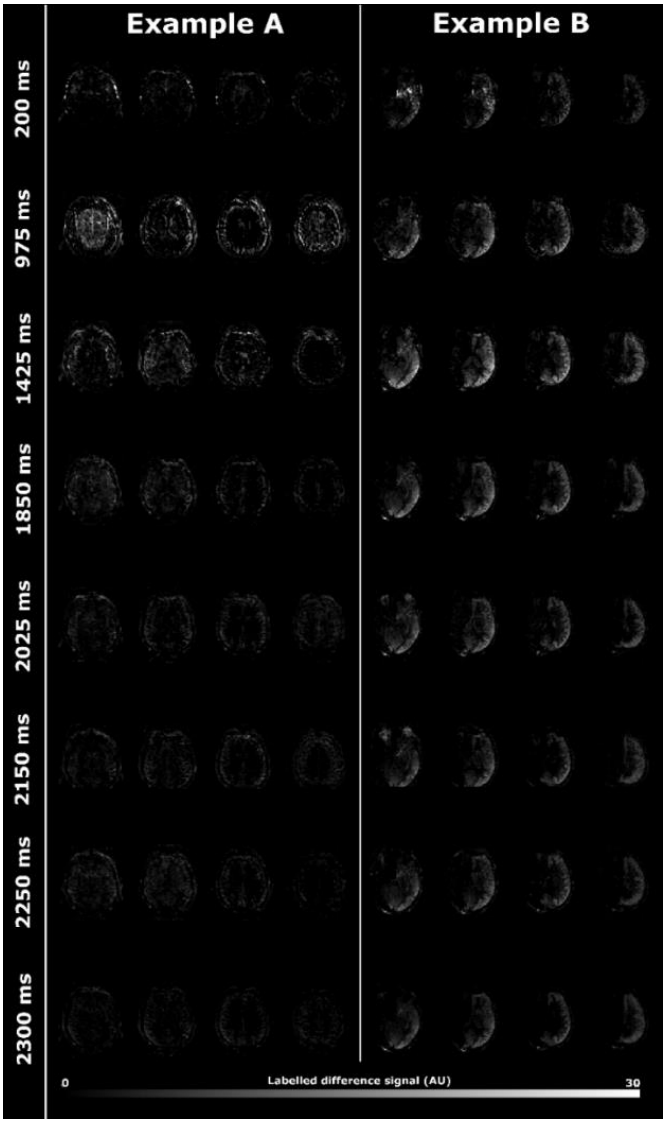

Figure 4.S2: Examples of arterial spin labelling difference maps at each post-labelling delay from two excluded participants. AU: arbitrary units.

Table 4.S1: Alternative analyses of associations between CBF_{GM} and LPA or MVPA, as executed by Bangen et al. (2023).

	Global		Frontal		Parietal		Temporal		Occipital		Motor		Cingulate	
CBF_{GM} (mL/100 g/min)	**β**	***P***	**β**	***P***	**β**	***P***	**β**	***P***	**β**	***P***	**β**	***P***	**β**	***P***
Age (years)	-0.24	0.111	-0.17	0.262	-0.15	0.312	-0.20	0.181	-0.17	0.261	-0.12	0.453	-0.12	0.447
Wear-time (mins/day)	-0.01	0.952	-0.04	0.785	-0.01	0.936	-0.02	0.909	-0.08	0.559	-0.03	0.849	-0.01	0.971
LPA (mins/day)	-0.05	0.690	-0.01	0.920	-0.01	0.929	-0.05	0.692	0.00	0.983	0.06	0.645	-0.04	0.784
MVPA (mins/day)	-0.20	0.181	-0.22	0.162	-0.18	0.247	-0.20	0.185	-0.14	0.374	-0.18	0.255	-0.19	0.215

β: standardised beta coefficient, CBF_{GM}: grey matter cerebral blood flow, LPA: light physical activity, MVPA: moderate-to-vigorous physical activity.

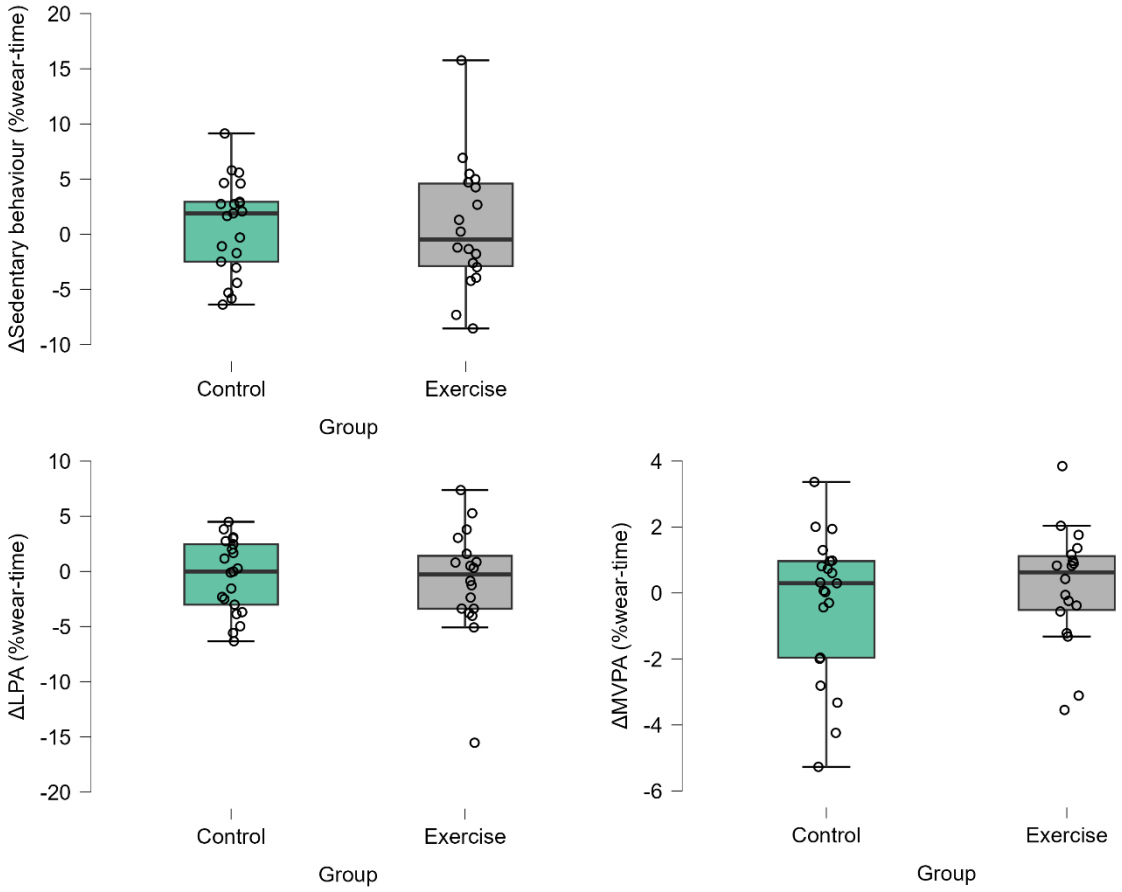

Figure 4.S3: Changes in sedentary behaviour (top left), light physical activity (LPA: bottom left), and moderate-to-vigorous physical activity (MVPA: bottom right) over the 6-month follow-up period, split by participants' randomised allocation to either a control (n=21: lifestyle maintenance) or exercise (n=18: thrice weekly, high-intensity interval training) group as part of a larger project. Changes calculated as the average of the three- and six-month measurements minus the pre-intervention measurement.

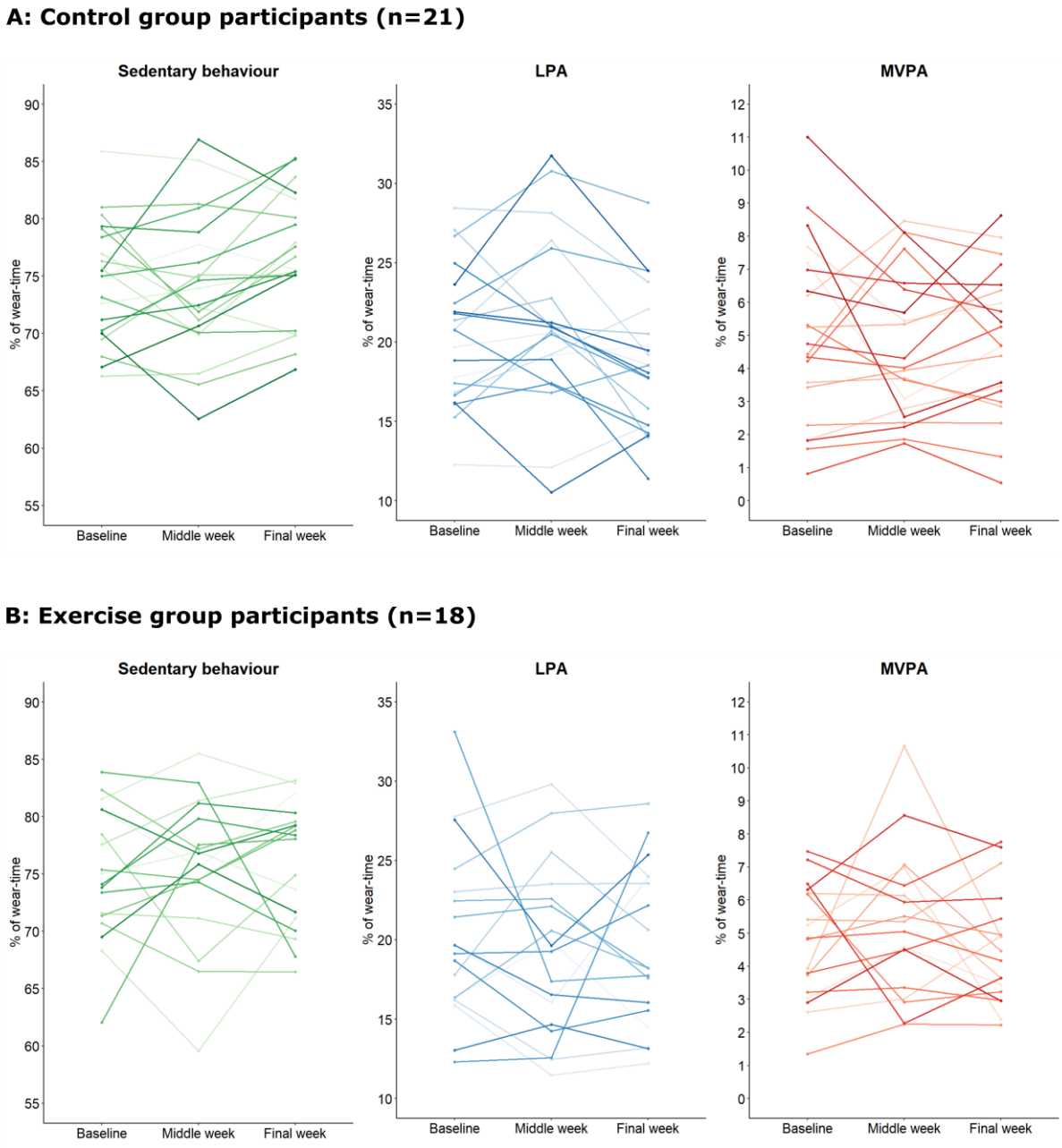

Figure 4.S4: Changes in sedentary behaviour, light physical activity (LPA), and moderate-to-vigorous physical activity (MVPA) from the pre-intervention, middle (+3 months), and final (+6 months) follow-up weeks, expressed as percentage of total accelerometer wear-time for participants randomised to the control and exercise group (panel A and B, respectively).

