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Arterial stiffness and brain health: investigating the impact of sex-related differences

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Résumé

Introduction: Il est bien établi que les maladies vasculaires, cérébrovasculaires et cardiovasculaires se manifestent différemment chez les hommes que chez les femmes. La rigidité artérielle (RA), un prédicteur indépendant de la maladie cardiovasculaire (MCV), a été associée à des changements de la réactivité cérébrovasculaire (RCV) et à un déclin cognitif lors du vieillissement. Plus précisément, les personnes âgées ayant une RA plus élevée présentent un déclin plus marqué au niveau des tâches exécutives. Une diminution des fonctions exécutives (FE) est également liée à une réduction de la RCV chez les personnes âgées. Cependant, il est important de noter que la relation entre la RA et la RCV est plus complexe. Certaines études montrent une diminution de la RCV associée avec une RA plus élevée, tandis que d'autres rapportent une RCV préservée avec une RA élevée. De plus, des travaux récents suggèrent que les différences de concentration en hématocrit (HCT) pourraient avoir une incidence sur les mesures de RA. Ici, nous avons étudié le rôle possible du sexe et de l'HCT sur ces relations hémodynamiques.

Méthodes: Des acquisitions ont été effectuées chez 48 adultes âgés en bonne santé (31 femmes, 63 ± 5 ans) dans un scanneur d'imagerie par résonance magnétique (IRM) 3T. Des données de marquage de spin artériel pseudo-continu utilisant des lectures à double écho ont été collectées pendant un défi d'hypercapnie (changement de CO_2 de 5mmHg, pendant deux blocs de 2 minutes). La RCV a été calculée comme étant le % de changement du signal de débit sanguin cérébral (% ΔCBF) par changement de mmHg dans le CO_2 à la fin de l'expiration. Les données de vitesse d'onde de pouls (VOP) aortique ont été acquises à l'aide d'une série de contraste de phase *cine* encodée par la vitesse durant 60 phases cardiaques avec un encodage en vélocité de 180cm/s dans le plan. La VOP dans l'arcade aortique a été calculée entre l'aorte ascendante et descendante. Les analyses statistiques ont été effectuées à l'aide de SPSS.

Résultats: Un test de modération contrôlant pour l'âge et le volume des hyperintensités de la matière blanche a révélé un effet direct significatif de la VOP sur la RCV (β = 1,630, IC à 95% [.654, 2,607), ainsi que de la VOP sur la FE (β = -. 998, IC 95% [-1,697, -,299]). Le sexe a modéré la relation entre VOP et RCV (β = -1,013, IC 95% [-1,610, -,4169]), et VOP et FE (β =

.447, IC 95% [.020, .875]). En outre, il existait un effet significatif de l'HCT sur les différences de sexe observées dans l'effet de modération (VOP * SEXE) sur la FE (β = -0,7680, SE = 0,3639, IC 95% [-1,5047, -0,0314], p = 0,0414).

Conclusion: Nos résultats indiquent que les relations entre la VOP, la RCV et la FE sont complexes et que le sexe et l'HCT modulent ces relations. L'influence des variations hormonales (p. ex. la ménopause) sur ces relations devrait être étudiée dans le futur et pourrait permettre de personnaliser les stratégies de prévention des MCV.

Mots-clés : rigidité artérielle, réactivité cérébrovasculaire, imagerie par résonance magnétique, marquage de spins artériels, cognition, maladie cardiovasculaire, sexe

Abstract

Introduction: It is well established that sex differences exist in the manifestation of vascular, cerebrovascular and cardiovascular disease. Arterial stiffness (AS), an independent predictor of cardiovascular disease (CVD), has been associated with changes in cerebrovascular reactivity (CVR) and cognitive decline in aging. Specifically, older adults with increased AS show a steeper decline on executive function (EF) tasks. Decreased EF is also linked with reduction in CVR among older adults. Interestingly, the relationship between AS and CVR is more complex, where some works show decreased CVR with increased AS, and others demonstrate preserved CVR with higher AS. In addition, recent work suggests that measurements of AS may be affected by differences in the concentration of hematocrit (HCT). Here, we investigated the possible role of sex and HCT on these hemodynamic relationships.

Methods: Acquisitions were completed in 48 healthy older adults (31 females, 63 ± 5 years) on a 3T MRI. Pseudo-continuous arterial spin labeling using dual-echo readouts were collected during a hypercapnia challenge (5mmHg CO2 change, during two, 2 min blocks). CVR was calculated as the %ΔCBF signal per mmHg change in end-tidal CO2. Aortic PWV data was acquired using a cine phase contrast velocity encoded series during 60 cardiac phases with a velocity encoding of 180cm/s through plane. PWV in the aortic arch was computed between ascending and descending aorta. Statistical analyses were done using SPSS.

Results: A moderation model test controlling for age and white matter hyperintensity volume revealed a significant direct effect of PWV on CVR (β=1.630, 95% CI [.654, 2.607), as well as PWV on EF (β=-.998, 95% CI [-1.697, -.299]). Sex moderated the relationship between PWV and CVR (β=-1.013, 95% CI [-1.610, -.4169]), and PWV and EF (β=.447, 95% CI [.020, .875]). In addition, there was a significant effect of HCT on the sex differences observed in the moderation effect (PWV*SEX) on EF (β=-0.7680, SE = 0.3639 ,95% CI [-1.5047, -0.0314], p=0.0414).

Conclusion: Together, our results indicate that the relationships between PWV, CVR and EF is complex and in part mediated by sex and HCT

. Future work should investigate the role of hormone variations (e.g., menopause) on these relationships to better personalize CVD prevention strategies.

Keywords : arterial stiffness, cerebrovascular reactivity, magnetic resonance imaging, arterial spin labeling, cognition, cardiovascular disease, sex

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List of Abbreviations

MRI:	Magnetic resonance imaging
WMH:	White matter hyperintensities
DTI:	Diffusion tensor imaging
FA:	Fractional anisotropy
RD:	Radial diffusivity
ASL:	Arterial spin labeling
AS:	Arterial stiffness
PWV:	Pulse wave velocity
cfPWV:	Carotid-femoral pulse wave velocity
GMV:	Grey matter volume
FLAIR:	Fluid-attenuated inversion recovery
MT:	Magnetization transfer
NODDI:	Neurite density and orientation dispersion
CHARMED:	Composite hindered and restricted model of diffusion
BOLD:	Blood-oxygen-level-dependent
fMRI:	Functional magnetic resonance imaging
MEG:	Magnetoencephalography
ADNI:	Alzheimer's disease neuroimaging initiative
TEdDI:	TE dependent diffusion imaging
MMSE:	Mini-mental state examination
MTL:	Medial temporal lobe
WML:	White matter lesion

CVR:	Cerebrovascular reactivity
CVD:	Cardiovascular disease
HCT:	Hematocrit
CSF:	Cerebrospinal fluid
VBM:	Voxel-based morphometry
PP:	Pulse pressure
CBF:	Cerebral blood flow
EF:	Executive function
PET:	Positron-emission tomography
TCD:	Transcranial doppler
PS:	Processing speed
MMSE:	Mini-mental state examination
CWIT:	Color-word interference test
TMT-B:	Trail making test part B
SNR:	Signal-to-noise ratio
pCASL:	Pseudo-continuous arterial spin labeling
HRT:	Hormone replacement therapy
RF:	Radiofrequency
CO_2:	Carbon dioxide
$EtCO_2$:	End-tidal carbon dioxide
$CMRO_2$:	Cerebral metabolic rate of oxygen
OEF:	Oxygen extraction fraction
CBV:	Cerebral blood volume
NO:	Nitric Oxide

Dedication

This thesis is dedicated to my mother, my father and my brother, for their continuous support, love and patience. This work would not have been possible without you by my side.

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I would like to take this opportunity to thank my supervisors Dr. Claudine Gauthier and Dr. Louis Bherer, for their guidance and mentorship throughout the years. I am extremely privileged and grateful you have given me the chance to be a graduate student in both of your labs. With both your expertise and dedication to your students, I have grown both professionally and personally. You have gone above and beyond the basic requirements of any research supervisor and have made this unlikely dream turn into a reality. I would also like to thank my committee members for accepting to evaluate this thesis.

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Chapter 1

Introduction

1.1 Imaging Modalities

Aging is associated with an increasing incidence of cerebrovascular, cardiovascular and neurodegenerative diseases (Niccoli and Partridge 2012; Harman 1990; Najjar et al. 2005). Recent advances in neuroimaging techniques have now begun to improve our understanding of the effects of aging on the brain, and how this is linked to the pathogenesis of diseases of aging. Age-related cerebrovascular alterations have been reported with the use of arterial spin labeling (ASL), a magnetic resonance imaging based-technique used for quantifying local perfusion in the brain (Leoni et al. 2017; Badji et al. 2019; Gauthier et al. 2013; Grade et al. 2015). Noninvasive ASL techniques directly estimates cerebral blood flow (CBF), the rate of delivery of arterial blood to the capillary bed in brain tissue, by applying a magnetic tag to inflowing arterial blood at the carotid level and measuring the delivery of tagged blood into each imaging voxel (Zhang et al. 2017). The magnetic tag is applied by inverting the magnetization of blood water using radiofrequency (RF) pulses that are similar to those used in the MR imaging process (Leoni et al. 2017; Williams; Intzandt et al. 2019; Grade et al. 2015). In addition, blood oxygenation level dependent (BOLD) MR imaging has been used in the aging literature as a common means of observing changes in the functional state of the brain (D'Esposito et al. 2003). This technique is based on the T2*-weighted signal, to indirectly measure flow changes by observing changes in the local concentration of oxygenated and deoxygenated hemoglobin in the brain (Golestani et al. 2016; Williams). The BOLD signal is however ambiguous in nature, as it simultaneously reflects changes in oxidative metabolism, blood flow and blood volume (Gauthier et al. 2013; Gauthier and Fan 2019). This ambiguity can be harnessed however to obtain measures of vascular health. Since the BOLD contrast is highly dependent on the local blood volume and flow, it can be used in combination with a vasoactive challenge such as breathholding or inhalation of carbon dioxide (CO_2) to map quantitative cerebrovascular reactivity (CVR), a measure of vascular elasticity in aging (Rostrup et al. 2000; Kastrup et al. 2001). Indeed, inhalation of CO_2 increases the concentration of CO_2 in the lungs, quantified by

end-tidal CO_2 ($EtCO_2$) which is at equilibrium with arterial CO_2 partial pressure in healthy people (Ibler and Lage 1984; Yezhuvath et al. 2009). Increased concentrations of CO_2 will then result in the dilation of small arteries, venous vessels, arterioles and increase CBF (Ito et al. 2003; Davis et al. 1998; Hoge et al. 1999; Yezhuvath et al. 2009). CVR is therefore an indirect measure of vasodilatory capacity as it measures the vascular response to a well-controlled vasodilation challenge. CVR is calculated as the percent change in BOLD signal per change in end-tidal partial pressure of CO_2 and expressed as %S/mm Hg.

1.2 Age-related changes in CBF and CVR

A decrease in whole brain CBF in older individuals in comparison to younger individuals has been demonstrated in several ASL studies (Ambarki et al. 2015; Amiri et al. 2014; Wagner et al. 2012). Similarly, previous work reports CBF declines at about 0.38 ~ 0.45% per year (Parkes et al. 2004; Biagi et al. 2007; Ambarki et al. 2015; Wagner et al. 2012; Vis et al. 2018; Zhang et al. 2018). Indeed, among healthy older adults, CBF reductions have been found to be more pronounced in the frontal, temporal and parietal lobes (Chen et al. 2011; Parkes et al. 2004). Moreover, aging is accompanied with impaired neuronal and glial mitochondrial metabolism (Tarumi and Zhang 2018). As such, the age related reductions in cerebral perfusion may be due to decreased cerebral metabolic rate (Marchal et al. 1992; Tarumi and Zhang 2018) and cerebrovascular dysfunction (Zhu et al. 2011; Tarumi and Zhang 2018). Indeed, age decreases the metabolic rates for oxygen and glucose by 5% per decade (Tarumi and Zhang 2018). Notably, the reductions in metabolic rate observed across the lifespan have been associated with decreases in CBF (Leenders et al. 1990; Petit-Taboué et al. 1998; Tarumi and Zhang 2018).

In addition, decreases in cerebrovascular responses to hypercapnia have been reported in the aging brain (Flück et al. 2014; Gauthier et al. 2013; Lu et al. 2011; Reich and Rusinek 1989; Yamaguchi et al. 1979; McKetton et al. 2018). For example, in a previous calibrated fMRI study using a 5mmHg hypercapnia block, it was found that older adults exhibited lower BOLD and flow responses in hypercapnia compared to younger adults (Gauthier et al. 2013). Similarly, De Vis *et al* found reduced BOLD CVR among older adults in cortical frontal, temporal and occipital grey matter, using two 10 mmHg hypercapnia blocks (Vis et al. 2015; McKetton et al. 2018). More importantly, the CVR BOLD signal is dependent on blood flow changes observed

in local T2* decay which reflects the interaction between cerebral metabolic rate of oxygen ($CMRO_2$), oxygen extraction fraction (OEF), CBF and cerebral blood volume (CBV) (Kim and Ogawa 2012). As such, a possible explanation for decreased CVR in elderly may be related to decreased $CMRO_2$, oxygen, OEF and CBF with age (Vis et al. 2015; McKetton et al. 2018). In addition, chronic dilation of cerebral vessels due to age-related inflammation can also be possible explanation for decreased CVR in the elderly. Nonetheless, CVR remains as one of the most reliable neuroimaging predictor of brain vascular health (Pillai and Mikulis 2015; Mandell et al. 2008).

1.3 Sex differences in CBF and CVR

Studies on structural brain development have shown that there are large sex differences in CBF across all ages. For instance, females show higher CBF than males during childhood, mid-puberty and mid-late adolescence (Tontisirin et al. 2007; Satterthwaite et al. 2014; Robison et al. 2019). Although previous work report a decline in CBF among box sexes during adulthood (Rodriguez et al. 1988; Robison et al. 2019), recent work suggests that females maintain a higher flow throughout adulthood. A study examining CBF among adults ranging from 18-72 years of age found that women consistently maintain a 11% increase in CBF compared to men across the lifespan (Rodriguez et al. 1988; Robison et al. 2019). This is also in line with previous work showing a 15% increase in CBF across young adulthood compared to males (Gur et al. 1982; Robison et al. 2019). Indeed, women have thinner blood than men, which allows the arterial blood to travel faster and has a shorter arterial transit time, increasing CBF (Robison et al. 2019). Furthermore, there is evidence that female sex hormones play a role in regulating CBF (Brackley et al. 1999; Diomedi et al. 2001; Robison et al. 2019). Indeed, a study examining the role of female sex hormones on cerebral hemodynamics found that post-menopausal women taking hormone replacement therapy (HRT) had increased levels of whole brain CBF compared to controls (Ohkura et al. 1995).

Sex differences have also been demonstrated in CVR using ultrasound imaging. Indeed, females exhibit greater cerebral autoregulation, the ability of the brain to maintain relatively constant CBF despite changes in blood pressure, during adolescence (Tontisirin et al. 2007; Robison et al. 2019) and older adulthood (Deegan et al. 2011). In addition, animal studies have shown that

middle cerebral arteries in female rodents are more dilated across a range of pressures compared to males. Similarly, it has been demonstrated that the response to angiotensin II, a vasoconstrictor that contributes to the development of arterial stiffness, is decreased in female human cerebral arteries in comparison to males (Ahnstedt et al. 2013). Similarily, estrogen has been associated with higher nitric oxide (NO) bioavailability and therefore lower vascular tone (Robinson et al. 2019). Taken together, these studies show strong evidence that sexual dimorphism exists in CVR.

With the overwhelming evidence that sex differences exist in the manifestation of vascular, cerebrovascular and cardiovascular diseases, a better understanding of the impact of sex on brain hemodynamics is needed to identify sex-specific preventive strategies to mitigate CVD risk. As such, this thesis first provides and overview of the current neuroimaging literature demonstrating the impact of arterial stiffness on the aging brain, followed by experimental work focusing on the impact of sex-related differences on the link between arterial stiffness, cognitive performance and cerebrovascular reactivity using magnetic resonance imaging in a group of healthy older men and women. Furthermore, the relative contribution of hematocrit on these relationships is investigated. We hypothesize that sex will moderate the relationships between pulse wave velocity and cerebrovascular reactivity and the relationship between pulse wave velocity and executive function. Furthermore, we anticipate that there will be asignificant effect of hematocrit on the sex differences observed in the moderation effects of sex on the relationships between pulse wave velocity and cerebrovascular reactivity and the relationship between pulse wave velocity and executive function

Chapter 2

Arterial Stiffness and Brain Integrity: a review of MRI findings

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2.1 Abstract

Background: Given the increasing incidence of vascular diseases and dementia, a better understanding of the cerebrovascular changes induced by arterial stiffness is important for early identification of white and grey matter abnormalities that might antedate the appearance of clinical cognitive symptoms. Here, we review the evidence from neuroimaging demonstrating the impact of arterial stiffness on the aging brain.