CHAPTER FIVE

EXPLORATORY ANALYSIS: PREDICTING CARDIORESPIRATORY FITNESS RESPONSES TO HOME-BASED EXERCISE TRAINING

5.1 Introduction

As previously outlined in Section 1.8.1 and shown in Figure 1.17, there is known to be considerable variation in cardiorespiratory fitness responses following a standardised exercise training programme, dictated by a combination of biological and methodological factors (Figure 5.1) (Meyler et al., 2021). Seminal work from The HERITAGE study indicated that ~20% of individuals do not experience considerable cardiorespiratory fitness improvements following endurance exercise training (Bouchard et al., 1999). Furthermore, heritability was found to account for nearly half of the variation in cardiorespiratory fitness responses (Bouchard et al., 1999). Indeed, this variation is also evident within the participants studied in this thesis and can be visualised in Figure 3.2, 3.S7, and 5.2. Genetics are clearly a very important factor in these responses which cannot be modified. However, there is still approximately half of the variation that is explained by other factors, some of which will be modifiable. Understanding which modifiable factors, such as exercise training volume or intensity (Meyler et al., 2021), can augment cardiorespiratory fitness responses to an exercise training intervention will inform exercise prescription guidelines and ultimately help optimise the well-established positive health outcomes elicited by regular exercise and physical activity.

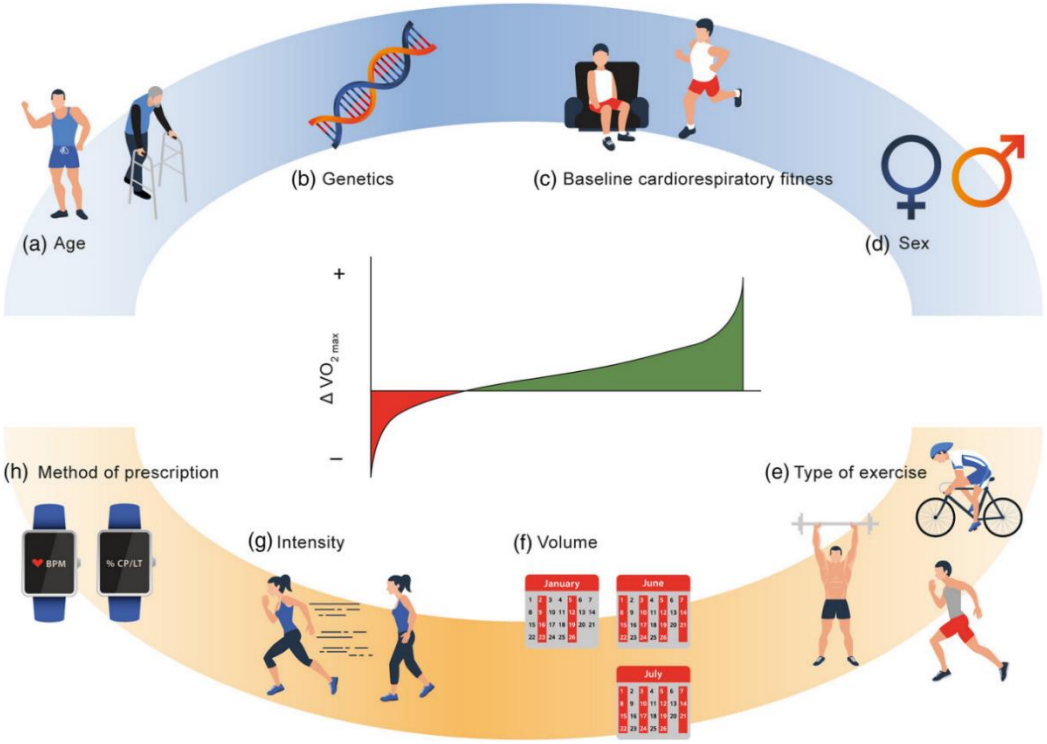

Figure 5.1: Biological and methodological factors that infuence cardiorespiratory fitness ($\dot{V}O_{2peak}$) responses to exercise trainng. From Meyler et al. (2021).

The exericse intervention used in this thesis was home-based for superior ecological validity. However, the lack of in-person supervision meant that it was important to objectively assess adherence throughout the intervention. In an ideal world, all participants would complete the exercise intervention exactly as prescribed, but the reality of home-based exercise interventions means this is nigh on impossible due to factors that are both avoidable (e.g., motivation or understanding) and unavoidable (e.g., illness, injury, holidays, or weather). Individual differences in adherence to the exercise intervention could have a large impact on the subsequent cardiorespiratory fitness responses. However, previous research investigating this response variability have generally focussed analyses on participants with high adherence, and these mostly consider only session attendance/completion (Ross et al., 2019). Furthermore, it may be that adherence to one specific aspect of the intervention is more important for cardiorespiratory gains than another (e.g., exercise intensity vs. volume). Therefore, the purpose of this exploratory

chapter was to assess whether adherence to the exercise intervention, baseline demographic characteristics, or pre-to-post intervention changes in demographic characteristics were associated with the observed changes in cardiorespiratory fitness within the exercise group.

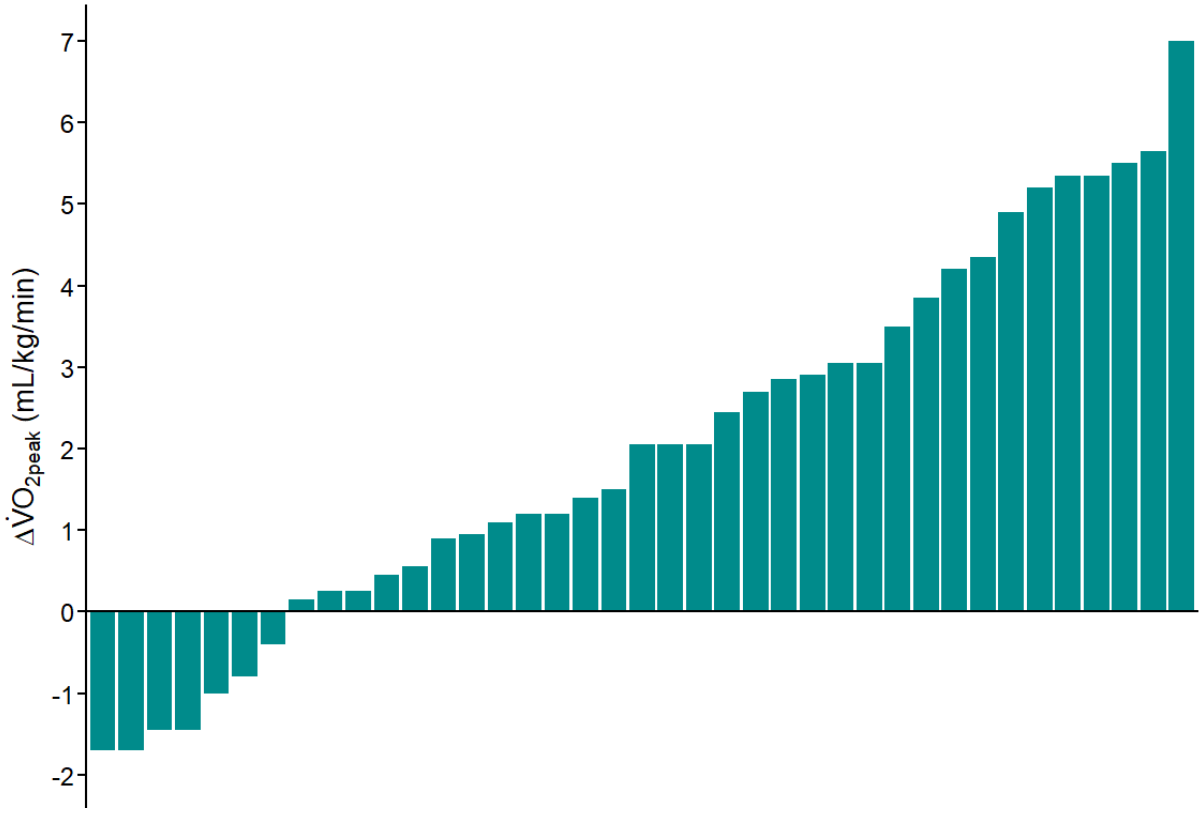

Figure 5.2: Individual participant changes in cardiorespiratory fitness ($\Delta\dot{V}O_{2peak}$) in response to the six-month exercise intervention (n=39: mean=2.0±2.3 mL/kg/min, range=-1.7–7.0 mL/kg/min).

5.2 Materials and methods

5.2.1 Participants

Of the 48 participants that were randomly allocated to the exercise group, 39 completed the intervention and returned for post-intervention tests. Reasons for drop outs from the intervention included lack of time (n=3), health issues (n=1), or personal issues (n=1). Participant characteristics are shown in Table 5.1.

Table 5.1: Participant characteristics.

	n = 39 (21M:18F)
Age (years)	65.7±4.7
BMI (kg/m^2)	26.5±2.7
Systolic blood pressure (mmHg)	138±13
Diastolic blood pressure (mmHg)	83±8
$\dot{V}O_{2peak}$ (mL/kg/min)	27.8±4.8
Sedentary behaviour (% wear-time)*	75.1±6.4
LPA (% wear-time)*	19.9±5.1
MPA (% wear-time)*	4.9±2.7
VPA (% wear-time)*	0.1±0.1

*Values are means± standard deviation. M: male, F: female, BMI: body mass index, $\dot{V}O_{2peak}$: peak oxygen consumption, LPA: light physical activity, MPA: moderate physical activity, VPA: vigorous physical activity. *: sample size=32.*

5.2.2 Outcome measures

Cardiorespiratory fitness

Methodology has been described previously in Section 3.3.5

Physical activity behaviours

Methodology has been described previously in Section 4.3.4. Briefly, physical activity behaviours were expressed as a percentage of wear-time and pre-to-post intervention changes were calculated as the average of values from the middle and final intervention weeks minus the pre-intervention value. For accuracy, the strict 13 hour minimum wear-time criteria was used as described previously (Section 4.3.4). Accelerometer data were only available for 32 participants. Of these 32, one participant had only pre-intervention and final week data available, and so final week minus pre-intervention week was used to calculate pre-to-post intervention change. Of the 32, 11 participants only met the 13 hour wear-time criteria for two of the three measurement weeks. To enable inclusion of these participants within analyses, the standard 10 hour wear-time inclusion criteria (Troiano et al., 2008) was used for the missing week to obtain physical activity behaviour values. The physical activity behaviours used in analysis were sedentary behaviour,

light physical activity (LPA), moderate physical activity (MPA), and vigorous physical activity (VPA) using established uniaxial cut-points (Troiano et al., 2008).

Exercise intervention adherence

Methodology has been described previously in Section 3.3.4 and 3.6.2. Briefly, adherence was objectively monitored using a training logbook in tandem with a fitness watch and chest heart rate monitor (Polar Unite and Polar H9). Useable heart rate data were available for 96±6% of sessions (only five participants had <90%). These data allowed accurate calculations of the volume and intensity of exercise completed for each participant over the 26-week exercise intervention. Adherence is expressed as percentage completion relative to what was prescribed and was calculated using only post-familiarisation data (i.e., from week five onwards). For the present exploratory analysis, the adherence metrics used were cumulative metabolic equivalent minutes (MET-mins), total intervention weeks, total session number, mean number of sessions completed each week, mean session duration, and high-intensity exercise minutes per session (i.e, ≥80% of peak heart rate (HR_{peak})). A composite adherence score was also used, calculated by summing individual participant Z-scores from each of the aforementioned metrics, excluding total intervention weeks which was not always in participant's control (e.g., illness, injury, or extensions for those finishing over University closed periods).

5.2.3 Statistical analysis

To identify potentially important predictors of cardiorespiratory fitness gains, bivariate Pearson correlations were run including adherence metrics, pre-intervention values of cardiorespiratory fitness, physical activity behaviours, and body mass index, and pre-to-post-intervention changes in physical activity behaviours and body mass index.

To fully assess the individual importance of adherence metrics to fitness gains, partial least squares regression (PLSR) analysis was used, which can handle the expected multi-collinearity

between adherence metrics (Table 5.S1) (Baumeister et al., 2024). PLSR analyses followed the same procedure as described in Section 4.3.5, with $\Delta\dot{V}O_{2peak}$ as dependent variable and total intervention weeks, cumulative MET-mins, total session number, weekly session number, session duration, and high-intensity exercise minutes as independent variables. PLSR provides selectivity fractions (SF) that indicate the fraction of total variance in $\Delta\dot{V}O_{2peak}$ explained by each independant variable.

5.3 Results

5.3.1 Adherence

Adherence to the exercise intervention was generally good (typically 80–110% of what was prescribed), but there was considerable variation within each metric, which can be visualised in Figure 5.3. This variation was most pronounced in adherence to exercise intensity. A summary of the adherence data is show in Table 5.2.

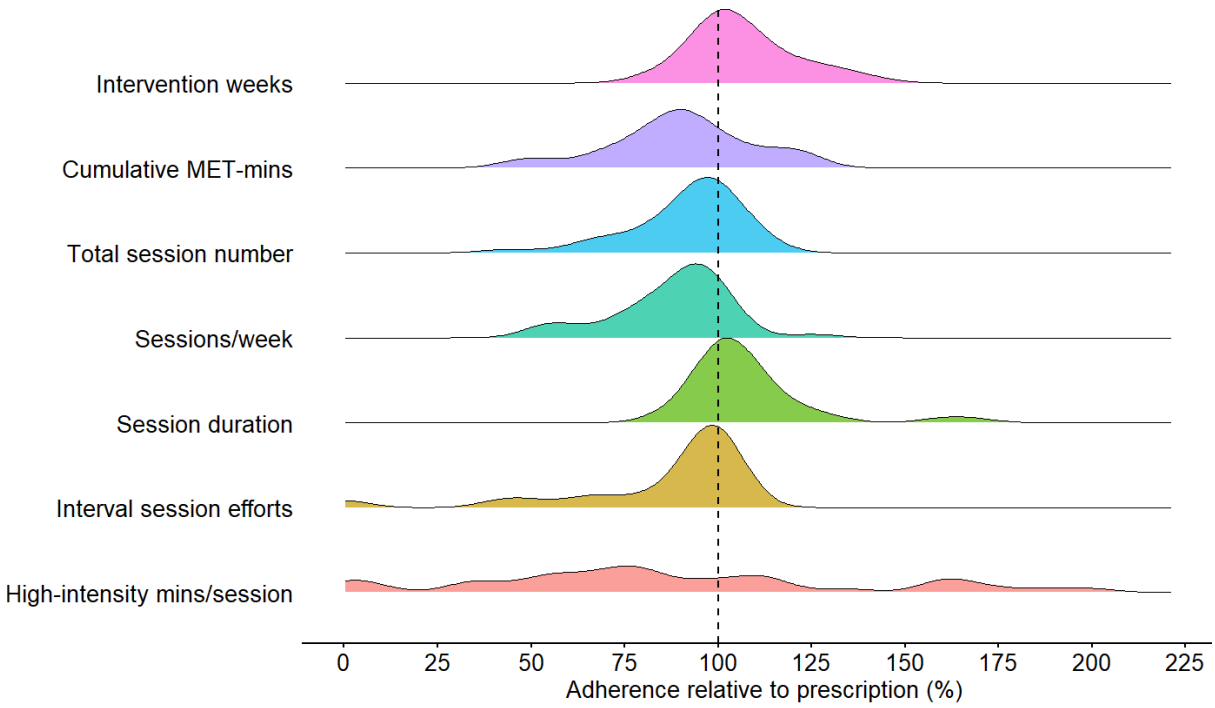

Figure 5.3: Distribution of adherence metrics to the exercise intervention (n=39).

Table 5.2: Adherence to the exercise intervention (n=39).

		Completed		
Adherence metric (n=39)	Planned	Mean	Range	%
Weeks	22	28±3	23–34	106±14
Cumulative MET-mins	8567	8163±1739	3743–11791	90±19
Session number	66	61±10	29–75	92±14
Sessions per week	3	2.6±0.5	1.6–3.8	88±15
Session duration (mins)	29.5	32±5	25–49	107±16
Interval session efforts/session	7	7±1	3–8	90±17
Minutes >80%HR_{peak}/session	10.5	9±5	0–21	87±52
Mean heart rate (%HR_{peak})	–	73±5	61–83	–

Values are means ± standard deviations. HR_{peak}: peak heart rate, MET: metabolic equivalents. Data is from post-familiarisation (weeks 5–26).

5.3.2 Predictors of cardiorespiratory fitness responses

Bivariate correlations found significant positive associations between $\Delta\dot{V}O_{2peak}$ and composite adherence score (Figure 5.4A; r_{37}=0.33, *P*=0.042) and total session number (Figure 5.4B; r_{37}=0.42, *P*=0.008). There were noteworthy associations between $\Delta\dot{V}O_{2peak}$ and Δsedentary behaviour (r_{37}=0.33, *P*=0.064) and ΔLPA (r_{37}=-0.33, *P*=0.070). The Supplemental Material (Section 5.5) contains the full correlation matrix (Table 5.S1).

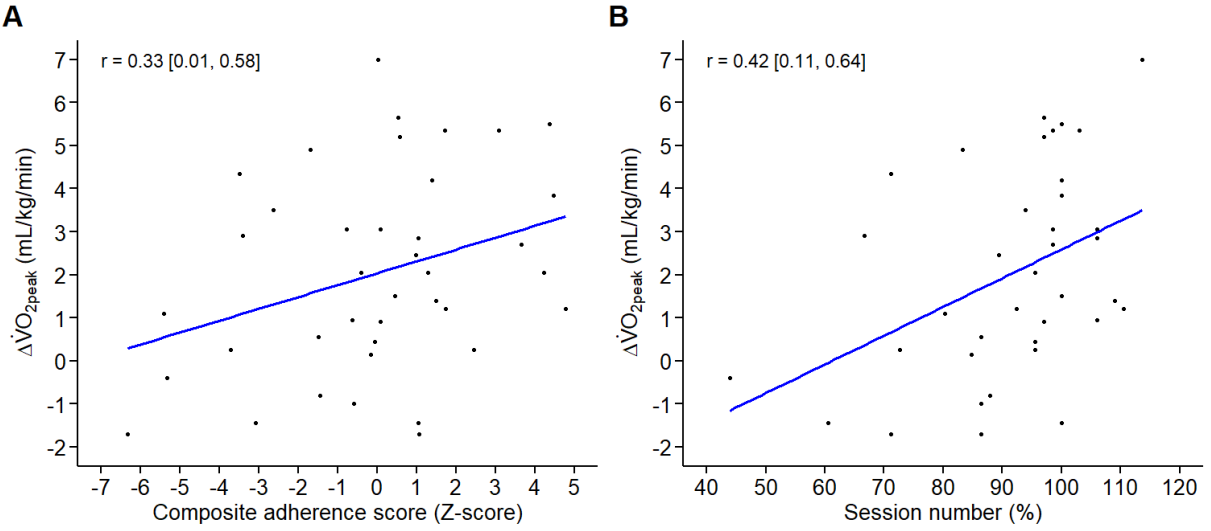

Figure 5.4: Bivariate correlations between changes in cardiorespiratory fitness ($\Delta\dot{V}O_{2peak}$) and composite adherence score (A) and session number adherence (B) (n=39).

Predicted cardiorespiratory fitness values were used for ten participants who completed a sub-maximal fitness test at pre- and/or post-intervention. To investigate whether these affected results, analyses were repeated using $\dot{V}O_{2peak}$ data from participants that completed 'true' maximal tests (n=29, RER=1.08±0.06, age-predicted $\%HR_{peak}$=106±7%, peak [lactate]=7.0±1.8 mmol/L). This did not affect the $\Delta\dot{V}O_{2peak}$–adherence composite or $\Delta\dot{V}O_{2peak}$–session number correlations but did abolish the noteworthy associations with physical activity behaviours, though the sample size for the latter was limited (n=23).

PLSR analyses identified significant positive associations between $\Delta\dot{V}O_{2peak}$ and adherence to cumulative MET-mins, total session number, and high-intensity exercise minutes (Figure 5.5). Adherence to total session number had a larger association with $\Delta\dot{V}O_{2peak}$ than high-intensity exercise minutes. Selectivity fractions are also shown in Table 5.S2. Figure 5.6 allows visualisation of how variations in composite adherence (A), cumulative MET-mins (B), session number adherence (C), and high-intensity adherence (D) are spread across the range of cardiorespiratory fitness responses observed within the full sample.