Method: This review presents findings from recent studies examining the association between arterial stiffness, cognitive function, cerebral hypoperfusion, and markers of neuronal fiber integrity using a variety of MRI techniques.

Results: Overall, changes associated with arterial stiffness indicates that the corpus callosum, the internal capsule and the corona radiata may be the most vulnerable regions to microvascular damage. Changes in gray matter structure have also been found to be associated with arterial stiffness and are present as early as the 4th decade. Moreover, decrease in cerebral perfusion has been associated with arterial stiffness as well as lower cognitive performance in age sensitive domains such as executive function.

Conclusion: Considering the established relationship between arterial stiffness, brain and cognition, this review highlights the need for future studies of brain structure and function in aging to implement measurements of arterial stiffness in parallel with quantitative imaging.

Keywords = arterial stiffness, diffusion, white matter microstructure, arterial spin labelling, cognition, cerebral blood flow

2.2 Introduction

Several cerebrovascular risk factors, such as hypertension and arterial stiffness have been associated with the pathogenesis of cognitive decline and dementia, in particular Alzheimer's disease and vascular dementia (Hanon et al., 2005; Henskens et al., 2008; Singer et al., 2014). Among those cerebrovascular risk factors, arterial stiffness is a common condition that arises with aging (Najjar et al., 2005) *figure 1* and is apparent as a 2-fold increase in aortic pulse wave velocity (PWV) in healthy individuals between the ages of 20 and 80 years (Vlachopoulos et al., 2011). Arterial stiffness refers to the loss of elasticity mainly in large arteries such as the aorta and carotids. The elasticity of large arteries allows the dampening of the arterial pressure waveform, transforming the pulsatile flow at the heart level into steady blood flow in the micro-vessels (Iulita et al., 2018; Scuteri et al., 2011). Consequently, arterial stiffening increases flow pulsations through the carotid and vertebral arteries which eventually extend deep into the microvasculature of the brain and may result in haemorrhages, endothelial denudation and thrombotic obstruction (Henskens et al., 2008; O'rourke and Hashimoto, 2007). Moreover, arterial stiffening elevates arterial pressure wave propagation, causing the reflected arterial wave to arrive back at the aorta during the systolic rather than the diastolic phase of the cardiac cycle (Laurent et al., 2005; Pase et al., 2012; Vlachopoulos et al., 2011), thereby escalating systolic blood pressure and contributing to a widening of pulse pressure (Pase et al., 2012).

Chronically elevated arterial stiffness and pulse pressure transmitted into the brain are known to contribute to cerebrovascular changes such as cerebral white matter parenchymal damage via an alteration of cerebral blood flow (Iulita et al., 2018; Mitchell, 2008; O'Rourke and Safar, 2005). In particular, areas perfused by arterioles supplied by the anterior and middle cerebral arteries are more vulnerable to cerebral hypoperfusion because of their geographic localization within areas with few interconnections (Badji et al., 2018; Rosano et al., 2013; Tarumi et al., 2015). For instance, it has been shown that abnormal elevations in central and cerebral pulsatility promote the development of white matter hyperintensities (WMHs), a marker of white matter degradation reflecting small vessel diseases (Singer et al., 2014). However, despite the acknowledged importance of arterial stiffness in the genesis of WMHs, little is known about the microstructural correlates of arterial stiffness. In addition, arterial stiffness has been also shown

to impact gray matter structure as well as cerebral perfusion, which in turn can impact cognitive function (Tarumi et al., 2011; Tarumi and Zhang, 2017).

Knowing how arterial stiffness is implicated in the pathogenesis of cognitive impairment lays the groundwork for devising better strategies to prevent cognitive decline. Thus, a better understanding of the cerebrovascular changes induced by arterial stiffness is important for early identification of the white and gray matter abnormalities that might antedate the appearance of clinical cognitive symptoms. Advances in neuroimaging techniques have now begun to improve our knowledge of the effects of arterial stiffness on the brain (Badji et al., 2018; Maillard et al., 2017; Tarumi et al., 2015, 2011; Tarumi and Zhang, 2017). For instance, diffusion tensor imaging (DTI) is particularly useful for the investigation of microstructural changes in white neuronal fiber tracts by means of semi-quantitative metrics such as Fractional anisotropy (FA) and Radial diffusivity (RD) (Mori and Zhang, 2006), while ASL can be used to estimate cerebral perfusion and cerebrovascular reactivity, a measure of vascular brain health in grey matter. Understanding the relationship between arterial stiffness and advanced neuroimaging markers such as DTI and ASL can help identify biomarkers of subclinical brain abnormalities which will in turn have tremendous public health implications considering the established relationship between these markers and the prognostic factors of cognitive decline and dementia (Dufouil et al., 2009; Pantoni, 2002; Pantoni et al., 2007; Rosano et al., 2005; Sachdev et al., 2005).

In this review, we analyze the latest MR literature using advanced neuroimaging techniques to investigate the impact of arterial stiffness in the white and gray matter of the brain. We summarize their main findings as well as bridge the gap between arterial stiffness, cerebral blood flow and microstructural integrity. Finally, we close this review with an overview of the pathological problem involving arterial stiffness, cognitive impairment and cutting-edge neuroimaging.

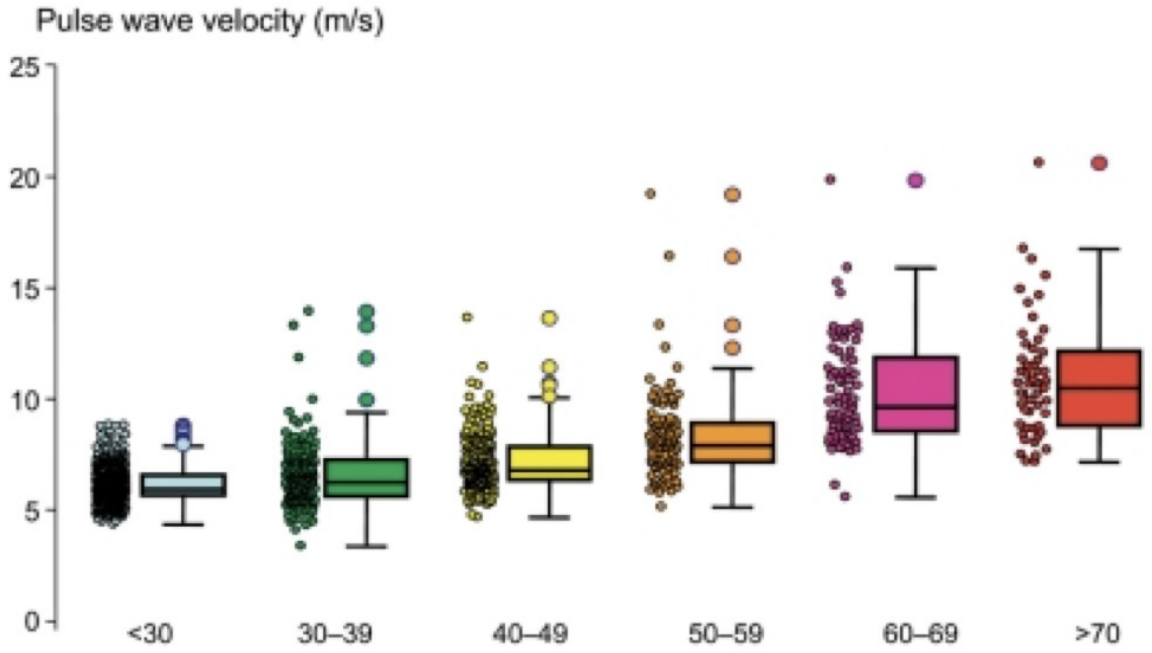

Figure 1 - Normal values for pulse wave velocity: average according to age (1455 subjects).

NB: Boxes contain 50% of the data and bars contain the remainder, horizontal lines indicate medians and the circle indicates outliers.

2.3 Arterial stiffness measurements

An extensive overview of all means to measure arterial stiffness and recommendation for their clinical application has been published by the European Network for Noninvasive investigation of Large Arteries (Laurent et al., 2006) and by an American Heart association panel (Townsend et al., 2015). Among all type of measurements, the methods used to establish a correlation with cognitive performance have been recently highlighted in the special issue on Vascular Dementia in the Journal of Neurochemistry (Iulita et al., 2018). The carotid-femoral pulse wave velocity (cfPWV) is currently the gold standard measure for central arterial stiffness. cfPWV reflects the time taken for the transmission of the arterial pulse wave from the carotid to the femoral artery andcan be assessed by the non-invasive procedure of applanation tonometry, following the Van Bortel protocol (Van Bortel et al., 2012). Briefly, cfPWV is measured twice by dividing arterial pulse traveling distance by the transit time and expressed in meters per second. The arterial pulse

traveling distance is measured as the straight distance between the carotid and femoral measurement sites using a tape ruler whereas the transit time is determined from the time delay between proximal and distal "foot" waveforms assessed by placing a tonometer both in the carotid and the femoral artery (Millasseau et al., 2005). The cfPWV is then calculated by taking the mean of the two measurements. However, other measures of arterial stiffening have been used in the literature. In *table 1* we provide an overview of the arterial stiffness measures used in all papers we refer to in the following sections of this review.

Table 1 - Arterial stiffness measures.

Methods	How it measured	How it is computed	Advantages	Limitations	Studies
cfPWV	Aplanation tonometry	Division of the arterial pulse traveling distance (between the carotid and femoral arteries) by the transit time	Gold standard, robust measure, non-invasive, measure or central artery stiffness, highly predictive of cardiovascular risk	Difficult in obese subjects, need accurate measurement of the two points across the body surface	Schmahmann et al. 2003, Tarumi et al. 2011, Mitchel et al. 2011, Rosano et al. 2013, Tarumi et al. 2013, Tarumi et al. 2014, Tarumi et al. 2015, Maillard et al. 2016, Lilamand et al. 2016, Maillard et al. 2017, Badji et al. 2018"
Aortic PWV	Cardiac magnetic resonance	Calculated as distance traveled across the aorta (m) divided by time delay in onset of velocity waves (seconds)	Able to accurately acquire blood flow velocity, measures the aortic length inside the central aorta, noninvasive assessment of cardiovascular risk.	Longer examination duration, not feasible yet for patients with certain cardiac devices, accurate transit time determination remains a challenge, CMR has low temporal resolution	Jefferson et al.2018
baPWV	Aplanation tonometry	Division of the arterial pulse traveling distance (between the brachial and ankle arteries) by the transit time	Non-invasive, high predictive value for CV events,	Difficult in obese subjects does not purely reflect central artery stiffness	Ohmine et al. 2008
AI	Aplanation tonometry	Calculated as the augmentation pressure divided by PP*100 to give a percentage	Simple, non-invasive, strong predictor of CV risk and mortality	Difficult in obese subjects indirect measure of arterial stiffness, weak correlation with PP and cfPWV, affected by other variables such as HR	Shrestha et al. 2009,
cSBP	Aplanation tonometry	Systolic blood pressure measured in the ascending aorta	Non-invasive	Difficult in obese subject	Shrestha et al. 2009,
PP	Brachial blood pressure	The difference between the systolic and diastolic pressure	Easy to calculate, strong predictor of CV risk, non invasive	Indirect measure, may reflect atherosclerosis, not predictive of arterial stiffness in young individuals	Tarumi et al. 2017 Lilamand et al. 2016, DuBose et al. 2018
Flow velocity	Doppler sonography	The difference between peak systolic velocity and end diastolic velocity divided by the mean flow velocity	Non invasive, provide morphology information	Limited resolution, needs experienced operator, mainly used in research settings	Michel et al. 2011
Carotid distension	Carotid sonography	The change in diameter in systole relative to the diastolic diameter during the cardiac cycle	Non invasive	High variability	Tarumi et al. 2013, Jochemsen et al. 2015

N.B. : cfPWV= carotid-femoral pulse wave velocity, baPWV= brachial ankle pulse wave elocity, AI= augmentation index, cSBP= central systolic blood pressure, PP= pulse pressure, CV= cardiovascular, CMR= cardiac magnetic resonance.

2.4 Overview of histopathological characteristics of cerebral white matter and gray matter aging

As we age, the dampening of pulsatile flow becomes less efficient (O'Rourke and Hashimoto, 2007). The progressive increase in collagen coupled with the degradation and fragmentation of elastin fibers contribute to stiffer blood vessels, leading to reduced microvascular reactivity through atherogenic, hypertrophic and inflammatory responses (Iulita et al., 2018; Pase, 2012). The effects of these changes on arterial walls are amplified by the early returns of pulse wave reflection leading to an increase in arterial pulse pressure which extends into smaller vessels and impacts the integrity of the brain and kidneys (O'Rourke and Safar, 2005). The brain and kidney vulnerability to central hemodynamic alterations could be accounted by their low resistance and impedance in addition to the short distance between arterioles and large arteries (Ito, 2012; O'Rourke and Safar, 2005).

Higher aortic stiffness is also associated with lower cerebral blood flow and global cerebrovascular reserve in humans (DuBose et al., 2018; Jefferson et al., 2018). This may be critical in brain regions more vulnerable to lower perfusion such as the white matter. Indeed, small white matter lesions are (WML) often localized in watersheds regions between the tissues supplied by the anterior, posterior and middle cerebral arteries, which correlate with the presence of symptomatic cerebrovascular insufficiency (Minkner et al., 2005). In addition, the deep white matter is essentially perfused by long medullary arterioles arising from the anterior and middle cerebral artery (Brown and Thore, 2011) which become tortuous with age (Brown et al., 2002; Hassler, 1965; Moody et al., 1991; Thore et al., 2007). This tortuosity appears as early as the 5th decade (Akima et al., 1986) and is present in all individuals above 80 years old (Beskow et al., 1971). In addition, arteries in the white matter arise from medullary arteries, which are end arteries coming from pial arteries that penetrate the cortical layers before entering the periventricular white matter regions (Takahashi and Others, 2013). Thus, it is not surprising that vascular changes affect both the white as well as gray matter in the brain.

Although cerebral aging is a complex process associated with a high degree of inter-individual variability, structural MRI can be used to identify non-disease related aging of the cerebral white and gray matter (Gunning-Dixon et al., 2009). On MRI, there is little doubt that the brain shrinks with age and that this shrinkage accelerates after the age of 50 (Gunning-Dixon et al.,

2009; Raz and Rodrigue, 2006). However, there is still a debate on whether the decline is more pronounced in white or gray matter. Indeed, several studies have shown a greater age-associated decline in white matter volume in the absence of gray matter loss (Allen et al., 2005; Bartzokis et al., 2003; Gunning-Dixon et al., 2009; Guttmann et al., 1998; Jernigan et al., 2001), whereas other studies have shown the opposite effect, with greater age-associated gray matter loss compared to the white matter (Blatter et al., 1995; Sullivan et al., 2004; Thompson et al., 2003). A number of factors could explain these conflicting results, such as the presence or absence of cardiovascular risk factors.