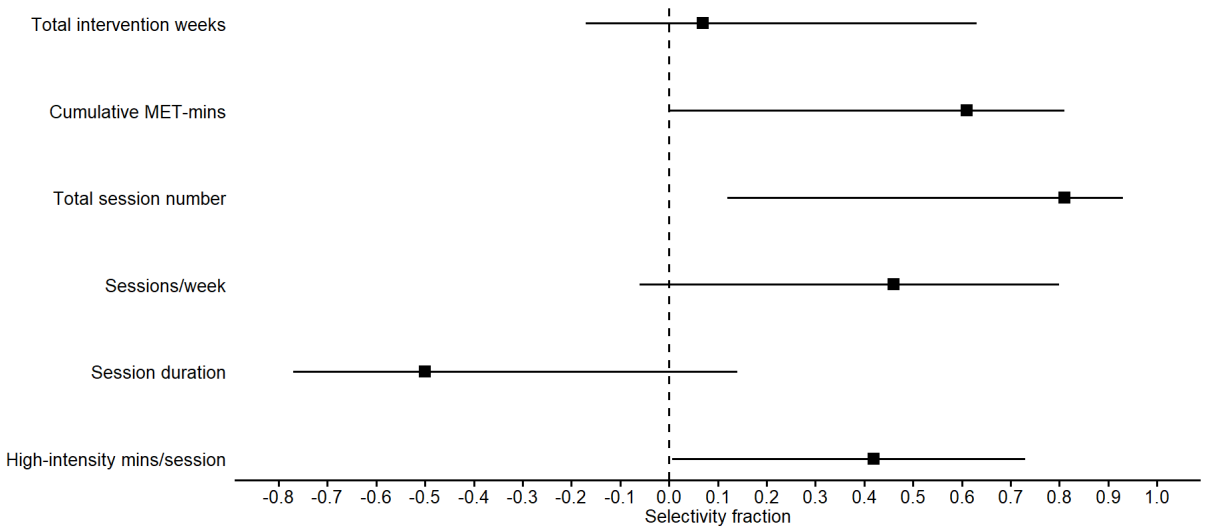

Figure 5.5: Selectivity fractions, a measure of explained predictive variance, between adherence metrics and changes in cardiorespiratory fitness ($\Delta\dot{V}O_{2peak}$). Values are medians with 95% confidence intervals. Determined using partial least squares regression (n=39).

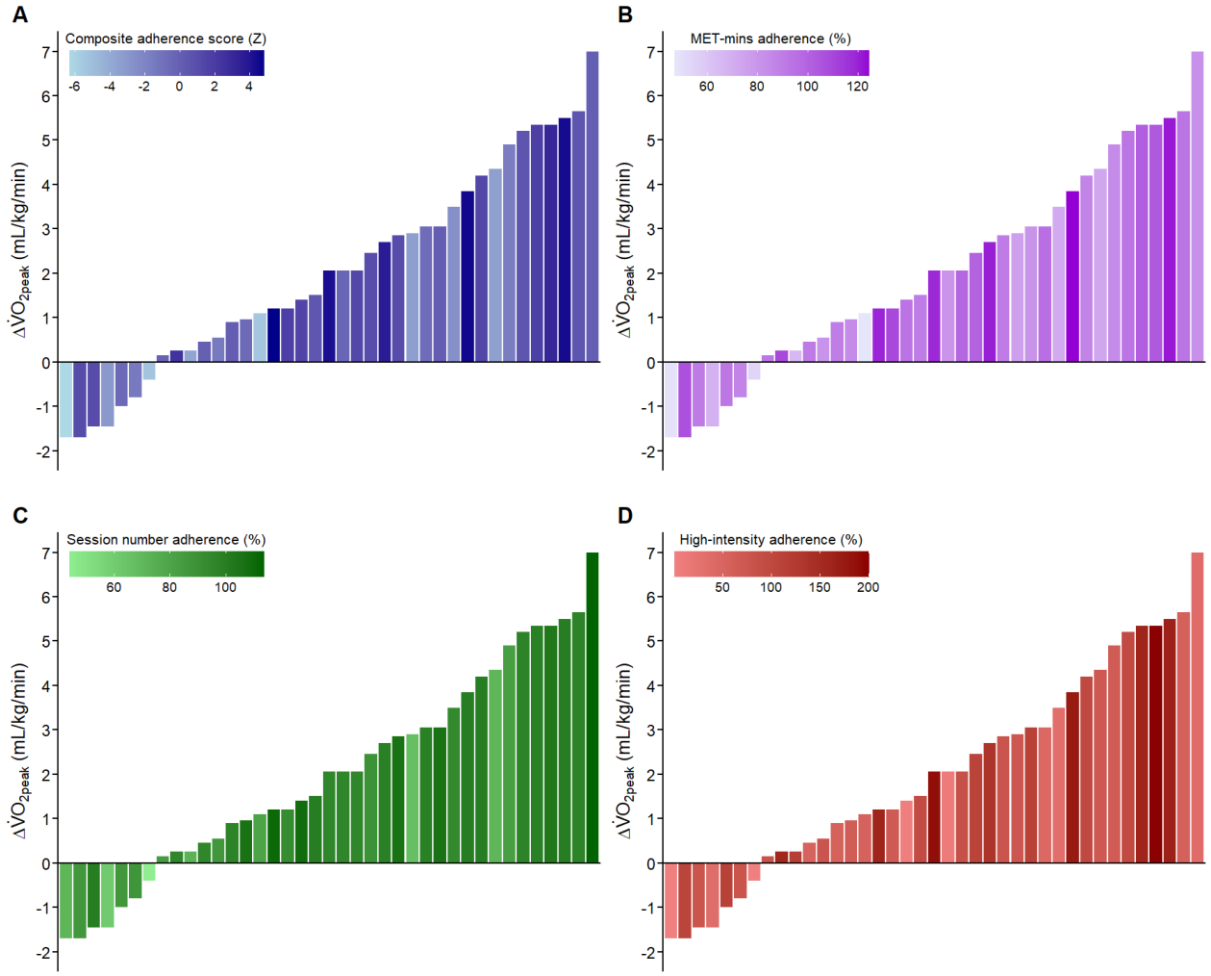

Figure 5.6: Individual participant changes in cardiorespiratory fitness ($\Delta\dot{V}O_{2peak}$) in response to the six-month exercise intervention (n=39), coloured corresponding to individual composite adherence score (A), cumulative MET-mins (B), session number adherence (C), and high-intensity adherence (D). Darker colour indicates superior adherence.

5.4 Discussion

The primary aim of this exploratory analysis was to assess whether adherence to a home-based exercise intervention was associated with subsequent cardiorespiratory fitness responses in older adults. A significant positive correlation between composite adherence score and $\Delta\dot{V}O_{2peak}$ provided general support for the hypothesis that superior adherence is beneficial for cardiorespiratory fitness gains. Furthermore, specific analyses of individual adherence metrics indicated that exercise volume and exercise intensity were the most important for

cardiorespiratory fitness gains, with adherence to the overall number of sessions completed having the strongest association. Changes in sedentary behaviour and LPA (increases and decreases, respectively), behaviours that are inherently linked, also appeared to correlate with greater fitness gains, though these did not reach statistical significance. Pre-intervention $\dot{V}O_{2peak}$, BMI, and physical activity behaviours, and ΔBMI were not correlated with $\Delta\dot{V}O_{2peak}$.

5.4.1 Exercise intensity and cardiorespiratory responses

Logically, it would be assumed that individuals that demonstrate the highest engagement with exercise training, performing the most sessions and/or exercising at a higher intensity, would experience superior cardiorespiratory fitness gains. The present exploratory analyses do offer some support for this hypothesis; however, the consensus within the previous literature regarding which aspect of exercise training (e.g., volume vs. intensity) is most important to cardiorespiratory fitness responses is unclear. High-intensity exercise training, as prescribed in the present study, is commonly cited to induce superior cardiorespiratory fitness gains compared to moderate-intensity continuous training. Indeed, evidence exists showing superior fitness responses and lower non-response rates to six-months of higher-intensity (n=31) versus lower-intensity exercise training (n=51) (matched for energy expenditure) in middle-aged adults (0% vs. 18%, respectively) (Ross et al., 2015). These findings agree with the present positive $\Delta\dot{V}O_{2peak}$–high-intensity adherence association (SF=0.42 [0.01, 0.73]). However, Ross et al. (2015) used intensities of either ~50%HR_{peak} or ~75%HR_{peak}, and so whilst this does indicate an effect on exercise intensity on fitness responses (i.e., low vs. moderate), it does not necessarily indicate a superior benefit of high-intensity exercise, classified as >77%HR_{peak} by American College of Sports Medicine guidelines (Liguori, 2020). The present analyses defined high-intensity exercise as ≥80%HR_{peak}, and this specific metric had the largest variability in adherence (Figure 5.3). In contrast, a recent meta-analysis dampens the idea that exercise intensity is the key to cardiorespiratory fitness

gains, reporting only a small-to-moderate, non-significant, effect that high-intensity training induces greater cardiorespiratory fitness gains compared with moderate-intensity continuous training (after adjustment for total exercise volume) (Fosstveit et al., 2024b).

5.4.2 Exercise volume and cardiorespiratory responses

Increases in exercise volume are also hypothesised to promote cardiorespiratory fitness gains. The present analyses included two indices of exercise volume, cumulative MET-mins and total session number, both of which were associated with superior fitness gains, but session number had the larger association. Cumulative MET-mins is ultimately derived from exercise intensity, exercise duration, and the number of exercise sessions. Variability in exercise duration was limited (Figure 5.3) and when using these three metrics as predictors of cumulative MET-mins in a multiple linear regression model, only session number and high-intensity exercise minutes were significant predictors (Table 5.S3). Therefore, in the present analyses, total session number should be considered the primary index of exercise volume, which appears to be more important to cardiorespiratory fitness gains than exercise intensity within this sample (Figure 5.5). In contrast to these findings, Ross et al. (2015) reported that adherence to the number of sessions completed had little impact on fitness responses. However, Ross et al. (2015) also assessed the impact of exercise volume more directly, reporting that, for the same exercise intensity, completing a higher exercise volume per session (i.e., energy expenditure) induced significantly greater cardiorespiratory fitness gains and reduced the proportion of non-responders from 38% to 18%. Another study reports similar findings in female older adults, but the effect weakened when exercising at a volume above recommended guidelines, potentially indicating a ceiling for the beneficial effects of exercise volume (Sisson et al., 2009). Furthermore, non-response rate was progressively reduced after six-weeks of higher volume training (81%, 50%, 0% for 60, 180, and 300 mins/week, respectively) and all the non-responders became responders by completing

an additional two exercise sessions per week (i.e., increasing exercise volume) (Montero and Lundby, 2017).

It was also possible that achieving a higher frequency of exercise sessions and thus a more consistent exercise exposure would promote superior fitness gains. In the present analyses, frequency was assessed as mean sessions per week, calculated by dividing total session number by total intervention weeks (including weeks with no exercise sessions), and was not correlated with $\Delta\dot{V}O_{2peak}$, although only four participants completed <2 sessions per week on average and thus there may not have been sufficient variability to detect effects. It should be considered that, overall, adherence to the exercise intervention was very good (90±19% to cumulative MET-mins, 80% of participants ≥80%), and 26% of participants actually achieved ≥100% cumulative MET-mins adherence. Therefore, the key message is that engaging with a greater exercise volume and/or higher intensity is important to fitness gains, rather than adherence to this specific exercise intervention.

5.4.3 Physical activity behaviours, demographics, and cardiorespiratory responses

Regarding physical activity behaviours, these analyses found no significant correlations between baseline values or pre-to-post intervention changes and $\Delta\dot{V}O_{2peak}$, although there were noteworthy associations indicating that greater cardiorespiratory fitness gains were associated with an increase or decrease in sedentary behaviour or LPA, respectively. These two behaviours are very closely related (Mansoubi et al., 2014) and the present analysis cannot isolate if the change in one or both of the behaviours is driving the association. In contrast, previous research finds a limited impact of these behaviours on cardiorespiratory fitness. For example, no associations were present cross-sectionally (O'Brien et al., 2022) and a six-month lifestyle intervention that reduced sedentary behaviour and increased LPA did not influence cardiorespiratory fitness levels (Norha et al., 2023). Higher-intensity physical activity (i.e., MPA or VPA) is generally considered more

important to cardiorespiratory fitness; however, cross-sectional research also reports a limited effect of MVPA on cardiorespiratory fitness, including that only ~5% of cardiorespiratory fitness variation is explained by MVPA levels (Raichlen et al., 2020) or that only VPA (not LPA, MPA, or sedentary behaviour) affect cardiorespiratory fitness (O'Brien et al., 2022). As outlined in Section 3.6.5 and 4.3.5, compared to control participants, exercise participants did not significantly alter their physical activity behaviours throughout the exercise intervention at the group-level, despite increasing cardiorespiratory fitness (Δ2.0±2.3 mL/kg/min). It should be considered that the present sample were quite physically active at baseline, with ~85% meeting current MVPA guidelines (Table 4.1), although this was not achieved through structured exercise. It is possible that MVPA experienced limited changes following the exercise intervention, and thus had limited associations with cardiorespiratory fitness responses, because participants stopped engaging with some of their habitual non-exercise MVPA (e.g., brisk walking) in order to keep up with the demands of the thrice weekly exercise intervention despite being asked to maintain their habitual behaviours.

Regarding participant demographics, the present analyses reported no significant correlations between baseline $\dot{V}O_{2peak}$ or BMI (baseline or change) and $\Delta\dot{V}O_{2peak}$. In agreement with the present findings, previous research reports no effects of baseline $\dot{V}O_{2peak}$ (Bouchard et al., 1999; Kohrt et al., 1991; Ross et al., 2015; Skinner et al., 2001), BMI (Ross et al., 2015; Sisson et al., 2009), or physical activity behaviours (Ross et al., 2015) on $\Delta\dot{V}O_{2peak}$. However, others have reported an inverse association with BMI in young adults (Pihlainen et al., 2020), a negative association with baseline $\dot{V}O_{2peak}$ in young adults and cardiac rehabilitation patients (Fuentes Artiles et al., 2023; Laddu et al., 2018; Pihlainen et al., 2020), or a positive association with baseline $\dot{V}O_{2peak}$ in middle-aged and older adults (Hautala et al., 2006; Sisson et al., 2009).

5.4.4 Limitations and future directions

These analyses are only exploratory, conducted in a small sample, and were not adjusted for multiple comparisons. These findings may lack generalisability because the present sample of older adults were generally quite healthy, physically active, and well-educated, potentially affecting their engagement with the exercise intervention and their cardiorespiratory fitness responses. To enable inclusion of as many participants as possible for this exploratory analysis chapter, approximately one third of participants with accelerometery data only had 13-hour wear-time validated data for two of the three measurement weeks. Replacing these missing weeks with 10-hour wear-time validated data could have influenced results. It should be considered that adherence associations may not be linear in nature, and that an upper limit exists for their beneficial effects on cardiorespiratory fitness, or even doing too much exercise or exercising at too high an intensity could have detrimental effects on outcome measures (i.e., inverted-U). In order to maximise cardiorespiratory fitness gains for all participants, repeated fitness assessments during the intervention period should be performed to identify those with a low response and thus increase their exercise volume and/or intensity accordingly. Finally, although having higher cardiorespiratory fitness has unequivocal health benefits (Lang et al., 2024; Tari et al., 2019), its high heritability means it may not be the best index of exercise intervention efficacy, particularly in older adults who perform limited activities that require high absolute levels of oxygen consumption. Interestingly, in older adults, exercise training appears to induce larger changes in lactate threshold than cardiorespiratory fitness (Fosstveit et al., 2024a) and thus changes in this measure could be more homogenous and have stronger associations with health outcomes. For example, carrying groceries is considered a strenuous activity of daily living, requiring a $\dot{V}O_2$ of ~20 mL/kg/min (Nayor et al., 2020), which corresponds to ~72% of $\dot{V}O_{2peak}$ in the present sample. This intensity is considerably lower than the intensity at which lactate threshold

occurs in older adults (89 [88, 91] %$\dot{V}O_{2peak}$), calculated in a larger but partly shared participant sample (Fosstveit et al., 2024a).

5.4.5 Conclusion

Although adherence to the home-based exercise intervention was generally very good, the present data highlight an importance of overall exercise volume and high-intensity exercise to exercise training-induced cardiorespiratory fitness gains in older adults. Exercise volume appeared to be strongest predictor of fitness gains and thus older adults attempting to improve cardiorespiratory fitness do not necessarily need to perform vigorous exercise (which may be unsafe for some) but can instead focus on increasing the number of their exercise sessions. There was not strong evidence that baseline participant characteristics (i.e., cardiorespiratory fitness, BMI, or physical activity behaviours) or changes in these measures were predictive of cardiorespiratory fitness responses. Future research should endeavour to objectively assess adherence, not only attendance, to exercise interventions to further improve understanding of which aspects are most beneficial for cardiorespiratory fitness and other health outcomes. Clearly, some individuals will need a greater exercise dose than others to achieve equivalent cardiorespiratory fitness gains, which highlights that adhering to general population-based guidelines may not be the most effective approach for some individuals. As such, it should be recognised that a single optimal exercise dose for all is not appropriate. Further work is needed to identify individuals that will have a low response to the general, population-based guidelines so that their exercise recommendations can be adjusted to optimise their response, enabling access to exercise-related health benefits.

5.5 Supplemental Material

Table 5.S1: Bivariate Pearson correlations between $\Delta\dot{V}O_{2peak}$, adherence metrics, and participant demographics (n=38).

		Adherence composite score	Intervention weeks	Cumulative MET-mins	Session number	Sessions /week	Session duration	High-intensity mins/session	Baseline VO_{2peak}	Baseline BMI	ΔBMI	Baseline SB#	Baseline LPA#	Baseline MPA#	Baseline VPA#	ΔSB#	ΔLPA#	ΔMPA#	ΔVPA#
ΔVO_{2peak}	r	**.327***	0.234	0.261	**.416****	0.190	-0.229	0.258	-0.214	0.040	0.003	-0.083	0.065	0.071	0.027	0.332	-0.325	-0.198	0.240
	P	**0.042**	0.151	0.108	**0.008**	0.246	0.162	0.112	0.190	0.811	0.986	0.652	0.725	0.701	0.883	0.064	0.070	0.276	0.186
Adherence composite score	r		-0.145	**.962****	**.736****	**.685****	**-.330***	**.693****	0.047	0.082	**-.359***	0.154	-0.130	-0.107	-0.177	-0.022	-0.038	0.138	0.237
	P		0.379	**0.000**	**0.000**	**0.000**	**0.040**	**0.000**	0.777	0.622	**0.025**	0.400	0.479	0.561	0.333	0.903	0.834	0.450	0.191
Intervention weeks	r			-0.236	0.153	**-.471****	-0.047	0.203	0.159	-0.004	0.063	-0.125	0.121	0.051	0.264	0.254	-0.185	-0.318	0.157
	P			0.147	0.352	**0.002**	0.778	0.214	0.334	0.979	0.705	0.497	0.509	0.783	0.144	0.161	0.309	0.076	0.391
Cumulative MET-mins	r				**.617****	**.637****	**-.323***	**.711****	0.065	0.110	**-.328***	0.233	-0.203	-0.153	-0.171	-0.139	0.096	0.158	0.124
	P				**0.000**	**0.000**	**0.045**	**0.000**	0.694	0.507	**0.042**	0.200	0.265	0.403	0.351	0.447	0.599	0.389	0.498
Session number	r					**.766****	**-.685****	**.324***	-0.021	-0.002	-0.267	-0.145	0.147	0.065	-0.080	**.386***	**-.432***	-0.099	**.384***
	P					**0.000**	**0.000**	**0.044**	0.898	0.990	0.101	0.429	0.422	0.724	0.662	**0.029**	**0.014**	0.589	**0.030**
Sessions/week	r						**-.644****	0.121	-0.168	0.020	-0.259	-0.059	0.045	0.064	-0.239	0.185	-0.249	0.061	0.215
	P						**0.000**	0.462	0.307	0.904	0.112	0.747	0.805	0.729	0.189	0.310	0.169	0.739	0.237
Session duration	r							-0.254	0.054	0.074	0.005	0.026	-0.009	-0.046	0.042	**-.365***	**.365***	0.171	-0.108
	P							0.119	0.743	0.656	0.975	0.886	0.960	0.804	0.818	**0.040**	**0.040**	0.348	0.556
High-intensity mins/session	r								0.198	0.023	-0.138	**.350***	-0.322	-0.210	-0.008	-0.105	0.101	0.054	0.005
	P								0.226	0.890	0.401	**0.050**	0.072	0.249	0.964	0.568	0.582	0.769	0.978
Baseline VO2peak	r									**-.335***	-0.078	0.257	-0.140	-0.338	0.052	-0.170	0.133	0.206	-0.215
	P									**0.037**	0.637	0.156	0.444	0.058	0.776	0.351	0.467	0.259	0.237
Baseline BMI	r										0.013	0.147	-0.180	-0.001	-0.048	-0.135	0.119	0.108	-0.052
	P										0.937	0.422	0.323	0.994	0.795	0.463	0.518	0.555	0.777
ΔBMI	r											0.076	-0.110	0.020	0.189	-0.060	0.088	-0.048	-0.004
	P											0.678	0.548	0.912	0.301	0.743	0.633	0.796	0.984
Baseline SB	r												**-.906****	**-.634****	**0.100**	**-.548****	**.585****	0.191	**-.353***
	P												**0.000**	**0.000**	**0.587**	**0.001**	**0.000**	0.296	**0.048**
Baseline LPA	r													0.248	-0.289	**.564****	**-.646****	-0.081	**.385***
	P													0.172	0.108	**0.001**	**0.000**	0.661	**0.030**
Baseline MPA	r														0.263	0.221	-0.157	-0.286	0.117
	P														0.145	0.225	0.392	0.113	0.525
Baseline VPA	r															-0.067	0.152	-0.142	**-.391***
	P															0.716	0.407	0.439	**0.027**
ΔSB	r																**-.954****	**-.609****	0.346
	P																**0.000**	**0.000**	0.053
ΔLPA	r																	0.346	**-.390***
	P																	0.052	**0.027**
ΔMPA	r																		-0.179
	P																		0.328

Bold indicates P<0.05. # indicates n=31. $\dot{V}O_{2peak}$: peak oxygen consumption, MET-mins: metabolic equivalent minutes, BMI: body mass index, SB: sedentary behaviour, LPA: light physical activity, MPA: moderate physical activity, VPA: vigorous physical activity.