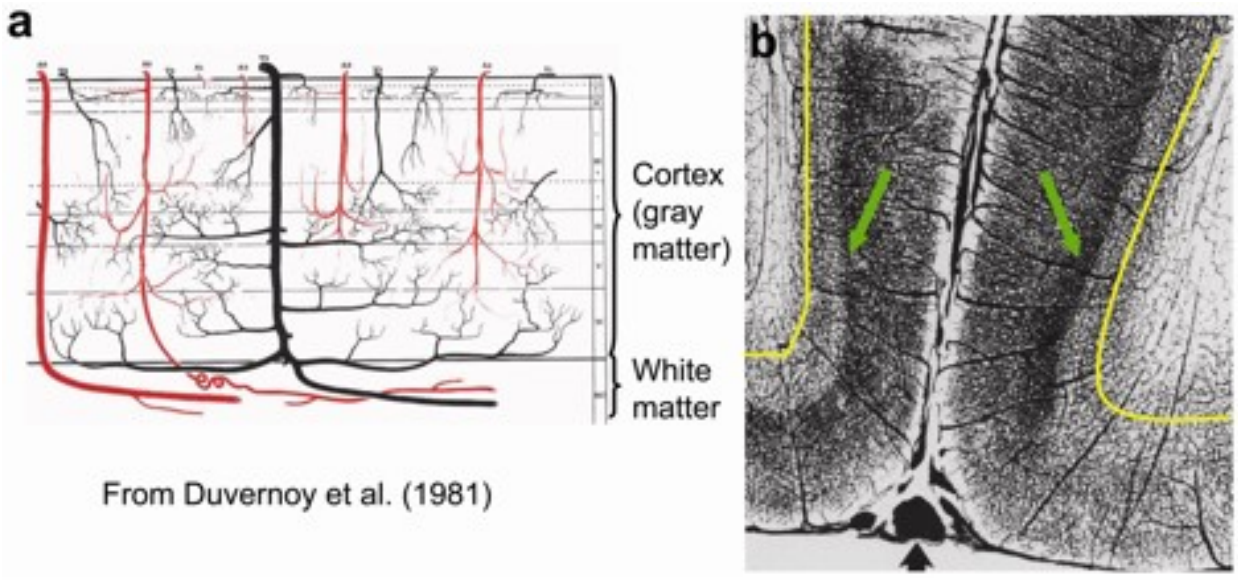

Figure 2 - Arteries and veins of the white and gray matter.

N.B.:

a) Illustration of intracortical arteries (red) and veins (blue) in relation to brain tissue. The arteries originate from the cortical surface. Some arteries penetrate the gray matter and perfuse white matter exclusively whereas other perfuse both the gray and white matter.

b) Ink-labelled vascular network in striate cortex illustrating reduced vasculature from gray to white matter. Layer IV of the gray matter has the most intensive vascular density (green arrows). Gray and white matter boundary is indicated by yellow lines.

2.5 Macro and Microstructural changes of the cerebral white matter related to arterial stiffness

Our understanding of the vascular pathogenesis of cognitive decline has improved in recent years. Arterial stiffness has previously been found to be associated with several neuroimaging features of small vessel disease such as silent cerebral infarcts (Tsao et al., 2013), lacunes (Hatanaka et al., 2011), microbleeds (Ochi et al., 2010) and WMHs , as assessed by T2-weighted or fluid-attenuated inversion recovery (FLAIR) images (Singer et al., 2014). For instance, Rosano *et al*. found that arterial stiffness, as assessed by cfPWV, is associated with WMH volume in the left superior longitudinal fasciculus in older adults (Rosano et al., 2013). This association remained significant after adjustment for blood pressure, the presence of diabetes mellitus and other markers of vascular conditions, suggesting that arterial stiffness is an independent predictor of white matter abnormalities. Moreover, Shrestha *et al*. reported an association between higher arterial stiffness and WMHs in periventricular and deep white matter (Shrestha et al., 2009) whereas Ohmine *et al*. found an association only in periventricular areas (Ohmine et al., 2008). These conflicting results could be due to the fact that both studies used different approaches to measure arterial stiffness. Shrestha *et al*. used measures of augmentation index which is an indirect measure of arterial stiffness that could be affected by ventricular ejection and peripheral hemodynamic changes, whereas Ohmine *et al*. used brachial-ankle PWV which reflects central artery stiffness (cf table 1).

Despite discordance in the spatial distribution of WMHs in the relationship with arterial stiffness, several brain regions have been highlighted in the reviewed DTI literature to be vulnerable to hemodynamic changes and to precede the development of WMHs. These regions include the corpus callosum, the internal capsule, the corona radiata, and the superior longitudinal fasciculus (Badji et al., 2018; Maillard et al., 2017, 2016; Tarumi et al., 2015). Indeed, conventional MRI techniques such as FLAIR only dichotomize white matter tissue into abnormal and normal tissue and do not quantitatively assess the white matter microstructure (Haller et al., 2013). In contrast, DTI provides a semi-quantitative evaluation of the underlying white matter tissue alteration at the voxel level (Wardlaw et al., 2015) by measuring water diffusion in white matter tracts. Interestingly, when the diffusion of water molecules is restricted by the presence of physical barriers (e.g axonal membranes and myelin), the signal becomes

imprinted with signatures of the confining geometry. Therefore, the diffusion signal carries valuable physiological information about tissue microstructure such as axonal membranes and myelin (Mori and Zhang, 2006; Palombo et al., 2018). For instance, FA measures the anisotropy of water diffusion (e.g. close to 0 if water diffuses equally in all directions like in pure water, close to 1 if water diffuses preferentially along a single direction like along white matter axons), while RD reflects the apparent diffusion coefficient orthogonal to the principal diffusion direction axis (e.g. perpendicular to the main axon bundle). In a study looking at DTI changes within WMHs over a three-year period, Maillard *et al*. showed significant DTI changes over time in these WMHs, but no change using FLAIR imaging (Maillard et al., 2014). This could indicate that microstructural white matter changes precede the development of WMHs visible in FLAIR imaging. In a more recent study, with 18 cognitively normal and 36 mild-cognitively impaired participants (55-80 years) showing no significant difference in DTI metrics, Tarumi *et al*. reported not only an association between PWV and WMHs but also an association between higher cfPWV, with lower FA and higher RD in the corpus callosum, the internal capsule, the corona radiata, and the superior longitudinal fasciculus (Tarumi et al., 2015). In another larger study, Maillard *et al*. reproduced the association between cfPWV and white matter injury as assessed by DTI in the corpus callosum, internal capsule and corona radiata (Maillard et al., 2016) in 903 participants from the Framingham Heart Study Third Generation (mean age, 48 ±8.7 years). Such associations were found to be accentuated by age and attenuated by antihypertensive treatment, and were present as early as the fifth decade (Maillard et al., 2016).

Recently, Maillard *et al*. investigated for the first time the potential of Free water imaging as a biomarker of subtle cerebrovascular injury. Free water reflects the fraction of water molecules that diffuse equally in all directions of space (an equivalent of FA=0). Using mediation analysis, Maillard *et al*. shed light on a potential pathophysiological cascade triggered by arterial stiffness and elevated blood pressure. Arterial stiffness and elevated blood pressure were associated with increased Free water content. Moreover, Free water had a direct effect on WMHs, but this effect was mediated by FA (Maillard et al., 2017). Interestingly, Tarumi *et al*. and Maillard *et al*. formulated two hypotheses: The first one is that alterations in white matter fiber tracts related to increased arterial stiffness are due to axonal demyelination (Maillard et al., 2017; Tarumi et al., 2015) and the second one is that the increase of free water following arterial stiffening may

result in axonal dispersion, lessening the constraint of water directionality along axons, as reflected by FA (Maillard et al., 2017). However, both groups used diffusion imaging for their experiment, which does not provide a specific measure of myelin integrity. Indeed, with an image resolution on the order of millimetres, diffusion MRI conflates information not only from thousands of neurons but also from various cell types such as myelin-producing cells (e.g. oligodendrocytes). Since the diffusion MRI signal averages, the contributions emerging from all compartments, the signal might reflect a number of physiological changes including demyelination (Assaf and Pasternak, 2008). Therefore, diffusion imaging provides only an indirect way to measure demyelination (Song et al., 2005), as this technique can be affected by confounding factors such as tract architecture and axon degeneration (Wheeler-Kingshott and Cercignani, 2009). As such, Tarumi *et al.* and Maillard *et al*. were not able to test their hypotheses.

In contrast, other MRI contrasts such as magnetization transfer (MT) imaging sensitize the MR signal to hydrogen atoms bound to macromolecules and thus provide a means to estimate myelin volume fraction (Edzes and Samulski, 1977; Kucharczyk et al., 1994; Wolff and Balaban, 1989). By using DTI and MT to assess axonal integrity and myelination of nerve fibers respectively, Badji *et al*. showed that arterial stiffness as assessed by cfPWV was associated with axonal degeneration rather than demyelination (Badji et al., 2018). Furthermore, despite their sensitivity to tissue microstructure, DTI metrics lack specificity for individual tissue microstructure feature (Zhang et al., 2012). Indeed, it can be hypothesized that the observation of a reduction in FA may be due to various microstructural tissue changes such as a reduction in neurite density or an increase in the dispersion of neurite orientation distribution (Beaulieu, 2009). In contrast, newer diffusion MRI techniques such as orientation dispersion and density imaging (NODDI) based on multi-shell protocol provide a model of white matter microstructure that consists of individual compartments for glial cells, axons and extracellular space that can be used to infer information about neurite density and orientation dispersion (Zhang et al., 2012).

Although NODDI has not been used yet to investigate the effects of arterial stiffness in the brain, Suzuki *et al*. recently compared NODDI metrics in hypertensive vs non-hypertensive participants and found a difference in both neurite density and orientation dispersion (Suzuki et

al., 2017). Moreover, Suzuki *et al*. also compared NODDI metrics between non-hypertensive and pre-hypertensive participants and found a higher orientation dispersion in the right superior longitudinal fasciculus and the right superior thalamic region. However, Suzuki *et al*. did not control for arterial stiffness, which may be the underlying parameter leading to alterations in white matter microstructure. This suggests that changes in orientation dispersion might be the earliest microstructural alterations related to hemodynamic changes. Further studies using the gold standard measure of arterial stiffness might need to rely on more advanced models to map the white matter microstructure (e.g. NODDI) in order to help researchers better understand the impact of arterial stiffness on axonal integrity.

Despite all these encouraging avenues, in-vivo probing of tissue microstructure with MRI has its caveats. Indeed, Novikov *et al*. raised concerns regarding the distinction between a biophysical model and its relevance to an actual physical phenomenon (Novikov et al., 2018). While more work is needed in the realms of microstructural modelling to accurately probe the white matter microstructure, these new biophysical models might help us better understand the effects of arterial stiffness on the white matter microstructure. Considering the fact that microstructural changes following arterial stiffening may be reversible as opposed to WMH (Fernando et al., 2006; Maillard et al., 2014), their early detection may be an important target for biomarker discovery.

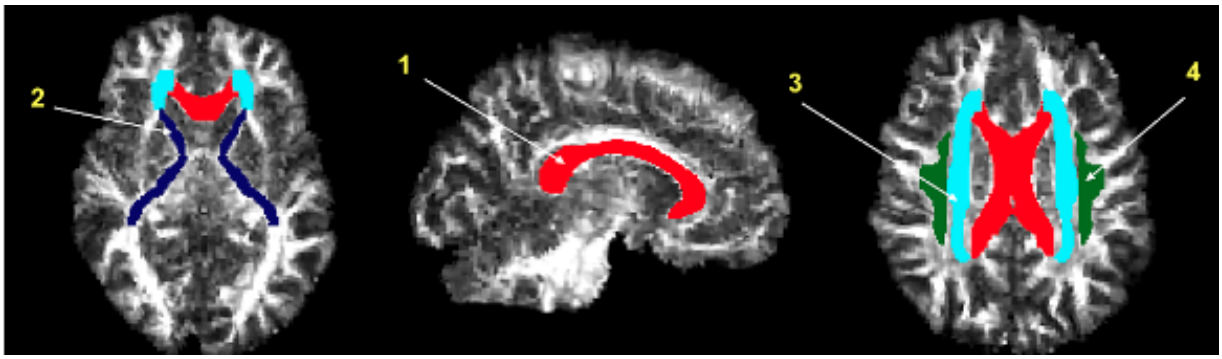

Figure 3 - White matter regions denoted vulnerable to arterial stiffness.

N.B.:

White matter regions based on the ICBM-DTI-81 atlas are overlaid on an FA map of one healthy older adult. 1: corpus callosum, 2: internal capsule, 3: corona radiata, 4: superior longitudinal fasciculus. These white matter regions have been identified as vulnerable to

arterial stiffness based on the reviewed literature. This means that these regions are more susceptible to microstructural changes following an increased arterial stiffness in the elderly.

2.6 Macro and Microstructural changes of the cerebral gray matter related to arterial stiffness

Several cross-sectional studies have shown a link between increased arterial stiffness and white matter changes in the brain. However, limited research has attempted to understand the impact of arterial stiffness on gray matter structure. Gray matter changes following arterial aging can be assessed using T1-weighted MRI and manifest essentially as reduced gray matter volume (GMV) or density. Schmahmann *et al*. and Maillard *et al.* found a significant association between higher PWV and lower gray matter density in young adults, particularly in the bilateral thalamic regions (Maillard et al., 2016; Schmahmann, 2003). This association was found to be accentuated by age and attenuated by antihypertensive treatment (Maillard et al., 2016). In addition, Maillard *et al.* found that these thalamic structural brain changes were present as early as the 5th decade (Maillard et al., 2016). By contrast, no relationship was found between higher cfPWV and GMV among older adults, in the Reykjavik Study (Mitchell et al., 2011). However, the authors looked also at the impact of carotid flow pulsatility on brain integrity using ultrasonography, and found that higher carotid pulsatility was associated with lower GMV(Mitchell et al., 2011). This result is consistent with the important role of carotid stiffness on brain health and the idea that excessive pulsatility following arterial stiffening leads to microvascular dysfunction and alteration in the brain (Mitchell et al., 2011). This hypothesis is further supported by work from Jochemsen *et al.* examining the associations between carotid distension, total brain volume, WML volume, and cortical volume among 526 older participants (Jochemsen et al., 2015). Jochemsen *et al*. found that lower carotid distension was related to a lower brain volume (both total and cortical) and a larger WML volume. However, after a mean follow-up of 4.1 years (in average), further stiffening of the carotid arteries did not lead to changes in total GMV or WML volume (Jochemsen et al., 2015). Although this is the first study to examine the relationship between arterial stiffness, the progression of brain atrophy and WML volume at the same time longitudinally, a potential explanation for these findings could be that changes over 4 years are modest compared to changes that occur across the lifespan to

older age. The authors further argue that arterial stiffness was mild in their population compared to other older cohorts and may not have been severe enough to lead to brain pathology (Jochemsen et al., 2015). In contrast, a previous study found that increased aortic stiffness was associated with larger WML volume in an older population 10 years later but the authors did not report on change in WML volume over time (Rosano et al. 2013).

However, it is still unclear what the local changes in T1-weighted signal intensity reflect at the microscopic scale (Tardif et al., 2016). Considering the fact that T1 is sensitive to several physiological features (e.g. myelin, fat, protein-rich fluid, melanin etc.), it is unlikely that a single cellular mechanism leads to the macroscopic brain changes detected using conventional T1-weighted images, making these images mainly qualitative and physiologically non-specific (Tardif et al., 2017, 2016). Furthermore, it has been shown that differences in grey matter volume could be caused by vasodilation or differences in blood volume (Tardif et al., 2017). This could be especially problematic in aging or diseases such as vascular conditions and dementia, where reductions in vascular density (and therefore blood volume) have been demonstrated (Montagne et al., 2016; Tardif et al., 2017). As such, GMV is biologically ambiguous and should be interpreted with caution as a marker of structural integrity (Tardif et al., 2016).

To address some of these issues, more quantitative MRI techniques have recently been used to better understand the link between arterial stiffness and gray matter integrity, in particular ASL. This technique provides a non-invasive, highly repeatable quantitative measure of human brain perfusion by manipulating the magnetic resonance signal of inflowing blood in feeding arteries before it is delivered to the capillary bed of different brain areas (Golay and Petersen, 2006). Using ASL, Tarumi *et al.* examined the association between cfPWV and regional cerebral perfusion within gray matter regions including the hippocampus, thalamus and caudate nucleus in 35 middle-age adults, but did not find evidence of significant associations (Tarumi et al., 2011). However, participants with higher cfPWV, defined as >1,090 cm/s, showed significantly lower cerebral perfusion in the hippocampus compared to participants with lower cfPWV (Tarumi et al., 2011). Considering the fact that participants from the Tarumi *et al.* study were between 40-60 years old, a potential explanation for the null results could be that changes in

gray matter structure following arterial stiffening follow a protracted time course and were thus not yet visible in this cohort (Maillard et al., 2016).