Table 5.S2: Selectivity fractions between adherence metrics and changes in cardiorespiratory fitness ($\Delta\dot{V}O_{2peak}$) using partial least squares regression analyses (n=39).

	Median	95%CI
Total intervention weeks	0.06	[-0.17, 0.63]
Cumulative MET-mins	0.61	[0.00, 0.81]
Total session number	0.81	[0.12, 0.93]
Sessions/week	0.46	[-0.06, 0.80]
Session duration	-0.50	[-0.77, 0.14]
High-intensity mins/session	0.42	[0.01, 0.73]

Values are medians with 95% confidence intervals (CI).

Table 5.S3: Predictors of cumulative MET-mins adherence.

	β	95%CI	P	VIF
High-intensity minutes	0.58	[0.37, 0.79]	<0.001	1.10
Session duration	0.23	[-0.05, 0.49]	0.100	1.98
Session number	0.59	[0.31, 0.86]	<0.001	2.01

MET-mins: metabolic equivalent minutes, CI: confidence interval, VIF: variance inflation factor.

CHAPTER SIX

GENEREAL DISCUSSION

6.1 Aims of the thesis

The aim of this thesis was to improve understanding of whether modifiable lifestyle factors can modify resting cerebral haemodynamics in older adults, and whether resting cerebral haemodynamics are important for cognitive function within this population. Multiple post-labelling delay arterial spin labelling (ASL) was used to accurately estimate resting cerebral blood flow and arterial transit time in grey matter (CBF_{GM} and ATT_{GM}, respectively), both globally and within lobular regions. The lifestyle factor of particular interest was cardiorespiratory fitness (i.e., $\dot{V}O_{2peak}$; peak oxygen consumption) because despite its unequivocal health benefits, the impact of cardiorespiratory fitness on cerebral haemodynamics (if any) is poorly understood. The key findings of this thesis, relevant mechanisms, and their practical implications will now be discussed. As with most research, more questions relevant to this field have been uncovered throughout this thesis and therefore directions for future research will also be outlined that can help build upon the foundations of this thesis.

6.2 Cardiorespiratory fitness and cerebral haemodynamics

Cross-sectional analyses within Chapter 2 found no association between cardiorespiratory fitness and global CBF_{GM} or ATT_{GM}. Further regional analyses also found no association between cardiorespiratory fitness and lobular CBF_{GM}. However, cardiorespiratory fitness was unexpectedly associated with longer ATT_{GM} in frontal, parietal, occipital, and motor lobes, although only parietal and occipital regions survived adjustment for multiple comparisons. Cardiorespiratory fitness-related lengthening of ATT_{GM} was an unexpected finding because ATT_{GM} is known to lengthen with age and cerebrovascular disease (Damestani et al., 2023; Yu et al., 2022), whereas

cardiorespiratory fitness declines with age and is associated with superior health outcomes (Lang et al., 2024; Letnes et al., 2020; Tari et al., 2019). Large artery cerebral blood velocity and vascular path length are key determinants of ATT_{GM} but are unlikely candidates to explain the observed lengthening because higher cardiorespiratory fitness tends to be associated with faster cerebral blood velocities (although this is not conclusive) (Smith et al., 2021) and reduced cerebral vessel tortuosity (i.e., shorter path length/less blood flow resistance) (Bullitt et al., 2009), effects that would theoretically shorten ATT_{GM}. An alternative explanation is that an increased number of small cerebral vessels associated with higher cardiorespiratory fitness levels (Bullitt et al., 2009) would increase total vessel cross-sectional area, thus causing a greater slowing of cerebral blood velocity through this, and downstream regions of the cerebrovascular tree. Only one other, smaller, study has investigated associations between cardiorespiratory fitness and ATT_{GM} in older adults, reporting no associations (Burley et al., 2021). Therefore, the present findings should be replicated in another sample to confirm the association.

Regarding CBF_{GM}, previous research has reported positive, negative, or complete lack of association between cardiorespiratory fitness and CBF_{GM} in older adults, primarily using single post-labelling delay ASL to measure CBF_{GM} (summarised in Section 1.6.1). Given that the present cross-sectional data indicate a lengthening effect of cardiorespiratory fitness on ATT_{GM}, interpretations and comparisons to previous research using single-delay ASL are limited because CBF_{GM} estimation could be considerably compromised. For example, considering two individuals that had identical ATT_{GM}-influencing traits apart from their cardiorespiratory fitness level, the timing of the single post-labelling delay used will affect the subsequent CBF_{GM} estimation. Specifically, using a longer delay (e.g., 2000 ms) would cause a greater underestimation of CBF_{GM} for the low-fit individual relative to the high-fit, because a shorter ATT_{GM} in the low-fit individual would mean more of the labelled blood would have already been delivered and then left the cerebral tissue by the time of imaging. In contrast, the opposite would be true if a shorter post-

labelling delay was used that was closer to the ATT_{GM} of the low-fit individual. The use of multiple post-labelling delay ASL is a key strength of the work described in this thesis because it allows for greater CBF_{GM} estimation accuracy and thus more definitive conclusions regarding the impact of cardiorespiratory fitness and other lifestyle factors on CBF_{GM}.

These aforementioned findings relating to cardiorespiratory fitness are from cross-sectional analyses for which it is easier to gather larger quantities of data, and which offer the potential to look at the longer-term relevance of various lifestyle factors, including cardiorespiratory fitness. However, because these analyses measure the dependent and independent variables simultaneously, the temporal relationship between these variables cannot be assessed and thus it is impossible to establish cause and effect. For this reason, Chapter 3 of this thesis included an exercise intervention in which the responses of CBF_{GM} and ATT_{GM} to a six-month, home-based, high-intensity interval training programme were assessed.

The randomised controlled trial within Chapter 3 found no group-level differences in CBF_{GM} or ATT_{GM}, globally or regionally, following the exercise intervention, despite a group-level increase in cardiorespiratory fitness compared to control group. However, as expected, there was large inter-individual variability in cardiorespiratory fitness responses within the exercise group, but also the control group, which could potentially dictate cerebral adaptations. To assess any impact of cardiorespiratory fitness, the exercise group was split in to a high and low cardiorespiratory fitness response group, relative to the cardiorespiratory fitness changes induced by lifestyle maintenance within the control group. Comparing pre-to-post intervention changes within the high and low cardiorespiratory fitness response groups revealed significant reductions in global CBF_{GM} within only the high response group, and no significant changes in ATT_{GM} within either the high or low response group. Furthermore, when assessing the exercise group as a whole, there

was a non-significant, but noteworthy, negative association between changes in cardiorespiratory fitness and changes in CBF_{GM}, but this association was not present in the control group.

Although there were no significant differences in adherence metrics to the exercise intervention between the high and low cardiorespiratory fitness response group, the biggest difference was to exercise intensity (rather than volume). Follow-up correlations in the whole exercise group revealed a negative association between CBF_{GM} changes and exercise intensity adherence, but no association with exercise volume adherence. Interestingly, rodent studies have demonstrated that exercise training-induced lactate exposure is a key mediator of subsequent cerebral capillarisation and cognitive improvements (El Hayek et al., 2019; Morland et al., 2017). Therefore, given that the high response group tended to spend more of their exercise sessions at higher intensities, and that greater engagement with high-intensity exercise was associated with CBF_{GM} reductions, these data highlight a potential role of repeated lactate exposure mediating the cerebral haemodynamic changes observed in the present data. The high-intensity exercise training used in the present data may therefore also help explain the differences in findings with previous research, which primarily used continuous, moderate-intensity exercise training (summarised in Section 1.6.2). Given the potential role of lactate exposure for these benefits, changes in lactate metabolism during sub-maximal exercise (i.e., lactate threshold) following exercise training may be a strong predictor of brain health benefits, because more significant changes in lactate threshold would be expected following greater exposure to lactate during exercise training. Compared with peak oxygen consumption, changes in lactate threshold are less influenced by genetics and exhibit greater responses to exercise training in older adults (Fosstveit et al., 2024a).

Indeed, cardiorespiratory fitness-dependant reductions in CBF_{GM} following exercise training were somewhat unexpected; however, there is no real basis for this surprise because the commonly

assumed and cited notion that exercise training increases blood flow to the brain has minimal robust experimental evidence supporting it, as outlined in Chapter 1 (Section 1.6). Although the cerebral tissue is not skeletal muscle, it is likely that it responds in a similar way to exercise training, whereby the ability of the tissue to extract and use the oxygen that is being delivered by the blood improves. Indeed, there is evidence of physiological adaptations to exercise training that would improve these processes in humans (e.g., erythropoiesis) and rodents (e.g., cerebral capillarisation and mitochondrial biogenesis) (Morland et al., 2017; Sellami et al., 2021; Steiner et al., 2011). This hypothesis is rarely considered within the literature but if true would have considerable effects on CBF_{GM}, whereby the blood flow requirements of the tissue would lower because the tissue is extracting and utilising oxygen at a greater efficiency. The present data support this theory, but concurrent measurements of cerebral oxygen extraction and/or metabolic rate are needed to confirm such speculations. These aforementioned potential mechanisms mediating CBF_{GM} reductions differ from those that are likely to contribute to normal age-related declines (e.g., vascular dysfunction or cerebral atrophy).

These intervention data somewhat contradict the previously discussed cross-sectional findings, whereby both sets of analyses report associations between cardiorespiratory fitness and cerebral haemodynamics, but not for the same haemodynamic measure. Regarding the cardiorespiratory fitness-dependant CBF_{GM} reductions following exercise training, it is possible that this is a short-term effect, mediated by changes, for example, in blood composition, blood pressure, or functional changes to central and cerebral vessels. With continued training, other structural changes are likely to follow in the longer-term that could affect cerebral haemodynamics, such as widening vessel lumens or neurogenesis. Furthermore, more chronic exposure to training-induced changes in blood pressure or cardiac output may affect the longer-term response of CBF_{GM} to exercise training. This short-term hypothesis is speculative but could explain why the cross-sectional analyses reported no associations between cardiorespiratory fitness and CBF_{GM},

assuming that participants cardiorespiratory fitness levels were relatively stable in recent years (i.e., alterations to CBF_{GM} in those with higher cardiorespiratory fitness levels may occur during the initial fitness gaining process but then normalise following continued training). Similarly, the same concept could explain the lack of cardiorespiratory fitness-dependant changes in ATT_{GM} following exercise training, but the possible lengthening effect observed within cross-sectional analyses. Specifically, ATT_{GM} may be influenced to a greater extent by structural cerebral changes (e.g., vessel tortuosity or angiogenesis) that occur over the longer-term. Some previous research has compared CBF_{GM} between masters athletes and sedentary peers, reporting no significant differences between groups, but a possible reduction in the severity of age-related declines within the masters athletes (Sugawara et al., 2020; Thomas et al., 2013). However, these used single-delay ASL to measure CBF_{GM} and thus estimation accuracy cannot be guaranteed when comparing young vs. older adults or high vs. low cardiorespiratory fitness groups.

6.3 Physical activity behaviours and cerebral haemodynamics

Using accelerometers to determine sedentary behaviour and physical activity levels offers a holistic view of an individual's movement (or lack of) patterns, irrespective of what structured exercise they may or may not engage in. Furthermore, unlike cardiorespiratory fitness, there is no genetic component to physical activity behaviours. Theoretically, physical activity behaviours have the potential to influence cerebral haemodynamics, possibly to a greater extent than cardiorespiratory fitness. Chapter 4 of this thesis aimed to address this question using both cross-sectional and longitudinal (six-months follow-up) analyses that appropriately controlled for the interplay between sedentary behaviour and physical activity levels. Neither of these analyses identified any associations between physical activity behaviours and CBF_{GM} or ATT_{GM} in older adults. Previous research generally reported that lower sedentary behaviour and higher physical activity levels were associated with higher regional CBF_{GM}, but these generally used single-delay

ASL, investigated behaviours in isolation, and were cross-sectional in nature (summarised in Section 1.9). The data presented in Chapter 4 are the first investigating associations with ATT_{GM}. Given the lack of longitudinal associations in Chapter 4 and the lack of group-level haemodynamic responses to exercise training in Chapter 3, collectively, the data within this thesis indicate that cardiorespiratory fitness gains, rather than physical activity behaviours, are an important factor for inducing cerebral haemodynamics changes in older adults, at least over the medium-term. For more definitive conclusions to be made, interventions targeting a specific physical activity behaviour (e.g., sedentary behaviour) or studies monitoring changes in physical activity behaviours and cerebral haemodynamics over the longer-term (i.e., years) are required.

6.4 Body mass index and cerebral haemodynamics

Cross-sectional analyses within Chapter 2 of this thesis identified that body mass index (BMI), an indicator of obesity, had strong associations with both CBF_{GM} and ATT_{GM} in older adults, whereby a higher BMI was associated with lower CBF_{GM} and a longer ATT_{GM}, both globally and regionally. Regarding CBF_{GM}, this finding agreed with previous research reporting that markers of obesity (i.e., BMI, waist circumference, waist-to-hip ratio, metabolic syndrome) are associated with lower resting CBF_{GM} (Birdsill et al., 2013; Knight et al., 2021; Leidhin et al., 2021). Regarding ATT_{GM}, this was a novel finding that had not been investigated in healthy older adults before. Both of these BMI associations follow the same direction as age-effects on CBF_{GM} and ATT_{GM}, indicating that maintaining a healthy body weight in later life may help reduce normal age-related changes in cerebral haemodynamics.

6.5 *Age and cerebral haemodynamics*

Although not a modifiable lifestyle factor, cross-sectional analyses described in Chapter 2 also identified that only ATT_{GM}, and not CBF_{GM}, was significantly associated with age (i.e., ATT_{GM} lengthened with age). This finding is not intended to imply that age-related declines in CBF_{GM} do

not exist, but instead indicate that ATT_{GM} may be more sensitive to age-related changes than CBF_{GM}. A lack of CBF_{GM} effect was likely caused by the limited age range of the sample and has also been reported by others using a sample with similar demographics (Juttukonda et al., 2022). Indeed, compared with CBF_{GM}, more widespread effects of age on ATT_{GM} have been reported previously (Damestani et al., 2023). Therefore, if changes in ATT_{GM} occur earlier and/or are more considerable than CBF_{GM}, ATT_{GM} could be used as an early indicator of declines in cerebrovascular health, which may be relevant for other brain health outcomes (e.g., cognitive function or cerebral atrophy). This information could be used to identify individuals potentially at risk of more substantial brain health declines in the future, enabling the opportunity to make lifestyle changes that could improve their prospects.

6.6 Cognitive function and cerebral haemodynamics

Regularly engaging with physical activity is undoubtedly good for general brain health. Surprisingly, however, the existing experimental evidence linking physical activity, or exercise training, or cardiorespiratory fitness to superior cognitive function is far from clear cut, though this is probably due to high between-study variability in the behavioural outcomes assessed, sample characteristics, and the type of intervention used, and not because regular physical activity or exercise has no impact on cognitive health. Furthermore, the underlying mechanisms of how exercise training may influence cognitive function are poorly understood. Analyses within Chapter 2 and 3 aimed to assess whether cerebral haemodynamics were an important factor for cognitive function in older adults. Cross-sectional analyses found no associations between CBF_{GM} or ATT_{GM} with cognitive function and, similarly, changes in CBF_{GM} or ATT_{GM} following exercise training were not associated with changes in cognitive function.

The lack of cognitive effects presented in this thesis may partly relate to the baseline characteristics of the sample. Participants passed a thorough health screen (i.e., ECG/blood

pressure/cognitive impairment/no history of serious disease) and were generally quite physically active (~85% meeting recommendations), highly educated (~40% achieving undergraduate or postgraduate degree), and towards the younger end of the older adult spectrum (~83% aged 60–69 years). Therefore, it is possible that the present sample had not yet experienced substantial age-related cognitive declines to which increased cardiorespiratory fitness or changes to cerebral haemodynamics could work against. Furthermore, compared with previous research reporting an effect of CBF on cognitive function over ~2 or ~7 years (van Dinther et al., 2023; Wolters et al., 2017), the exercise intervention was relatively short and therefore the observed changes to CBF_{GM} (and the potential mediating cerebral adaptations) may not have been present for long enough to meaningfully affect behavioural outcomes. For example, possible exercise training-induced increases in cerebral oxygen utilisation (in turn reducing resting CBF_{GM}) may require more time to affect the structure and function of the cerebral tissue. Finally, using only one task per cognitive domain and/or analysing the domains separately may have made the data more susceptible to individual variability and hidden potential associations. Performing several tasks for each domain and creating a composite score for each domain or a general composite score using all domains may better reflect an individual's cognitive function within that domain and subsequent changes following an intervention. Indeed, using this technique has shown cognitive benefits following a six-month aerobic training intervention (Jonasson et al., 2017) and has demonstrated significant associations between CBF and cognitive function in older adults (De Vis et al., 2018; Leeuwis et al., 2018; Moonen et al., 2021; van Dinther et al., 2023).

It should also be considered that the focus of research investigating healthy cognitive ageing is not to improve cognitive function, but instead to maintain, or simply reduce the rate of inevitable decline. A short-term improvement in a specific cognitive task is likely to lack real-world relevance. These questions can only be answered with longer-term investigations that allow for natural decline to occur, and then either assess the effectiveness of an intervention (e.g., physical

activity, diet, social stimulation, or cognitive training) at improving rates of decline or attempt to identify modifiable characteristics of individuals that demonstrate the best cognitive prospects (e.g., cerebral haemodynamics, cardiorespiratory fitness, sedentary behaviour, BMI, blood pressure, or social activities). Although not obvious in the present data, the theoretical basis for the importance of cerebral haemodynamics to cognitive function remains and has indeed been supported by previous research (van Dinther et al., 2023; Wolters et al., 2017). Notably, these previous investigations have looked at the capacity of CBF_{GM} or volumetric CBF to predict changes in cognitive function over the longer-term (i.e., years). Both studies report that lower baseline blood flow predicts greater rates of cognitive declines, therefore it is not currently obvious how the cardiorespiratory fitness-dependant CBF_{GM} reductions evident in the present data fit within this picture.

6.7 Modifiable factors affecting cardiorespiratory fitness gains

Given the discussed importance of cardiorespiratory fitness to general and brain health, and the considerable variation in exercise training-induced fitness responses, apparent in the present data and existing literature, understanding how to maximise this response in older adults should be a priority. Exploratory analyses within this thesis indicate that objectively measured adherence to an exercise intervention is indeed an important modifiable factor affecting cardiorespiratory fitness gains in older adults, particularly adherence to the overall volume and intensity of exercise. Of these two metrics, overall volume appeared to have the strongest effect and thus older adults aiming to improve their cardiorespiratory fitness levels can attempt to increase the number of times they exercise, rather than focussing on exercising at a higher intensity, which may be difficult or unappealing for some. However, although seemingly efficacious, increasing exercise volume has barriers of its own, with lack of time commonly cited as a key barrier to exercise participation (Godin et al., 1994). To maximise cardiorespiratory fitness gains in older adults, an individualised

approach to exercise training prescription is likely the best option – potentially finding that optimal combination of exercise volume and intensity.

Furthermore, although an individual may lack a considerable increase in their cardiorespiratory fitness following exercise training, this does not necessarily mean they have not experienced beneficial physiological adaptations. For example, despite no group-level changes in cardiorespiratory fitness, significant improvements in measures of body composition and blood lipids were observed in older adults following exercise training (Nielsen et al., 2022). Finally, using markers other than peak oxygen consumption to assess intervention efficacy that are less affected by heritability, such as changes in lactate threshold, may have stronger associations with subsequent health outcomes.

6.8 Future directions

Potential future directions have somewhat been outlined thus far in this discussion but will be summarised here. Longer-term interventions or prospective studies are required to draw firm conclusions regarding how cardiorespiratory fitness, exercise training, and physical activity behaviours affect cerebral haemodynamics in older adults. The same can be said for determining the importance of cerebral haemodynamics to cognitive function in older adults. Utilising comparisons between masters athletes and sedentary peers offers an alternative time and resource efficient approach that could provide some initial insights into these questions. Importantly, these longer-term studies should not just consider how baseline characteristics affect subsequent changes in brain health variables, but also whether the way in which baseline characteristics change (or do not) over time dictates future brain health. Investigating these individual differences and understanding what characteristics are most beneficial for brain health is essential to enabling a healthy ageing process.

Future studies investigating cerebral haemodynamics should not just focus on the blood flow to the cerebral tissue in isolation, but also consider how the cerebral tissue is using the oxygen being delivered by the blood (i.e., cerebral oxygen extraction and/or utilisation), as this will impact interpretations. On this point, the presumption that more always means better should be avoided, as indicated by the CBF data within this thesis and others identifying the prevalence of compensatory hyperperfusion. For studies using arterial spin labelling to measure CBF, multiple post-labelling delays should be used in order to correct for the impact of individual and regional differences in ATT, improving CBF estimation accuracy and thus the reliability of subsequent conclusions. This is particularly important if the variable or intervention of interest may indeed affect ATT. Furthermore, expanding the use of multiple post-labelling delays will improve understanding of the potential prognostic value of ATT, which appears to have greater age-related sensitivity than CBF.