Additionally, in a more recent study, Jefferson *et al.* (Jefferson et al., 2018) quantified thoracic aortic stiffening from cardiac magnetic resonance and found reduced cerebral blood flow as assessed by ASL in cognitively normal older adults free from clinical stroke and dementia. More specifically, higher aortic PWV was related to lower frontal cerebral blood flow and higher cerebrovascular reactivity (CVR), in the frontal, temporal and occipital lobes, as well as in the whole brain (Jefferson et al., 2018). The latter suggests that aortic stiffening may be associated with cerebral hypoperfusion in the presence of preserved CVR (Jefferson et al., 2018). Interestingly, APOE-ε4 carriers were found to have higher PWV, lower cerebral blood flow in the whole brain and in the temporal lobe, as well as a higher CVR in the temporal lobe compared to non APOE-ε4 carriers (Jefferson et al., 2018). This is particularly relevant as individuals who inherit the APOE-ε4 allele have a genetic predisposition making them at higher risk of developing Alzheimer's disease (Liu et al. 2013). Moreover, the relationship between CVR and PWV was regionally specific to the temporal lobes, where Alzheimer's disease pathology first appears (Braak and Braak, 1991). Furthermore, Lilamand *et al.* looked at the association between PWV, and medial temporal lobe (MTL) atrophy in older adults (Lilamand et al., 2016). By categorizing participants of their study into three groups (no atrophy, mild atrophy, severe atrophy), the authors found that PWV was significantly associated with severe MTL atrophy (Lilamand et al., 2016), suggesting that arterial stiffness can potentially increase hypoperfusion of the MTL.

Despite these results, the relationship between cfPWV and cerebral blood flow remains unclear with some studies showing no reductions with cerebral blood flow, in particular in young individuals (Tarumi et al., 2014, 2011). Similarly, in a subset of older adults, aortic stiffness, brachial systolic blood pressure and pulse pressure were not correlated with resting global cerebral blood flow even after adjusting for age and sex (DuBose et al., 2018). While this could be indicative of a lack of association between arterial stiffness and cerebral blood flow in some populations at least, ASL sequences also suffer from some technical limitations. The contrast afforded by the subtraction of tagged images is only a fraction of a percent of the functional MRI contrast, providing a limited signal-to-noise ratio (SNR). Furthermore, the more commonly

used single delay ASL cannot measure transit times directly which further limits SNR (Golay and Petersen, 2006). As such, most ASL measurements are unable to determine whether the changes detected are true reflections of changes in flow or the result of alterations in transit times. Multi-delay implementations can, however, alleviate some of these problems (Golay and Petersen, 2006), and recommendations from a recent paper on ASL in aging and disease can help optimize parameter selection for the population studied (Alsop et al., 2015). Finally, coverage of the brain is typically incomplete, limiting the ability to draw conclusions on the entire brain. Multi-band approaches are however promising in this regard (Li et al., 2015). Taken together, it is important to note that ASL measurements should be interpreted with some caution, taking into account the methodological quality of the implementation (e.g. what post-label delay times were used, whether there are multiple delays, etc.). As such, to better understand the relationship between arterial stiffness and cerebral blood flow, longitudinal studies using the gold standard measure of arterial stiffness and multi-delay ASL are necessary.

2.7 Bridging the gap between arterial stiffness, cerebral blood flow and microstructural integrity.

As mentioned in previous sections, arterial stiffness has been consistently associated with white as well as gray matter changes in the elderly population (Gunning-Dixon et al., 2009; Singer et al., 2014; Tarumi and Zhang, 2017). Interestingly, the interplay between cerebral blood flow and white matter health have also been investigated. In general, a good agreement in the literature exists, highlighting a strong interplay between cortical blood flow and white matter microstructure (Chen et al., 2013; Tarumi and Zhang, 2017). In particular, Tarumi *et al*. have shown that participants with a higher 24h ambulatory measurement of pulse pressure had a lower diastolic cerebral blood flow, which accounted for 15-20% of the reduction in white matter fiber integrity as assessed by DTI (Tarumi et al., 2017). In addition, white matter cerebral blood flow has also been shown to be significantly associated with white matter microstructural integrity (Aslan et al., 2011; Giezendanner et al., 2016). However, conflicting results exist in the literature regarding the direction of the correlation. Aslan *et al*. showed that cerebral blood flow measured in various white matter tracts was inversely correlated with FA (Aslan et al., 2011) whereas Giezendanner *et al*. found a positive correlation between cerebral blood flow in the white matter and FA (Giezendanner et al., 2016). A potential mechanism for the observed negative

correlations found by Aslan *et al*. is that white matter cerebral blood flow may be related to the axonal diameter of white matter tract (Aslan et al., 2011). Indeed, Aslan *et al*. showed that the cerebral blood flow of these white matter tracts was negatively correlated with FA and Axial diffusivity, but was positively correlated with RD (Aslan et al., 2011). However, as mentioned previously, DTI is not well suited to disentangle the exact underlying microstructural properties that contribute to the observed relationships between anisotropy and cerebral blood flow (Beaulieu, 2002; Giezendanner et al., 2016; Miller et al., 2007). Advances in diffusion models such as NODDI (Zhang et al., 2012), CHARMED (composite hindered and restricted model of diffusion) (Assaf and Basser, 2005) or TE dependent Diffusion Imaging (TEdDI) (Veraart et al., 2018) might be of great interest to probe the nature of the complex relationship between cerebral blood flow and the underlying microstructural characteristics in the white matter.

Considering the fact that white and gray matter share a common vascular blood supply, a potential explanation underlying the mechanisms between arterial stiffness, white matter microstructural integrity and cerebral blood flow is that arterial stiffening of large arteries impacts both white and gray matter structure based on their respective cerebral blood flow. Indeed, sustained inadequate cerebral blood flow may reduce the delivery of oxygen and glucose to neuronal cells, thereby slowly initiating a pathway of progressive alteration in brain integrity, neuronal metabolism and cognitive decline (de la Torre, 1999, 1997, 1994; de la Torre and Mussivand, 1993). Studies with arterial stiffness measures (cfPWV), advanced diffusion model (NODDI, CHARMED, TEdDI etc), and measures of cerebral blood flow both in the white and gray matter (ASL) in healthy individuals are needed to test this hypothesis.

However, one should note that despite the recent advances in ASL technologies, the SNR of ASL in the white matter is known to be relatively poor due to small perfusion fraction and long transit times (Van Gelderen et al., 2008). Experimental data shows that it might be possible to measure white matter perfusion by using an appropriate tagging duration and post-labelling delay in healthy individuals (Wu et al., 2013) but a further extension of pulse labelling delay might be needed when ASL is acquired in the elderly populations and/or individuals experiencing ischemia as prolonged transit time is expected (Wu et al., 2013). Another alternative to improve the accuracy of the quantification of the white matter cerebral blood flow is the use of multiple pulse labelling delays which however requires longer scan time.

2.7 Arterial stiffness and brain integrity: Insight from other modalities

Although MRI appears to be the modality of choice to study the impact of arterial stiffness on brain integrity, other modalities such as positron emission tomography (PET), and transcranial Doppler (TCD) have been used (Jefferson et al., 2018; Zhu et al., 2013). For instance, Dubose *et al.* quantified global cerebral blood flow in 205 adults using quantitative [^{15}O] water PET to look at its relationship with cfPWV, as well as cerebrovascular reserve measured as the change in global cerebral blood flow after intravenous infusion of acetazolamide. Interestingly, Dubose *et al.* reported not only a lower global cerebral blood flow in older adults compared with younger adults but also an association between higher cfPWV and lower cerebrovascular reserve. This association remained significant after adjusting for age, sex, and mean arterial pressure. In contrast, aortic stiffness (as assessed by cfPWV) was not related to global cerebral blood flow but the authors argue that increased arterial stiffness may impair the cerebrovasculature's ability to dilate in response to a vasodilatory challenge and therefore reduce its ability to increase global cerebral blood flow in older adults (DuBose et al., 2018). In another study, Jaruchart *et al.* replicated these findings in 28 healthy sedentary young (mean age, 25±1 years) and older adults (mean age,67±1 years) using brachial-ankle PWV and TCD (Jaruchart et al., 2016) which was used to measure cerebrovascular conductance and reactivity in response to a physiological CO2 stimulus, and basal CBF. The authors reported a reduction in cerebral blood flow velocity and cerebrovascular conductance in older adults compared with younger adults. In addition, arterial stiffness was significantly and inversely associated with cerebral blood flow velocity and cerebrovascular conductance among older adults (Jaruchart et al., 2016). These findings suggest that CVR may be an important measure to implement in future studies to better understand the physiological changes underpinning the relationship between higher arterial stiffness and brain integrity. However, one must note that the TCD technique also suffers from several limitations. For instance, although this modality assesses CVR by measuring the change in cerebral blood flow velocity in the middle cerebral artery following a hypercapnic challenge, it assumes that hypercapnia does not induce a diameter change in the middle cerebral artery (Coverdale et al., 2014). As such, TCD may underestimate cerebral blood flow velocity.

Other modalities than PET and TCD and have been used to study the impact of arterial stiffness in the brain such as resting-state magnetoencephalography (MEG). For instance, Nieboer *et al*.

examined the association between carotid stiffness (as assessed by ultrasound imaging) and functional connectivity, computed from the phase lag index of resting-state magnetoencephalography (MEG) in 230 young healthy adults (mean age= 42±0.7) (Nieboer et al., 2016). The phase lag index estimated the asymmetry in the distribution of phase differences between two-time series and was assessed in six frequency bands (δ–γ)(Nieboer et al., 2016). Interestingly, carotid stiffness was found to be significantly associated with increased functional connectivity in the α2 band in men and β band in woman after adjusting for a set of covariates (participants' height, mean arterial pressure, body fat percentage and level of triglyceride to HDL-C)(Nieboer et al., 2016). Although the authors did not find a significant association between cognitive performance and functional connectivity, they suggested that the increase in connectivity following arterial stiffening might be due to: i) either the effect of a compensatory mechanism in order to maintain brain homeostasis such as the one reported in individuals with mild cognitive impairment (Bajo et al., 2012) or ii) the effect of a pathological mechanism leading to neurodegeneration (de Haan et al., 2012; Nieboer et al., 2016). Given the absence of other literature on the link between connectivity and arterial stiffness using MEG or other, MRI-based measures of connectivity (BOLD connectivity, structural connectivity etc), future longitudinal studies looking at the relationship between changes in arterial stiffness and brain connectivity are needed to confirm these hypotheses.

Taken together, other imaging modalities than MRI have provided valuable knowledge towards a better understanding of the underlying mechanisms underpinning brain changes associated with vascular aging. However, contradictory results also exist, in particular related to the relationship between PWV and CVR. Indeed, some authors have reported reductions in CVR among older adults with greater aortic stiffness using PET (DuBose et al., 2018) and TCD (Jaruchart et al., 2016) while others demonstrate preserved CVR in the presence of higher aortic PWV using ASL (Jefferson et al., 2018; Zhu et al., 2013). Although these modalities (TCD, PET, ASL) measure CVR differently, these results indicate that the relationship between PWV and CVR is highly complex. As such, additional studies using different imaging modalities should further investigate the impact of other markers of cerebral health to better understand the impact of hemodynamic changes in the aging brain.

2.8 Arterial stiffness and cognition

In general, there is a good agreement in the literature showing that higher PWV is associated with alterations in cognitive performance and cognitive decline independently of other cardiovascular risk factors (Cui et al., 2018; Iulita et al., 2018; Pase et al., 2012; Singer et al., 2014). Cross-sectional evidence suggests that greater arterial stiffness is associated with poorer performance in age-sensitive domains such as processing speed, executive skills, working memory, and episodic memory (Iulita et al., 2018; Pase et al., 2012; Singer et al., 2014). However, some discrepancies exist in the literature, mainly due to different study designs, target populations and heterogeneity of cognitive screening tools (Iulita et al., 2018). For instance, Hanon *et al.* (Hanon et al., 2005) looked at the relationship between arterial stiffness and global cognitive function by means of the Mini-Mental State Examination (MMSE) in a population of elderly adults experiencing memory loss and found PWV to be inversely correlated with MMSE. Although these results are in line with other studies (Benetos et al., 2012; Fukuhara et al., 2006; Scuteri et al., 2005; Zhong et al., 2014), the Rotterdam study provides contradictory findings (Poels et al., 2007). Indeed, this longitudinal population-based cohort study ongoing since 1990 investigates the factors that determine the occurrence of cardiovascular and neurological diseases in the elderly and did not reveal any associations between PWV and cognitive decline as assessed by the MMSE (Poels et al., 2007). However, after adjustment for cardiovascular risk factors, an association between increased PWV and poorer performance on the Stroop test was found (Poels et al., 2007). While the MMSE is a useful screening tool for advanced cognitive decline and dementia, it is a non-specific indication of the general mental state that has important limitations such as the lack of standardization and sensitivity to mild cognitive impairment (J. Larner, 2012). Thus, it should be interpreted with caution as it may not be sensitive enough to measure subtle cognitive changes. In contrast, the literature has shown associations between arterial stiffness and individualized domains of cognitive function including executive function, memory, processing speed, verbal learning, and visual-spatial function. For instance, although the Baltimore study did not find aortic stiffness to predict MMSE scores, significant associations were found between PWV and a decline in verbal learning and memory performance (Waldstein et al., 2008). In addition, Elias *et* al (Elias et al., 2009) found PWV to be associated with poorer performance in visual-spatial organization, and

memory and verbal episodic memory after adjusting for cardiovascular risk factors. These results are also strengthened with other cross-sectional studies showing progressively lower performance on processing speed and executive function in relation to increased arterial stiffness (Tarumi et al., 2013; Tsao et al., 2013; Waldstein et al., 2008).

Taken as a whole, these results suggest that arterial stiffness impacts multiple brain regions as it has been associated with a decrease in various cognitive domains. However, the pathophysiological mechanisms through which arterial stiffness impacts the brain are complex and poorly understood. A variety of neuroimaging techniques such as DTI and ASL allow us to gain some insight into the functional and structural brain changes related to arterial stiffening, and interestingly the literature has already provided evidence of a strong interplay between white matter changes and cognitive performance (Badji et al., 2018; Tarumi et al., 2015). Indeed numerous studies have reported that WMH burden correlates with poorer performance in age-sensitive domains, including executive skills and memory (Cook et al., 2004; Gunning-Dixon and Raz, 2000; Kramer et al., 2007; Pase et al., 2012; Singer et al., 2014). Moreover microstructural integrity of the regions known to be vulnerable to the effects of arterial stiffness (e.g.., corpus callosum, internal capsule, superior longitudinal fasciculus) have also been found to be associated with cognitive performance. For instance, Sasson *et al*. reported that executive function is correlated with DTI changes in the superior longitudinal fasciculus (Sasson et al., 2013) and Madden *et al.* reported that the best predictor of response time in a visual task was a higher FA in the corpus callosum for older adults and a higher FA in the internal capsule for the elderly (Madden et al., 2004). In addition, Tarumi *et al.* (Tarumi et al., 2015) and Badji *et al*. (Badji et al., 2018) showed that cognitive flexibility as assessed by the Trail making test B-A was positively correlated with FA and negatively correlated with RD in the regions known to be vulnerable to age-related hemodynamic changes, in particular the corpus callosum and the corona radiata (Badji et al., 2018). These results are not surprising considering the established relationship between higher arterial stiffness, white matter changes and cognitive decline (Singer et al., 2014). Furthermore, MRI measures of brain gray matter structure as assessed by T1-weighted have also found a strong association between GMV and cognitive function. For instance thalamic volume, which has been denoted to be vulnerable to arterial stiffness (Maillard

et al., 2016; Schmahmann, 2003), has been found to predict performance on tests of cognitive speed in healthy aging (Van Der Werf et al., 2001).

In addition, promising results have shown that measuring cerebral blood flow using ASL could also have important implications in predicting both cognitive performance and decline in cognitively normal individuals. For instance, by means of longitudinal ASL and neurocognitive testing over 18 months, Xekardaki *et al*. were able to distinguish participants with a stable cognitive function from participants with deteriorated cognitive function, whose cerebral blood flow measured by ASL resembles that of individuals with mild cognitive impairment (Xekardaki et al., 2015). In addition, in a recent study using over 7,700 brain images and tens of plasma and cerebrospinal fluid biomarkers from the Alzheimer's Disease Neuroimaging Initiative (ADNI), Iturria-Medina *et al*. have shown that vascular dysregulation is an early pathological event during disease development and advised that imaging brain hypoperfusion through cerebral blood flow quantification should be considered the primary predictive biomarker for Alzheimer's disease (Iturria-Medina et al., 2016). Other evidence gathered from the literature further highlights that imaging cerebral blood flow can be used not only to study the prognosis of cognitive decline but also predict conversion to Alzheimer's disease (Alsop et al., 2000; de la Torre, 2018; Johnson et al., 2005). As such, considering the bulk of evidence presented in this review, measuring and reducing arterial stiffness may offer a realistic chance to identify early changes to better devise interventions that can significantly delay abnormal brain function before irreversible structural damage occurs.