Identifying the type of exercise that produces the optimal brain health benefits is an important next step so that population-level recommendations can be updated. High-intensity exercise, as used in this thesis, appears to be a strong candidate for inducing meaningful brain health benefits in a time-efficient manner. Lactate might play a key role mediating these benefits and so future research should objectively assess if lactate exposure is a determinant of any changes, and the optimal (or lowest) amount needed to induce benefits. Any exercise intervention studies should endeavour to objectively monitor adherence, not only attendance, as this could have a substantial impact on outcome measures, as demonstrated in this thesis.

This thesis has focussed on resting cerebral haemodynamics, with a particular focus on CBF given the current inconsistencies within the literature. However, functional cerebrovascular measures may exhibit greater responses to exercise training or have a more substantial impact on cognitive function. Furthermore, changes in cerebrovascular function could well be involved with exercise

training-induced changes in cerebral haemodynamics, such as those seen in this thesis. Future research should include functional cerebrovascular measures alongside resting cerebral haemodynamics, such as arterial stiffness (central and cerebral), cerebrovascular reactivity, or cerebral flow-mediated dilation.

6.9 Conclusion

The primary purpose of this thesis was to investigate the impact of modifiable lifestyle factors on resting cerebral haemodynamics (cerebral blood flow and arterial transit time) in older adults and assess whether cerebral haemodynamics were important for cognitive function within this population. Data from this thesis indicate that a lower BMI may help limit normal age-related changes in cerebral haemodynamics whereas having higher cardiorespiratory fitness or experiencing high cardiorespiratory fitness gains may induce changes to cerebral haemodynamics in the direction of normal age-effects (i.e., lower cerebral blood flow and longer arterial transit time). Evidence from this thesis did not support a substantial involvement of blood pressure, accelerometer-derived physical activity behaviours, or physical function to differences in cerebral haemodynamics. Furthermore, the present data found no associations between resting cerebral haemodynamics and cognitive function in older adults. This thesis highlights the need for an improved longer-term understanding of how cerebral haemodynamics interact with modifiable lifestyle factors and cognitive function, including the need to assess brain health using a more holistic approach instead of investigating brain health metrics in isolation.

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APPENDICES

Participant Information Sheet

Fitness, Ageing and Bilingualism (FAB): The benefits of regular physical activity for language abilities in healthy ageing

We would like to invite you to take part in a research study conducted at the University of Birmingham. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish to. Ask the researchers if there is anything that is not clear or if you would like more information. Take your time to decide whether you wish to take part or not.

What is the purpose of the study?

The way we process information changes as we grow older. In the present study, we investigate whether cognitive language processing is related to physical fitness in healthy older adults.

Why have I been invited?

You have been invited to take part in this study because you are between 60 and 85 years old and are a native English speaker. To be eligible to participate, you should have normal or corrected-to-normal vision and hearing and have no diagnosed cognitive impairments or language impairments such as dyslexia or stuttering. As we are interested in measuring the effects of increased physical activity you should currently do less than 150 mins moderate exercise per week.

What will I be asked to do?

A. Screening measures.

You will first be asked to sign a consent form so that we can be sure that you are happy to be involved in this research. Next we will ask you to complete a short screening questionnaire, which will ask you about your language profile, your general health and your physical activity levels. We will also monitor your heart activity at rest with an electrocardiogram. These are screening measurements which will allow us to decide whether you can safely participate in the remainder of our study. Exclusion criteria for our study are: a heart condition, uncontrolled high blood pressure, diabetes or another metabolic disorder, respiratory disease (e.g. chronic obstructive pulmonary disease), chronic liver or kidney disease, or a neurological disorder.

Your electrocardiogram will be checked by a clinician; if we observe any abnormal heart rhythms, we will send this information to you so that you can take it to your GP. In case you do not meet our eligibility criteria, you will take no further part in the study and any information collected about you so far will be destroyed.

Lastly, you can only participate in this study once you are vaccinated against COVID-19 (i.e., have had either 2 doses of a double dose COVID-19 vaccination or a 1 single-dose vaccination. See here for more information on the types of vaccination currently approved in the UK: https://www.nhs.uk/conditions/coronavirus-covid-19/coronavirus-vaccination/coronavirus-vaccine/). You must also have had booster vaccinations if these are required. The latest vaccination dose must be over 3 weeks prior to you visiting campus. You can only participate if you are able to show the researcher your vaccine status upon arrival to campus. We will send you information about how to do this through the NHS website or app.

B. Main study measures and exercise programme

If you meet our eligibility criteria, we can invite you to participate in our main study. The main study consists of multiple testing sessions which we will schedule when it suits you. Completing all questionnaires, computer tests and the fitness and physical function tests, will take around 8 hours, divided across at least four days - both before and after a six-month physical exercise programme.

You will be asked to do a couple of computer tasks. For example we will give you words and ask you to make up sentences using the words, or show you pictures of objects and ask you to name the objects, or repeat numbers or letters.

You will also be invited to complete an exercise fitness test to determine your current aerobic capacity (i.e., VO2max). This is a progressive walking exercise test that involves walking on a treadmill where the incline of the treadmill is increased in stages (e.g. every 4 minutes) until you cannot keep going. The actual incremental test should last no more than 20 minutes, including an active 'cool down' period. Before completing this test, we will ask you to abstain from heavy physical exercise and alcohol for 24 hours. You will also be asked not to consume food for 2 hours prior to reporting to the laboratory.

As well as this fitness test, we will ask you to complete a number of physical function tasks, such as a hand grip strength test, an 'up and go' test, as well as balancing. These tests will allow us to determine how the exercise intervention has improved your general physical function.

Before and after the intervention, we will also ask you to take part in an MRI-scanning session. For the MRI measurements you will receive an additional participant information sheet which we would like to ask you to read.

The six-month exercise programme will involve a home-based intervention. We will provide you with a range of online exercise sessions that you can complete in or around (e.g. walking in a

nearby park) your home. You will be asked to complete at least 3 sessions per week and we will provide you with a heart rate monitor and activity tracker to monitor your exercise sessions and general physical activity across this 6-month period.

If you are allocated to the control group, we will provide you with the heart rate monitor and activity tracker to monitor your normal activity levels, without providing any of the online resources to increase your physical activity patterns over this period. Allocation to the exercise versus control group is randomized, and we cannot tell you before the start of the study to which group you will be allocated. This means that you may not be allocated to partake in the exercise intervention. If you are allocated to be part of the control group then we will offer access to all intervention materials at the end of your study participation.

If you decide you would like to take part in this study we will go over what you will be doing in more detail and there will be opportunity for you to ask questions. You are encouraged to ask questions prior to and throughout your visits with us.

Do I have to take part?

Your participation in this study is completely voluntary. If you decide to take part and then later change your mind, either before you start the study, during it or afterwards, you can withdraw without giving your reasons, and, if you wish, your data will be destroyed. The deadline for withdrawal after participation to the study is one month after the last session you participated in.

You will receive £7.50 per hour for participation in all our screening and testing sessions.

If you withdraw before the experiment starts, you will not receive any compensation. If you withdraw during the experiment, compensation will be prorated by the time participated. If you withdraw after you participated, this would not affect your compensation.

What are the possible benefits of taking part?

Your contribution will help us learn more about cognitive and language processing abilities throughout the lifespan and the relationship between these and physical fitness.

What are the possible risks of taking part?

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

• Sensations of fatigue and physical exhaustion – this will be short-lived and will subside in a few minutes upon stopping exercise

• Fainting – often related to physical exhaustion and then suddenly stopping, this will be mitigated by the inclusion of a cool-down period immediately after the formal exercise test is complete to gradually bring you back to normal

• Cardiovascular event (e.g., myocardial infarction or 'heart attack') – this is a small risk, particularly for healthy individuals and because we are using a submaximal test. Anyone who may be at risk is likely to be excluded from the study after our initial screening. We will also ensure that you are warmed-up and cooled down appropriately around exercise sessions. A First Aid qualified researcher or trainer will always be present, and in the unlikely event of a medical emergency, standardised protocols will be followed to ensure appropriate medical attention provided straight away. Medical experts are located at the Queen Elizabeth Hospital. Our research facilities at the University of Birmingham are situated closely to the main hospital.

What happens to the answers I give?

The results may produce publishable research of interest to a wide scientific community and lead to new grant applications and collaborations. Your data are stored anonymously. You will not be identified in any report or publication.

Will my taking part in this study be kept confidential?

All information collected as part of this study is fully confidential. There will be a special code on all of the data provided by you. This is only used if you decide to withdraw from the study and we need to destroy your answers. Otherwise, your answers are never linked with your name. The data will not enable any individual to identify you and only those concerned with the research project will have access to the information you have provided. All information collected as part of this study will be kept in locked filing cabinets and on password protected computers. Your data will be stored for at least 10 years after the study is finished.

Who is organising the research?

This study is organised by the School of Psychology and School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham and has been approved by the University of Birmingham Ethics Committee.

What if something goes wrong? Who can I complain to?

In case you have a complaint on your treatment by a member of the research team (contact information listed below) or anything to do with the study, you can approach one of the investigators listed at the bottom of this page. In the unlikely event that you suffer injury to yourself or damage to your property as a result in taking part in this research, the University does have an insurance policy to cover harm arising as a result of the defect in the design of the study.

Who can I ask for further information about the study?

This research is financed by the Norwegian Research council and supervised by a team of Academics at the University of Agder (Professor Linda Wheeldon: [redacted] and the University of Birmingham (Dr. Katrien Segaert: [redacted] Dr. Sam Lucas: [redacted].

The study is run by our Postdoctoral researcher (Dr. Foyzul Rahman [redacted] and research assistant (name: e-mail).

Consent form

ID:	Researcher initials

☐ I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐ I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. I understand that I can withdraw my data at any time during the experiment and for the duration of one month after my completion of the study.

☐ I understand that data collected during the study will be looked at by researchers from the University of Birmingham. I give permission for these individuals to have access to my data. Upon completion of the study, the data may be placed on an appropriate repository for data-sharing and be accessed by researchers not affiliated with the University of Birmingham. I understand that all my data will be stored anonymously.

☐ I confirm that I am fully vaccinated against COVID-19 (including having received a booster), as confirmed by the Researcher through the participant's NHS records.

☐ As part of the screening procedure for this study, we will monitor your heart activity with an electrocardiogram. If we observe abnormal heart rhythms, we will send you a print-out of the electrocardiogram, so that you can take this information to your GP. For this purpose, please write your address below (BLOCK LETTERS):

☐ I agree to take part in the study.

Participant Name (BLOCK LETTERS): _____

Participant Signature: _____ Date _____

Researcher Name (BLOCK LETTERS): _____

Researcher Signature: _____ Date _____