2.9 Conclusion

Taken together, there appears to be a strong relationship between arterial stiffness and declines both in brain structure and function. Considering the strong interplay between arterial stiffness, gray matter, and white matter changes in the aging brain, and the accumulating evidence that arterial stiffness impacts the trajectory of cognition later in life, the results reported in this review highlight the need for novel interventions to prevent or reverse arterial stiffness. Since arterial stiffness measurements are non-invasive, cheap and easily implemented, our review emphasizes the need to include arterial stiffness measurements not only in routine clinical assessments but also in future imaging studies of brain structure and function in aging.

Promising future research directions include the use of white matter microstructural models (NODDI, CHARMED, TEdDi etc) to better understand the impact of vascular changes on white matter microstructure, in particular in regions that have been consistently identified as impaired in the earliest stages of Alzheimer's type dementia (e.g. corpus callosum). Future longitudinal studies looking at the impact of vascular aging in white as well as gray matter cerebral blood flow are also needed in order to obtain a better picture of the earliest changes in cerebral blood flow following hemodynamic changes. Important technical challenges must however be overcome for these studies to become routine, such as the improvement of the quantification of the white matter cerebral blood flow. Studying changes in gray matter structure will also contribute to our understanding of how gray matter changes following vascular aging translate into cognitive impairments. One promising approach is the use of diffusion to map the gray matter myeloarchitecture (Ganepola et al., 2018). Finally, future studies should also implement multi-band ASL approaches to maximize brain coverage and detect blood flow reductions in all regions of the brain susceptible to microvascular damage.

Chapter 3

Sex moderates the relationship between aortic stiffness, cognition and cerebrovascular reactivity in healthy older adults

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3.1 Introduction

Cardiovascular diseases (CVD) are the leading cause of death worldwide in men and women, and on average, someone dies of CVD every 38 seconds resulting in 2,303 deaths per day due to CVD (Members et al. 2010). Cardiovascular risk is highly sex-dependent, as men exhibit greater incidences and prevalence than women (Petrea et al. 2009; Appelros et al. 2009). However, the risk of heart disease is often underestimated in women due to the misperception that females are protected against CVD. This could be explained, in part, by the findings of an epidemiological study indicating that premenopausal women are relatively protected from CVD when compared to age-matched men (DuPont et al. 2019). Yet, the incidence of CVD increases disproportionately in women after menopause (Coutinho 2014; Ellekjaer et al. 1997; DuPont et al. 2019), typically 7 to 10 years later, and is the most common cause of death in women over the age of 65 years (Maas et al. 2010). This calls for further understanding of the underlying mechanisms for sex differences involved in the development of cardiovascular diseases in women over the age of 65 years. The pathophysiology underlying CVD differs depending on the presence of sex hormones leading to differences in vascular properties, including differences in vascular tone (DuPont et al. 2019). Notably, estrogen is thought to have a positive effect on the inner layer of the artery wall, helping to keep blood vessels flexible, and allowing them to relax and expand to accommodate blood flow (Maas et al. 2010; Towfighi et al. 2009). Consequently, a decline in estrogen in postmenopausal women may lead to arterial stiffening and thus contribute to the increased prevalence of CVD in women in later life (Pepine et al. 2006; Orshal and Khalil 2004; Shaw et al. 2006). Moreover, recent work suggests a potential protective role of testosterone against arterial stiffness as well, specifically, low testosterone levels in male is associated with increased arterial stiffness (Kyriazis et al. 2011) and increased augmentation index, an indirect measure of stiffness (Corrigan et al. 2015).

It is well established that elevated AS is an independent predictor of CVD (Mitchell 2009). The elasticity of large arteries allows for the dampening of the arterial pressure waveform, transforming the pulsatile flow at the heart level into steady blood flow into the micro-vessels (Scuteri et al. 2011; Iulita et al. 2018; Badji et al. 2019). Unfortunately, during aging, large arteries (e.g the aorta, the carotids etc) become stiffer and show a reduced capacity to dampen the arterial pressure waveform (Badji et al. 2019). With aging, the elastic properties of blood

vessel walls are known to deteriorate (Novak 2012). In particular, the ratio between elastin and collagen changes in favor of collagen, making the vessel stiffer (Novak 2012). Considering the impact of AS on vascular health, non-invasive methods have been developed to measure it, among which pulse wave velocity (PWV) is considered to be the gold standard (Van Bortel et al. 2012).

The impact of AS differs considerably between males and females because of numerous endogenous factors such as the previously mentioned sex hormones and biochemical properties of the arteries (Rossi et al. 2011; Segers et al. 2007). For instance, it has been shown that aged male and female monkeys develop similar levels of AS but a decrease in elastin was noted only in male monkeys (Qiu et al. 2007). In addition, dyslipidemia and glucose contribute to a modest increase in arterial stiffness only in females (Kim et al. 2014). Moreover, it has been shown that the association between AS and mortality is almost two-fold higher in females compared to males (Regnault et al. 2012).

However, some of the sex-related differences observed may be in part due to differences in how sex-specific physiological factors (e.g HCT) affect the measures themselves. For example, recent work suggests that hemodynamic measures, such as PWV, may be affected by differences in the concentration of HCT. Indeed, males and females typically have different HCT levels (Yip et al. 1984; Vahlquist and Others 1950; Garn et al. 1975), and whole blood viscosity (hematocrit and plasma viscosity) has been shown to be positively correlated with certain cardiovascular disease factors and measures of vascular function (Parkhurst et al. 2012; Bonithon-Kopp et al. 1993; Levenson et al. 1987). Thus, this raises the possibility that a portion of the sex effects observed in the literature could be mediated, in part, by differences in HGB levels.

AS is also associated with downstream organ damage, especially in high-flow organs such as the brain (Pase et al. 2016; Iulita et al. 2018; Mitchell 2009; O'Rourke and Safar 2005; Badji et al. 2019). Indeed, high pulsatile flow following AS may damage cerebral microvessels, thus, leading progressively to changes in cerebral blood flow (CBF) (Singer et al. 2013; Tarumi et al. 2013; Tarumi et al. 2011). Increased stiffness is also associated with changes in cerebrovascular reactivity (CVR), defined as the ability of microvessels to increase blood flow in response to a vasodilatory stimulus (DuBose et al. 2018; Jefferson et al. 2018). It can be hypothesized that

increased stiffening of the aorta and higher pulse pressure may lead to damage in downstream vessels from the damaging effects of the pressure amplification caused by large artery stiffness. Therefore, higher artery stiffness may contribute to the impaired ability of cerebrovasculature to dilate maximally to augment cerebral blood flow (CBF) in older adults. Yet, the literature has found conflicting results, where some have reported reductions in CVR among older adults with greater aortic stiffness using positron-emission tomography (PET) and transcranial doppler (TCD) (DuBose et al. 2018; Jaruchart et al. 2016), while others demonstrate preserved CVR in the presence of higher aortic PWV (Jefferson et al. 2018; Zhu et al. 2013) using arterial spin labeling (ASL), an MRI technique for noninvasive quantification of CBF. Because these imaging modalities (TCD, PET, ASL) are sensitive to CVR arising from different vessel sizes, these results indicate that the relationship between PWV and CVR is complex. With the evidence that sex differences exist in the manifestation of AS, it is unclear if the conflicting results could partly be due to sex-specific characteristics, especially given the known impact of estrogen on vascular elasticity, tone and nitric oxide signaling.

Another consequence of a stiffer vascular network is a change in cognitive function (Singer et al. 2014). There is accumulating evidence from cross-sectional studies that AS is associated with the pathogenesis of cognitive decline in both males and females (Singer et al. 2014; Elias et al. 2009; Fukuhara et al. 2006) in age-sensitive domains such as processing speed (PS), verbal memory, and executive functions (EF) (Poels et al. 2007; Watson et al. 2011). Interestingly, associations between PWV, impaired CVR and severity of dementia have also been established (Silvestrini et al. 2006). Indeed, reduced reactivity has been linked to decreased executive functioning, memory, global cognition, and attention outcomes (Haratz et al. 2015). Interestingly, evidence for sex differences has been reported in a number of specific cognitive domains (Halpern and LaMay 2000). Yet, no study to date has investigated the role of sex on the relationship between cognition and neuroimaging markers of brain hemodynamics. Thus, the purpose of this study is to clarify the impact of sex-related differences on the link between PWV, cognitive performance and CVR and the relative contribution of HCT on these relationships.

3.2 Methods

3.2.1 Participants

Fifty-four healthy older adults (17 males, mean age 63 ± 5 years) were recruited for this study and completed a magnetic resonance imaging (MRI) session. Participants were recruited through a participant database at the Centre de recherche de l'Institut universitaire de gériatrie de Montréal and from Laboratoire D'Etude de la Santé cognitive des Ainés. Inclusion criteria for participation included being in the age range of 55 to 75 years, approval by a geriatrician to participate, non-smoker, no evidence of cognitive impairment as determined through cognitive tests conducted by a neuropsychologist, and MRI compatibility. Exclusion criteria included individuals taking prescription medication known to be vasoactive (e.g. hypertension, statins, etc.), presence of cardiac disease, hypertension (including use of anti-hypertensive medication), neurological or psychiatric illnesses, diabetes, asthma, thyroid disorders, smoking within the last 5 years, or excessive drinking (more than two drinks per day). In addition, the Mini Mental Status Examination (MMSE) was administered, a global cognitive screening tool used for dementia. Participants with scores less than 26 (out of 30) were excluded (Kurlowicz and Wallace 1999; Gauthier et al. 2015). All procedures were approved by Comité mixte d'éthique de la recherche du Regroupement Neuroimagerie/Québec and were conducted according to the Declaration of Helsinki. All participants provided written informed consent. From all participants that were recruited, a total of 48 older adults (17 males, mean age 63 ± 5 years) were included in the analysis, 6 were excluded as they were deemed outliers since they were more than 2.5 standard deviations above or below the mean of PWV values.

3.2.2 Cognitive Composite Score

Cognitive functioning was assessed with a comprehensive neuropsychological battery consisting of the following cognitive tests: Similarities, Digit Span Backwards, Digit Span forward, Digit Symbol, Color-word interference test (CWIT), and Trail Making Tests, parts A and B (Gauthier et al. 2013; Intzandt et al. 2019).

A composite score for executive function (EF) was calculated using four cognitive tests from the neuropsychological battery that included the CWIT Inhibition and switching conditions, trail making test part B and the digit span backwards. The trail making test part B (TMT-B), the CWIT inhibition condition and the CWIT switching condition, was timed in seconds where a

low score (faster response) indicates better functioning. The digit span backwards was calculated as the number of successful trials where a higher score indicates better EF.

Individual raw scores for each test were transformed into z-scores. The scores that were response time were multiplied by -1 to reflect poor performance. Indeed, a higher composite score indicates better cognitive functioning. Cronbach's alpha was used as a test of reliability to look at the internal consistency for the group of variables. A Cronbach alpha of 0.789 was computed for executive function showing good internal consistency.

3.2.3 Hypercapnia

As previously described (Gauthier et al. 2015; Gauthier et al. 2013; Intzandt et al. 2019), the hypercapnic manipulation was completed with a computer-controlled gas system with a sequential gas delivery circuit (Respiract™, Thornhill Research Inc., Toronto, Canada). The hypercapnic manipulation consisted of two, 2-minute blocks of hypercapnia, with 2 minutes of air before and after each hypercapnia block. End-tidal partial pressure of CO2 (ETCO2) was targeted at 40mmHg at baseline and 45 mmHg during the hypercapnia blocks. End-tidal partial pressure of O2 (ETO2) was targeted to be 100 mmHg throughout the experiment. Participants breathed through a soft plastic mask that was firmly placed on their face with adhesive tape (Tegaderm 3M Healthcare, St. Paul MN) to ensure that no leaks were present. Participants completed the breathing manipulation once prior to being in the scanner to ensure comfort levels, and once during the MRI session.

3.2.4 MRI Acquisition

All acquisitions were completed on a Siemens TIM Trio 3T MRI system (Siemens Medical Solutions, Erlangen, Germany). A 32-channel vendor-supplied head coil was used for all acquisitions. An anatomical $1mm^3$ MPRAGE acquisition (TR/TE/flip angle = 300ms/3ms/90°, 256x240 matrix) was acquired for the registration process from native to standard space, and to measure grey matter volume. A fluid attenuation inversion recovery (FLAIR) acquisition with the parameters: TR/TE/flip angle 9000 ms/107 ms/120° and an echo train length of 15, an inversion time of 2500 ms, 512 x 512 matrix for an in-plane resolution of 0.43 x 0.43 mm and 25 slices of 4.8 mm was used to estimate the presence and severity of white-matter hyperintensities. In addition, a pseudo-continuous arterial spin labeling (pCASL) acquisition

was acquired, providing simultaneous BOLD contrast using dual-echo readouts (TR/TE1/TE2/flip angle = 2000ms/10ms/30ms/90°) with 4x4x7mm voxels, 64 x 64 matrix and 11 slices, post-label delay = 900ms, tag duration=1.5s, and a 100mm gap during a hypercapnia challenge (5mmHg end-tidal CO2 change, iso-oxic during two, 2min blocks).

3.2.5 Aortic Exam

As previously described (Gauthier et al. 2015; Intzandt et al. 2019), during the MRI session a thoracic aortic exam was also acquired using simultaneous brachial pressure recording (Model 53,000, Welch Allyn, Skaneateles Falls, NY USA) using a 24- element spine matrix coil. Black blood turbo spin echo sagittal oblique images were acquired to visualize the aortic arch (TR/TE/flip angle: 700 ms/6.5 ms/ 180, 1.4 x 1.4 mm in-plane resolution, 2 slices at 7.0 mm). A perpendicular plane to the ascending and descending aorta was defined from these images. A cine phase-contrast velocity encoded series was collected (TR/TE/flip angle: 28.6ms/1.99ms/30, 1.5 x 1.5 mm x 5.5 mm) during 60 cardiac cycles in three segments, with velocity encoding of 180 cm/s through plane. A series of cine FLASH images were acquired within the same plane with the following parameters: TR/TE/flip angle: 59ms/3.44ms/15, with 1.2 x 1.2 in-plane resolution and a single slice of 6mm, 60 cardiac phases, acquired in 8 segments.

3.2.6 Data Analysis

Preprocessing of TI-weighted MPRAGE images were done using voxel based morphometry (VBM) in SPM's Computational Anatomy Toolbox (CAT) 12 (Penny et al. 2011; Ashburner and Friston 2000; Gaser 2016) to segment grey matter, white matter and cerebrospinal fluid (CSF). The registration matrix from T1 space to MNI space was calculated as part of the VBM pipeline Co-registration of native CVR data was done using a non-linear rigid registration with ANTS (Avants et al. 2008) with a b-spline interpolation to bring them from native to individual T1 space. CAT12 was then used to register from T1 to standard space using a Gaussian smoothing kernel of 8mm and a non-linear registration with 12 degrees of freedom as previously described. (Intzandt et al. 2019).

3.2.7 Resting CBF Analysis

Resting CBF was calculated as previously described (Intzandt et al. 2019). CSF masks were created individually for each older adult to use as a CSF M0 for CBF quantification. 10 voxels

were manually chosen in the same axial slice for each participant, within the lateral ventricles. The M0 was then estimated from the control time series and estimated using a monoexponential recovery with a T1 value of 1.65s. Due to varying anatomical structures, each CSF mask was visually inspected to ensure that the region of interest was located in the ventricles. The Bayesian inference for arterial spin labeling MRI toolbox (BASIL) was used for CBF quantification with the following parameters: labeling: cASL/pcASL; bolus duration: constant (1.5s), post label delay: 0.9s; calibration image: average of the control images; reference tissue type: CSF; mask: CSF mask for each participant; CSF TI: 4.3s; TE:10ms; T2: 750ms; blood T2: 150 ms; arterial transit time: 1.3s, T1:1.3s, TI blood: 1.65s, inversion efficiency: 0.85 (Intzandt et al. 2019)

3.2.8 CVR Analysis

CBF-CVR was processed using Neurolens2 (www.neurolens.org) (Gauthier et al. 2013; Intzandt et al. 2019). Preprocessing of all raw images included motion correction and spatial smoothing using a 6mm Gaussian kernel. The CBF signal was isolated from the first series of echoes using linear surround subtraction (Liu and Wong 2005; Gauthier and Hoge 2012; Gauthier et al. 2012; Intzandt et al. 2019). The CBF fractional change during hypercapnia was obtained by fitting a general linear model to the CBF signal and dividing the estimated effect size by the estimated constant term. Glover's parameters (1999) (Glover 1999) for a single-gamma hemodynamic response function were used when fitting the linear models, which included linear, quadratic, and third order polynomials representing baseline signal and drifts. The CBF percent change obtained was then divided by the average end-tidal CO2 change during the hypercapnia manipulation for each participant to yield CBF-CVR. The baseline CBF was then used to compute absolute values of CBF-CVR.

3.2.9 Vascular Lesion Quantification

White matter hyperintensity volume (WMH) for the whole brain was quantified semi-automatically. As previously described (Gauthier et al. 2015; Intzandt et al. 2019) visual identification on FLAIR images were completed by a single rater who was blinded to clinical information, which were then delineated using Jim image analysis package, version 6.0 (Xinapse Systems Ltd, Northants, UK). WMH volume for the whole brain was quantified using tools from the FMRIB Software Library (FSL).

3.2.10 Pulse Wave Velocity Data

The aortic data was analyzed using the ARTFUN software (Herment et al. 2010), where pulse wave velocity in the aortic arch was computed between the ascending and descending aorta from cine phase contrast images. The aortic lumen contours of the ascending and descending aorta were automatically segmented using amplitude images of cine phase contrast series where flow profiles were also estimated. PWV was calculated as described in (Gauthier et al. 2015; Intzandt et al. 2019)

3.2.11 Blood Tests

Before the MRI exam, participants underwent a blood draw. The blood samples were used to test the concentration of hemoglobin and hematocrit (Gauthier et al. 2015).

3.2.12 Statistical Analysis

Statistical analysis of all data was done using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY). Descriptive statistics for age, education, MMSE scores, WMH, CBF-CVR, PWV and executive functioning scores are reported in the whole sample and compared between males and females in *Table 1*. Statistical comparisons between males and females were done using independent samples *t* tests. Moderation analyses were performed using the PROCESS Macro for moderation analyses (Hayes 2017). The analyses were bootstrapped to amend any shortcoming in power by simulating greater data based on an algorithm to maintain the current pattern. By default, bootstrapped samples were set to simulate 5,000 samples (Hayes 2017). Moderation effects of sex on the PWV-CVR, PWV-EF, and CVR-EF relations were tested controlling for age and WMH volume. In addition, a moderated moderation analysis was conducted to see if the sex effects in our moderation could be explained by differences in hemoglobin (Fig. 9, Fig. 10).

3.3 Results

A total of 48 older adults (31 Females, 17 Males) participated in this study. Subject characteristics are summarized in *Table 2*. It was found that females had a significantly higher resting CBF ($p < 0.05$) in whole brain grey matter, higher composite scores for executive functioning ($p < 0.05$) and lower hemoglobin than males ($p < 0.05$). There was no difference

between males and females for PWV ($p > 0.05$) and CBF-CVR ($p > 0.05$) in whole brain grey matter. able 2 - Participant Demographics.

Demographic	All (n=48)	Males (n=17)	Females (n=31)
Age (years)	63.35 ± 4.86	64 ± 4.37	63 ± 5.14
Education (years)	16.29 ± 3.56	16.18 ± 3.32	16.3 ± 53.74
EF* (composite score)	0.003 ± 0.74	-0.31 ± 0.87 *	0.177 ± 0.60 *
PWV (m/s)	8.70 ± 2.89	8.89 ± 2.99	8.59 ± 2.88
MMSE (out of 30)	28.79 ± 0.94	28.53 ± 1.32	28.94 ± 0.63
Log WMH volume	0.35 ± 0.15	0.36 ± 0.13	0.35 ± 0.17
Resting CBF (ml/100g/min)*	42.46 ± 10.05	35.74 ± 8.71*	46.14 ± 8.85*
CBF-CVR (ml/100g/min/mmHg CO2)	4.64 ± 2.39	4.50 ± 2.52	4.71 ± 2.36
HBG (g/L)*	139.49 ± 11.27	148.41 ± 11.24 *	134.45 ± 7.77 *
HCT *	0.421 ± 0.03	0.447 ± 0.321*	0.406 ± 0.241*

*$p < 0.05$

3.3.1 Moderation Analysis

Results revealed a significant standardized direct effect of PWV on CVR (β=1.6307, SE = 0.4839, 95% CI [0.654, 2.607], p=0.0016) as depicted in *Figure 4*. The moderation effect (SEX *PWV) was also a significant predictor of CVR (β=-1.013, SE = 0.2957, 95% CI [-1.610, -0.4169], p=0.0014) showing that the effect of PWV on CVR was a function of sex. Further analysis revealed that the effect of PWV on CVR was significantly positive in males (β=0.6170, SE = 0.2184 ,95% CI [0.1762, 1.0577], p=0.0072) and significantly negative in females (β=-0.3967, SE = 0.1902 ,95% CI [-0.7805, -0.0129], p=0.0431) (*see figure 5*).

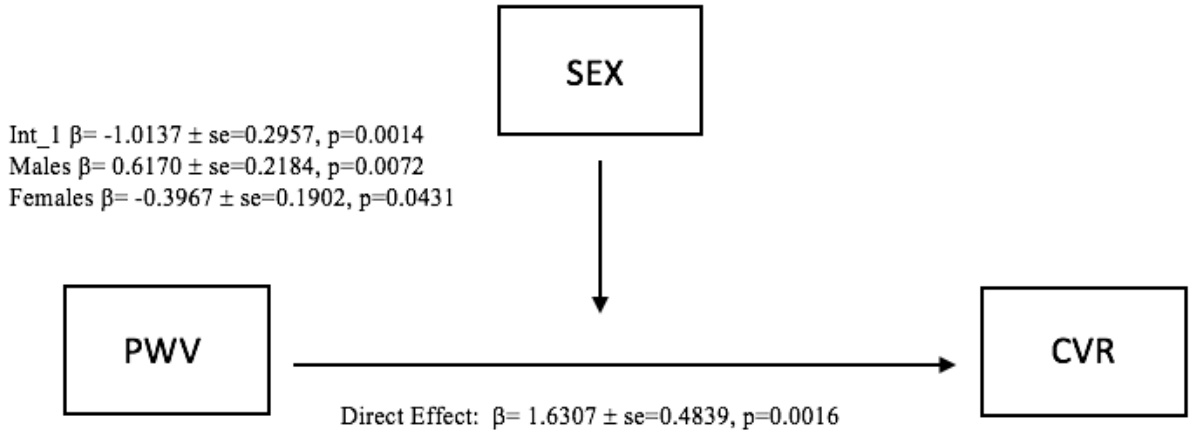

Figure 4 - Moderation Effect (PWV*SEX) on CVR.

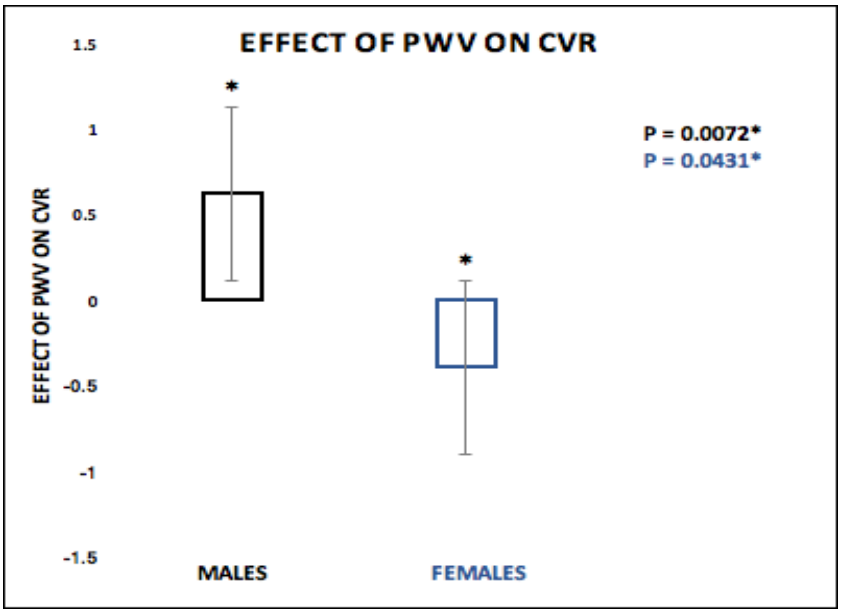

Figure 5 - Effect of PWV on CVR.

N.B.:

CVR was significantly positive in males (p=0.0072)* and significantly negative in females (p=0.0431)*.

Results further revealed a significant standardized direct effect of PWV on EF (β=-0.9980, SE = 0.3463, 95% CI [-1.6970, -0.2990], p=0.0062). The moderation effect (SEX *PWV) was also a significant predictor of EF (β=0.4479, SE = 0.2117, 95% CI [0.0207, 0.8751], p=0.0403) showing that the effect of PWV on EF was a function of sex (*figure 6*). As shown in *figure 7* the effect of PWV on EF was significantly negative in males (β=-0.5501, SE = 0.1563 ,95% CI [-0.8656, -0.2346], p=0.0011) but not significant in females (β=-0.1022, SE = 0.1361 ,95% CI [-0.3769, 0.1725], p=0.4569).

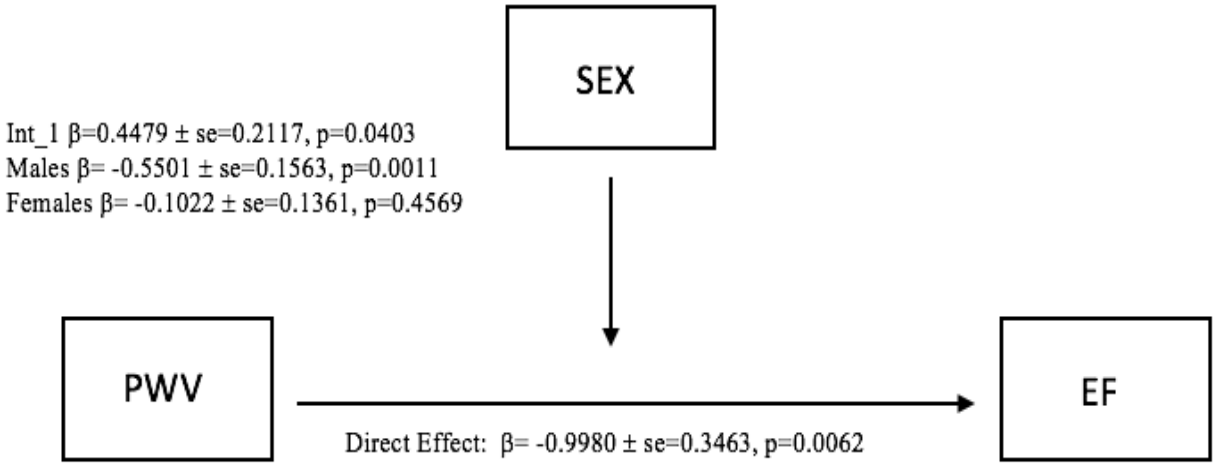

Figure 6 - Moderation Effect (PWV*SEX) on EF.

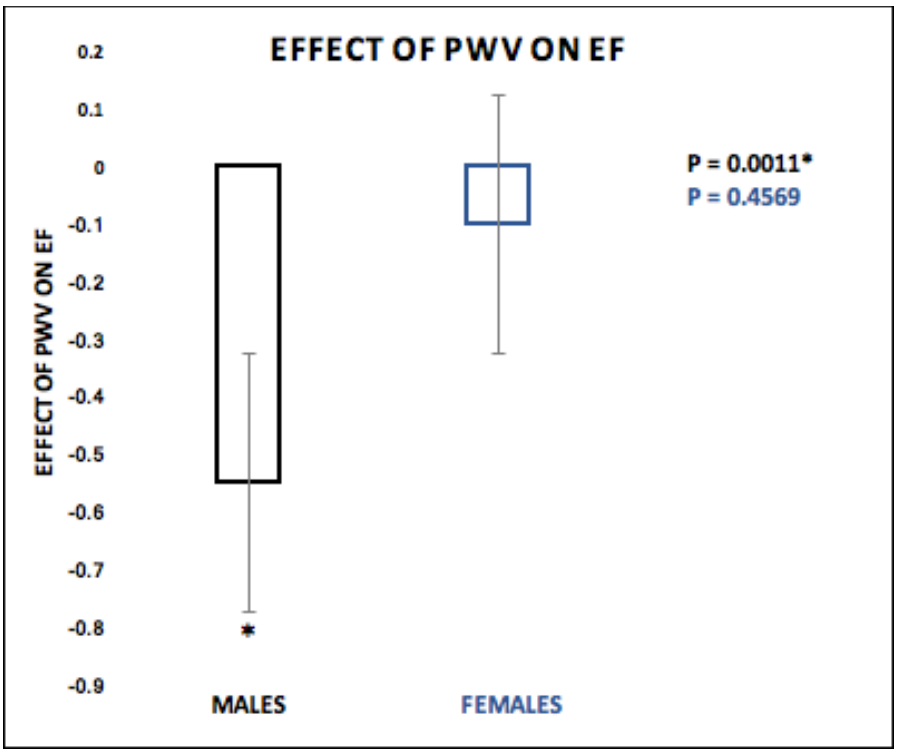

Figure 7 - Effect of PWV on EF.

N.B.:

The effect of PWV on EF was significant negative in males (β=-0.5501, SE = 0.1563 ,95% CI [-0.8656, -0.2346], p=0.0011) but not significant in females (β=-0.1022, SE = 0.1361 ,95% CI [-0.3769, 0.1725], p=0.4569).

Finally, a moderation analysis also revealed a significant standardized direct effect of CVR on EF (β=-0.8472, SE = 0.3332 ,95% CI [-1.5195, -0.1748], p=0.0148). However, the moderation effect (SEX *CVR) did not predict EF (β=0.3438, SE = 0.1990 ,95% CI [-0.0579, 0.7455], p=0.0914) *(figure 8)*.

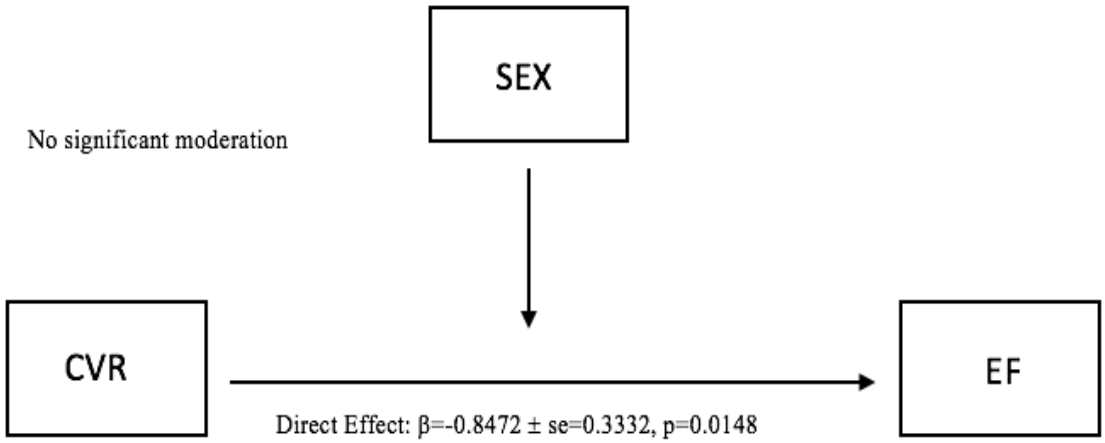

Figure 8 - Moderation model showing the effect (CVR*SEX) on EF.

3.3.2 Moderated Moderation Analysis

A moderated moderation analysis was conducted to see if the sex effects in our moderation could be explained by differences in hemoglobin, given the sex differences in hematocrit concentration shown in *Table 2*. Results revealed that there was no effect of HCB on the sex differences observed in the moderation effect (PWV*SEX) on CVR (*figure 9*)

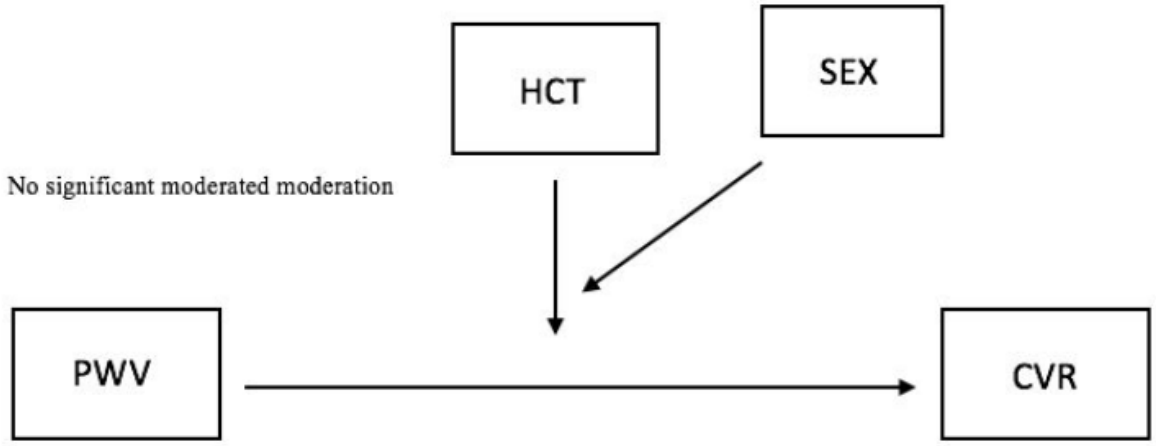

Figure 9 - Moderated moderation model depicting the effect of HCT on the relationship PWV on CVR among sexes.

In addition, through a moderated moderation analysis we tested whether sex moderated the moderation effect of HCT on the relationship between PWV and EF. It was found that the moderated moderation effect (PWV*HCT*SEX) was a significant predictor of EF (β=0.5554, SE = 0.2500, 95% CI [.0492, 1.0615], p=0.0324) (*Figure 10).*As shown in *Figure 11* the effect of PWV on EF was negative in males (β=-0.3097, SE = 0.4479 , 95% CI [-1.2164, 0.5970], p=0.4934) and significantly negative in females (β=-0.4797, SE = 0.1985, 95% CI [-0.8815, -0.0779], p=0.0206) with low HCT levels (0.3884 g/L). In addition, the effect of PWV on EF was negative for both males (β=-0.4394, SE = 0.2936, 95% CI [-1.0338, 0.1549], p=0.1427) and females (β=-0.0834, SE = 0.1273, 95% CI [-0.3411, 0.1742], p=0.5161) with medium HCT levels (0.4200 g/L) but did not reach significance. Lastly, it was found that the effect of PWV on EF was significantly negative for males (β=-0.5626, SE = 0.1774, 95% CI [-0.9216, -0.2035], p=0.0030) and positive for females (β=0.2928, SE = 0.2042, 95% CI [-0.1207, 0.7063], p=0.1599) with high HCT levels (0.4500 g/L).

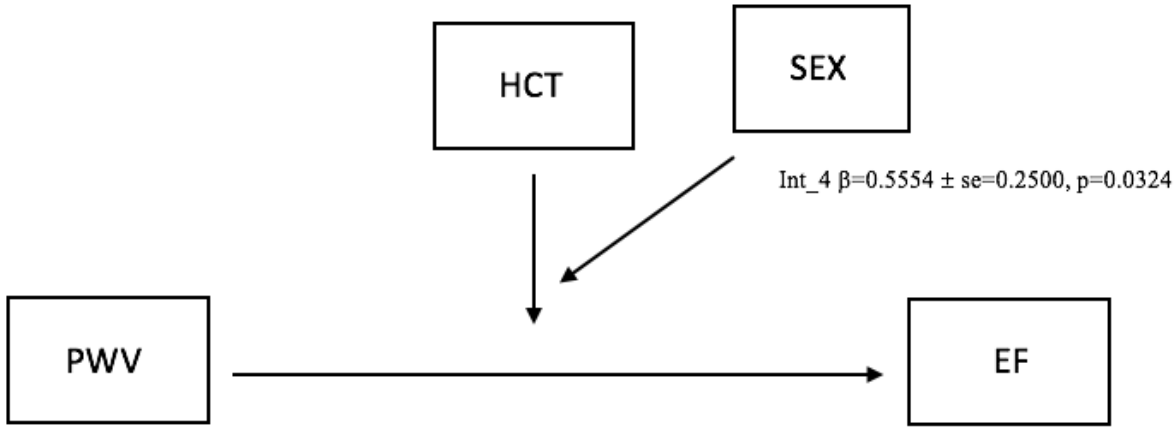

Figure 10 - Moderated moderation model depicting the effect of HCT on the relationship PWV on EF among sexes.

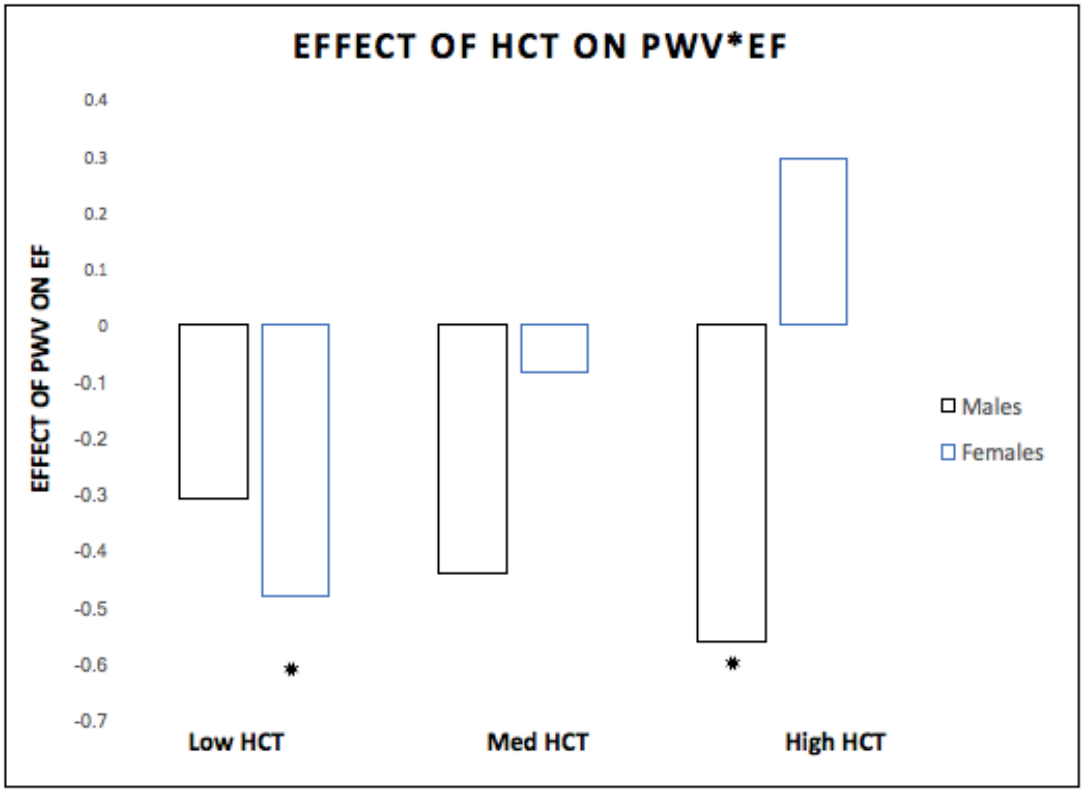

Figure 11 - The effect of HCT on the relationship PWV on EF among sexes.

N.B.:

The effect of PWV on EF was significantly negative (p=0.0206)* in females (blue font) with low HCT levels and significantly negative (p=0.0030)* in males (black font) with high HCT levels

3.4 Discussion

3.4.1 Main Results

In this study, we investigated the impact of sex on the link between PWV, cognitive performance and CVR. An important finding is that sex moderates the relationship between: i) PWV and CVR; and ii) PWV and EF; but not between iii) CVR and EF. Specifically, the effect of PWV on CVR was significantly positive in males and significantly negative in females. Additionally, the effect of PWV on EF was significantly negative in males but not significant in females. Furthermore, results from the moderated moderation analysis revealed that there was no effect of HCT on the sex differences observed in the moderation effect of sex on the

relationship between PWV and CVR however there was a significant effect of HCT on the sex differences observed in the moderation effect of sex on the relationship between PWV and EF. Together, our results indicate that some of the complex relationships between PWV, CVR and EF shown in the literature are being driven by sex and that HCT may be involved in driving some of these sex effects.

3.4.2 Sex Differences

The sex differences identified in this study are consistent with the existing literature, including differences in CBF, HCT and EF performance. In this sample, females have higher rates of resting CBF than males. These findings are similar to previous studies of normal subjects indicating that women typically display greater resting global CBF (Rodriguez et al. 1988; Esposito et al. 1996) and higher CBF velocities compared to males (Vriens et al. 1989; Martin et al. 1994; Oláh et al. 2000; Tegeler et al. 2013). In addition, several studies have reported data suggesting that males and females tend to present with differential performance in certain cognitive domains (Burstein et al. 1980; Kennison 2003; Castonguay et al. 2015). Indeed, our findings of decreased executive function scores in older males compared to older females are also in line with previous research (Halpern and LaMay 2000). Finally, in our population, females had significantly lower hemoglobin levels than males, consistent with previous findings showing that women have mean levels approximately 12% lower than males (Murphy 2014).

3.4.3 Arterial Stiffness and Cerebrovascular Reactivity

Overall, there is substantial evidence supporting sex hormone effects on vascular stiffness (Ogola et al. 2018; DuPont et al. 2019). Over the lifespan, arterial stiffness increases linearly in both men and women however there is a more rapid increase in stiffness associated with depletions in estrogen levels postmenopause (Mitchell 2014; DuPont et al. 2019). Indeed, several studies have shown that hormone receptors, including estrogen and testosterone are cardio-protective (DuPont et al. 2019; Wu et al. 2014; Karas et al. 1994; Dockery et al. 2003). Previous work highlighting the protective effects of estrogen have shown that arterial stiffness, measured using cfPWV, is increased in postmenopausal women taking hormone replacement therapy (HRT) when compared to matched women not taking HRT (Rajkumar et al. 1997; DuPont et al. 2019). In addition, testosterone was once perceived to play a role in promoting

CVD among males (Thompson et al. 1989; Sullivan et al. 1998), however recent epidemiological studies point to the contrary. Indeed, testosterone deficiency has been associated with increased cfPWV among healthy older males compared to age-matched males (Vlachopoulos et al. 2014; DuPont et al. 2019). In addition, low testosterone has also been associated with impared microvascular function and arterial elasticity, measured using augmentation index. (Corrigan et al. 2015; DuPont et al. 2019). Overall, ample data suggest that sex hormones substantially impact the manifestation of arterial stiffness among males and females. As such, a thorough understanding of the underlying mechanisms that contribute to these sex differences could aid in developing sex-secific strategies to reduce or prevent CVD risk.

Although quantitative measurements of CBF and CVR in relation with arterial stiffness have been performed by others (DuBose et al. 2018; Jefferson et al. 2018), the literature shows conflicting results. While more recent reports suggest a preserved CVR with increased aortic stiffness (Jefferson et al. 2018; Zhu et al. 2013), earlier studies using PET and TCD rather than MRI did not find such an association, but rather reductions in CVR among adults with greater aortic stiffness (DuBose et al. 2018; Jaruchart et al. 2016). It is possible however that these counterintuitive findings could stem from differences in study designs, heterogeneity of target populations (males vs females) and/or differences in imaging modalities. For example, one previous TCD study describing sex-related differences in cerebral vasomotor reactivity has shown increased vasodilatory response in females compared with male subjects (Matteis et al. 1998). More importantly, these studies did not control for menstrual phase or account for sex hormones, which are known to acutely alter CBF and cerebrovascular responsiveness (Brackley et al. 1999; Krejza et al. 2001; Krejza et al. 2003; Krejza et al. 2013; Nevo et al. 2007).

Following these results, our work presents the first evidence concerning a sex moderation between PWV and CVR. Our findings of low CVR among females and high CVR among males with increased arterial stiffness could be explained by a few hypotheses. The first, by Jefferson et al (2018) is that microcirculatory remodeling occurring in response to higher pulsatile energy, caused by arterial stiffness, may lead to increased resistance (Jefferson et al. 2018). Secondly, prior research relating arterial stiffness to cerebral hemodynamics have shown sex homornes can directly modulate CVR and CBF (Krause et al. 2006). In fact, estrogens enhance sensitivity

to vasodilatory factors and are well known for their systemic vasoactivity (Goldman et al. 1976; Magness et al. 1993). Indeed, several studies have demonstrated that estrogens improve vascular flow and arterial pulsatility (Sarrel 1990; de Ziegler et al. 1991; Cust et al. 1990). Thus, the possibility of a direct effect of estrogens on the arterial wall must also be considered in the context of these results. For instance, Adams *et al*. have previously shown that the presence of estrogen receptors in the arterial wall may influence artery wall metabolism (Adams et al. 1987). In addition, a recent study using transcranial doppler ultrasonography found a large reduction in CVR in postmenopualsal women compared to premenopausal women (Matteis et al. 1998). These observations support the notion that findings from the present study could be explained by different hormonal changes in males and females. It is important to note however that given the age range included in this study, very few if any of the female participants are expected to be pre-menopausal. Therefore, estrogen levels are expected to be low in our female sample. On the other hand, males retain the abilty to synthesize sex hormones later in life, and testosterone is to some extent converted to estrogen, so that the beneficial effects of estrogen on the brain may in fact be most present in our male sample (Schulster, 2016). However, these possibilities remain to be tested in a future study including measurement of sex hormones.

3.4.4 The Association between pulse wave velocity and cognitive function relative to sex

In the present study the relationship between arterial stiffness and EF is in part driven by differences in sex. We also demonstrate that higher arterial stiffness, as measured by PWV, is associated with poorer performance on EF tasks among males only. Our findings are in line with prior studies that have demonstrated sex differences in the relationship between arterial stiffness and cognitive function. For instance, Singer et al. found no significant association between arterial stiffness and global cognition, however negative associations between PWV and composite global cognition were observed in males only when stratifying the sample by sex (Singer et al. 2013). Similarly, Waldstein et al reported men as scoring significantly lower than women on tests of verbal memory at higher levels of pulse pressure, an alternative measure of arterial stiffness (Waldstein et al. 2008). In addition, there is a good agreement in the literature showing that arterial stiffness and related central hemodynamics are associated with reductions in cognitive performance on memory, processing speed, and executive function tasks (Iulita et

al. 2018; Singer et al. 2014; Badji et al. 2019). However, these studies are limited because they have used a nonspecific measure of cognition (i.e MMSE). Here, we extend their findings to include executive function domains that are especially susceptible to cardiovascular disease (Gorelick et al. 2012). Indeed, our results are consistent with other studies pointing to a general association between PWV and executive dysfunction (Lim et al. 2016; Poels et al. 2007; Hajjar et al. 2016). For example, Hajjar et al found that subjects with higher PWV had the greatest 4-year risk of decline in executive function (Hajjar et al. 2016). It has been hypothesized that this may be due to the fact that the dorsolateral prefrontal cortex, essential for executive function tasks, is situated in a watershed region of the brain (Suchy 2015; de la Torre 2002). Thus, one could speculate that with increased arterial stiffness, these regions may be deprived of oxygen and perfusion (Suchy 2015; de la Torre 2002).

Our results showed no significant association between arterial stiffness and EF among females. Moreover, females in this cohort displayed significantly better performances as compared to males on the cognitive tasks assessed. It is well known that there are significant differences between sexes in regard to vascular function and cognitive performance among older adults (Castonguay et al. 2015; Narkiewicz et al. 2006). Indeed, across the lifespan, males have a higher risk for CVDs where as females are relatively protected until menopause after which they catch up to males, likely due to estrogen depletion (Singer et al. 2013; Narkiewicz et al. 2006). In addition, males have a higher prevalence of mild cognitive impairment compared to females (Petersen et al. 2010; Brodaty et al. 2013; Singer et al. 2013). A possible hypothesis for these sex differences is that men experience cognitive decline earlier in life, while women transition from normal cognition to impaired cognition in the decades following menopause (Petersen et al. 2010).

3.4.5 The Association between cognitive function and cerebrovascular reactivity relative to sex

While the vessel reactivity-cognition link has been investigated in cardiovascular and neurodegenerative disorders, the association between CVR and cognition in healthy aging is unclear. Most reports indicate that blood vessel reactivity in the brain decreases with healthy aging (Reich and Rusinek 1989; Lu et al. 2011; Gauthier et al. 2015; Gauthier et al. 2013; Gauthier et al. 2012; Bhogal et al. 2016; De Vis et al. 2015), The mechanisms underlying the

decreasing reactivity of cerebral vessels with aging are postulated to be related to local vascular stiffening (Desjardins 2015). An interesting finding of our study is that sex did not moderate the relationship between CVR and EF among older adults suggesting that other hemodynamics mechanisms may be at play. Interestingly, recent work published by our group, has shown that CVR, which could be interpreted as an indirect measure of vascular elasticity in the brain, may not be uniquely dependent on brain-based vascular properties and may be parly dependent on more global properties such as changes in chemosensitivity and cerebral autoregulation (Intzandt et al. 2019). Indeed, the lack of moderation between CVR and cognition could be attributable to the fact that this indirect measure of stiffness in the brain may be biased by unknown physiological changes and may be underestimating the effects on cerebral hemodynamics. As such, CVR should be interpreted with caution as a measure of stiffness in the brain. Nonetheless, future work aiming to disentangle the relationships between CVR and cognition should implement more robust and direct measures of vascular elasticity in the brain (Baraghis et al. 2011; Warnert et al. 2016).

3.4.6 The effect of Hematocrit

Additionally, we aimed to assess if the sex effects observed in the moderation effect (PWV*SEX) on EF were driven by differences in hemoglobin. Our findings suggest that HCT may be in part driving these sex differences. Interestingly, it has previously been shown that males and females have different levels of hemoglobin which affect MR measures of flow (Kimberly et al. 2013;Yip et al. 1984; Vahlquist and Others 1950; Garn et al. 1975). Since PWV, a surrogate marker for stiffness, is dependent on the differences in pressure which in turn is dependent on radius of vessels and blood viscosity, it is plausible that our results reflect a blood viscosity effect (Stojadinović et al. 2015; Painter 2008). We reasoned that PWV as a measure of stiffness may not be pure. Interestingly, most published studies have ignored the effect of blood viscosity on PWV measurement and MR-CBF estimates, since the measure is based on the underlying assumption that blood viscosity is constant across subjects (Stojadinović et al. 2015; Parkhurst et al. 2012). It has been previously shown that there might be some relation between blood flow and vascular wall, and that blood viscosity as a mechanical property of blood flow might affect measures of PWV (Kim et al. 2013).

3.4.7 Limitations

The results from this study should be viewed in light of some limitations. First, menopausal status and hormonal levels were not acquired at the time of the study. It is assumed that our female population is predominantly postmenopausal given that the age range was 55-75 years, with a mean age of 63 and the average age of naturally occurring menopause in Canada 51(Rossi et al. 2011). Nonetheless, this assumption is speculative, and other studies should account for menopausal status to replicate and provide clarity on the mechansims that underlie the present results. Future work should include younger subjects (40-55 years of age) to better study hormonal status in females

While a strength of the current study is the use of non-invasive quantitative perfusion imaging to calculate cerebral blood flow and CVR, one must note that the ASL technique suffers from several limitations. Indeed, the contrast afforded by the subtraction of tagged images is only a fraction of a percent of the functional MRI contrast, providing limited signal-to-noise ratio (SNR) (Golay and Petersen 2006; Badji et al. 2019). Furthermore, coverage of the brain is typically not possible without advanced multi-band approaches, limiting the ability to draw conclusions on the entire brain (Badji et al. 2019; Golay and Petersen 2006). Finally, most ASL imaging methods are unable to determine whether the changes detected are true reflections of changes in flow or the result of alterations in transit times (Badji et al. 2019). Indeed, there are potential sex bias in transit times since women and men have different head sizes. For instance, since women are shorter, it would be assumed that this would lead to a shorter transit time in women compared to men. Given our short post labeling delay, this would would mean that we would have a more appropriate PLD for women. This could indeed partly explain the higher CVR in men since the flow during hypercapnia would be less PLD-related limited than in women. This is especially problematic in the case of single delay ASL studies, such as the one presented here. Despite these limitations of ASL, it has consistently been shown that CBF-CVR is a more specific measure of vascular health (Halani et al. 2015). Indeed, it is most sensitive to vascular elasticity, and may be the best choice when sensitivity is desired (Halani et al. 2015). In addition to the general limitations of ASL, the post-labeling delay chosen in this study is suboptimal for older adults since it was optimized for a younger population (Badji et al. 2019;Intzandt et al. 2019). As such, with our limited SNR, it is possible that our CBF data before

and during hypercapnia was underestimated. Thus, to better understand the relationship between arterial stiffness, cognition and CVR, longitudinal studies using multi-band approaches and multi-delay implementations with optimized post-labeling delays are necessary.

Another limitation to this study is our sample size. Indeed, our subset of female and male participants is small, limiting our ability to infer our findings on the general population. Indeed, it may be plausible that due to our small sample size we were unable to detect the mediating effect of sex in some of our models. In addition, our ratio of males (n=17) to females (n=31) in this sample may be biased toward females, underrepresenting our male population. As such, our study may lack adequate statistical power to detect an effect size of practical importance, especially in males. Nonetheless, our analyses were boostrapped which can overcome the power problem of small samples. Also, our sample included predominantly very healthy older adults, limiting our ability to generalize our findings to other older adults, who often suffer from cardiovascular risk factors. We speculate that associations reported here would likely be stronger in a cohort with worse cardiovascular health. Moreover, a follow-up study with better statistical power is needed to confirm our findings.

Finally, this study has a cross sectional design and our findings are retrospective in nature making it difficult to draw general conclusions on the population. Although this study provides valuable knowledge on the impact of sex on the relationship between aortic stiffness, cognition and CVR, a longitudinal study is needed to better understand the sex differences among those hemodynamic measures across the lifespan to better personalize CVD prevention strategies.

3.5 Conclusion

The findings from this study add to a growing body of research that underscores the interrelationship between cardiovascular function and brain health among aging adults. Overall, this paper identified a sex moderation between PWV and CVR, PWV and EF but not between CVR and EF in a sample of healthy older adults. Our data also demonstrated that HGB may play a role in driving some of these sex effects. These findings could be the results of different sex hormones, such as estrogen and testosterone, that are known to alter cerebrovascular measures of brain health. Finally, as the key oxygen-carrying molecule in the body, hemoglobin may play a direct role in influencing vascular measures of stiffness such as PWV and affect MR

measures of flow. More importantly, understanding these hemodynamic associations may lead to earlier detection and targeted interventions to prevent or lessen the onset of cardiovascular diseases linked with higher aortic stiffening. Thus, future longitudinal studies that explore sex differences should include evaluation of the role of hemoglobin and investigate the role of hormone variations (i.e. sex menopause).

Chapter 4

Conclusion

Overall, the work presented as part of this thesis advances the knowledge in the field of brain imaging of arterial stiffness and leaves room for future studies that should further characterize the impact of sex on different hemodynamic measures in older adults. Based on the finding that sex moderates the relationships between PWV and CVR as well as PWV and EF, future studies should further investigate the exact mechanisms (i.e. hormone variations, menopause) that may be driving these relationships. To do so, future longitudinal studies tracking vascular aging in women across the lifespan are warranted to determine the mechanisms that play a role in vascular stiffening and how these may differ from aging men. For instance, having a longitudinal study design with a population ranging from young adulthood to older adulthood will allow us to isolate and investigate separate components of vascular aging. In addition, acquiring menopausal status will also shed light of the previously mentioned protective role of sex hormones testosterone and estrogen. In turn, this will help to better characterize the impact of CVD on brain health.

Secondly, many studies using PWV as a measure of arterial stiffness neglect the potential effects of HGB. Recently, high HGB concentrations were shown to be associated with higher PWV and an elevated risk of CVDs (Lee et al. 2018; Zhang et al. 2019). Furthermore, recent work suggests that there might be some relation between blood flow and vascular wall stiffness, and that the characteristics of blood flow might influence measurements of PWV (Kim et al. 2013). Similarly, our work shows an effect of HGB on the sex differences observed, suggesting that blood properties differ between males and females and might be influencing PWV. Indeed, according to the Poiseuille-Hagen Equation, HGB may affect measurements of PWV in two ways: by influencing the viscosity of blood (Kim et al. 2013; Fossum et al. 1997), and by affecting the caliber of peripheral arterioles (Fossum et al. 1997). As a result, future studies that explore sex differences using measurements of arterial stiffness should include multiple assessments of hemoglobin levels.

Furthermore, the relationship between measures of brain health (CVR) and arterial stiffness should be further investigated. Indeed, it has consistently been shown that sex differences exist among these measures of cerebral hemodynamics. Yet, it is not clear what the direction of these relationships are in cross-sectional studies due to conflicting findings. Based on our results, sex could be driving these conflicting relationships. In addition, as shown by our previous work, quantitative measures of CVR could be biased by other physiological outcomes including autoregulation and chemo sensitivity (Intzandt et al. 2019). Indeed, studies using CVR in aging may underestimate their effects on cerebral hemodynamics. Thus, future studies targeting sex differences among quantitative measures of brain health should implement more robust measures of vascular elasticity to replicate these findings. For example, using novel techniques such as optical coherence tomography (OCT) to evaluate flow pulsatility and vessel compliance is a promising tool to measure vascular dysfunction (Baraghis et al. 2011).

Although there are many opportunities for future studies, this work presents novel findings in that it provides the first evidence of sex being a moderator among markers of vascular aging. Those findings are extremely relevant to today's increasing older population and the increasing evidence of sex differences in the manifestation of CVDs. Indeed, understanding the mechanisms driving sex differences in measures of vascular stiffness and cerebral health has potential to identify novel sex-specific therapies to mitigate CVD risk and better personalize CVD preventive strategies.

References

Adams, M. R., Kaplan, J. R., Koritnik, D. R., & Clarkson, T. B. (1987). Pregnancy-associated inhibition of coronary artery atherosclerosis in monkeys. Evidence of a relationship with endogenous estrogen. *Arteriosclerosis* , *7*(4), 378–384.

Ahnstedt, H., Cao, L., Krause, D. N., Warfvinge, K., Säveland, H., Nilsson, O. G., & Edvinsson, L. (2013). Male-female differences in upregulation of vasoconstrictor responses in human cerebral arteries. *PloS One*, *8*(4), e62698.

Akima, M., Nonaka, H., Kagesawa, M., & Tanaka, K. (1986). A study on the microvasculature of the cerebral cortex. Fundamental architecture and its senile change in the frontal cortex. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, *55*(4), 482–489.

Allen, J. S., Bruss, J., Brown, C. K., & Damasio, H. (2005). Normal neuroanatomical variation due to age: the major lobes and a parcellation of the temporal region. *Neurobiology of Aging*, *26*(9), 1245–1260; discussion 1279–1282.

Alsop, D. C., Detre, J. A., Golay, X., Günther, M., Hendrikse, J., Hernandez-Garcia, L., … Zaharchuk, G. (2015). Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *73*(1), 102–116.

Alsop, D. C., Detre, J. A., & Grossman, M. (2000). Assessment of cerebral blood flow in Alzheimer's disease by spin-labeled magnetic resonance imaging. *Annals of Neurology*, *47*(1), 93–100.

Ambarki, K., Wåhlin, A., Zarrinkoob, L., Wirestam, R., Petr, J., Malm, J., & Eklund, A. (2015). Accuracy of Parenchymal Cerebral Blood Flow Measurements Using Pseudocontinuous Arterial Spin-Labeling in Healthy Volunteers. *AJNR. American Journal of Neuroradiology*, *36*(10), 1816–1821.

Amiri, M., Pouliot, P., Bonnéry, C., Leclerc, P.-O., Desjardins, M., Lesage, F., & Joanette, Y. (2014). An Exploration of the Effect of Hemodynamic Changes Due to Normal Aging on the fNIRS Response to Semantic Processing of Words. *Frontiers in Neurology*, *5*, 249.

Appelros, P., Stegmayr, B., & Terént, A. (2009). Sex differences in stroke epidemiology: a systematic review. *Stroke; a Journal of Cerebral Circulation*, *40*(4), 1082–1090.

Ashburner, J., & Friston, K. J. (2000). Voxel-Based Morphometry—The Methods.

NeuroImage, *11*(6), 805–821.

Aslan, S., Huang, H., Uh, J., Mishra, V., Xiao, G., van Osch, M. J. P., & Lu, H. (2011). White matter cerebral blood flow is inversely correlated with structural and functional connectivity in the human brain. *NeuroImage*, *56*(3), 1145–1153.

Assaf, Y., & Basser, P. J. (2005). Composite hindered and restricted model of diffusion (CHARMED) MR imaging of the human brain. *NeuroImage*, *27*(1), 48–58.

Assaf, Y., & Pasternak, O. (2008). Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. *Journal of Molecular Neuroscience: MN*, *34*(1), 51–61.

Avants, B. B., Epstein, C. L., Grossman, M., & Gee, J. C. (2008). Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Medical Image Analysis*, *12*(1), 26–41.

Badji, A., de la Colina, A. N., Sabra, D., Karakuzu, A., Bherer, L., Lamarre-cliche, M., … Girouard, H. (2019). The relationship between cognitive function, cortical blood flow and sub-cortical white-matter health in the elderly. *JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM*, *39*, 29–30. SAGE PUBLICATIONS INC 2455 TELLER RD, THOUSAND OAKS, CA 91320 USA.

Badji, A., Noriega de la Colina, A., Karakuzu, A., Duval, T., Desjardins-Crépeau, L., Joubert, S., … Cohen-Adad, J. (2019). Arterial stiffness and white matter integrity in the elderly: A diffusion tensor and magnetization transfer imaging study. *NeuroImage*, *186*, 577–585.

Badji, A., Sabra, D., Bherer, L., Cohen-Adad, J., Girouard, H., & Gauthier, C. J. (2019). Arterial Stiffness and Brain Integrity: A review of MRI findings. *Ageing Research Reviews*. https://doi.org/10.1016/j.arr.2019.05.001

Bajo, R., Castellanos, N. P., Cuesta, P., Aurtenetxe, S., Garcia-Prieto, J., Gil-Gregorio, P., … Maestu, F. (2012). Differential patterns of connectivity in progressive mild cognitive impairment. *Brain Connectivity*, *2*(1), 21–24.

Baraghis, E., Bolduc, V., Lefebvre, J., Srinivasan, V. J., Boudoux, C., Thorin, E., & Lesage, F. (2011). Measurement of cerebral microvascular compliance in a model of atherosclerosis with optical coherence tomography. *Biomedical Optics Express*, *2*(11), 3079–3093.

Bartzokis, G., Cummings, J. L., Sultzer, D., Henderson, V. W., Nuechterlein, K. H., & Mintz, J. (2003). White matter structural integrity in healthy aging adults and patients with Alzheimer disease: a magnetic resonance imaging study. *Archives of Neurology*, *60*(3), 393–398.

Beaulieu, C. (2002). The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR in Biomedicine*, *15*(7-8), 435–455.

Beaulieu, C. (2014). Chapter 8 - The Biological Basis of Diffusion Anisotropy. In H.

Johansen-Berg & T. E. J. Behrens (Eds.), *Diffusion MRI (Second Edition)* (pp. 155–183). San Diego: Academic Press.

Benetos, A., Adamopoulos, C., Bureau, J.-M., Temmar, M., Labat, C., Bean, K., … Guize, L. (2002). Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation*, *105*(10), 1202–1207.

Benetos, A., Watfa, G., Hanon, O., Salvi, P., Fantin, F., Toulza, O., … PARTAGE Study Investigators. (2012). Pulse wave velocity is associated with 1-year cognitive decline in the elderly older than 80 years: the PARTAGE study. *Journal of the American Medical Directors Association*, *13*(3), 239–243.

Beskow, J., Hassler, O., & -O. Ottosson, J. (1971). CEREBRAL ARTERIAL DEFORMITIES IN RELATION TO SENILE DETERIORATION. *Acta Psychiatrica Scandinavica*, Vol. 47, pp. 111–119. https://doi.org/10.1111/j.1600-0447.1971.tb02143.x
Bhogal, A. A., De Vis, J. B., Siero, J. C. W., Petersen, E. T., Luijten, P. R., Hendrikse, J., … Hoogduin, H. (2016). The BOLD cerebrovascular reactivity response to progressive hypercapnia in young and elderly. *NeuroImage*, *139*, 94–102.

Biagi, L., Abbruzzese, A., Bianchi, M. C., Alsop, D. C., Del Guerra, A., & Tosetti, M. (2007). Age dependence of cerebral perfusion assessed by magnetic resonance continuous arterial spin labeling. *Journal of Magnetic Resonance Imaging: JMRI*, *25*(4), 696–702.

Blatter, D. D., Bigler, E. D., Gale, S. D., Johnson, S. C., Anderson, C. V., Burnett, B. M., … Horn, S. D. (1995). Quantitative volumetric analysis of brain MR: normative database spanning 5 decades of life. *AJNR. American Journal of Neuroradiology*, *16*(2), 241–251.

Bonarjee, V. V. S. (2018). Arterial Stiffness: A Prognostic Marker in Coronary Heart Disease. Available Methods and Clinical Application. *Frontiers in Cardiovascular Medicine*, *5*, 64.

Bonithon-Kopp, C., Levenson, J., Scarabin, P. Y., Guillanneuf, M. T., Kirzin, J. M., Malmejac, A., & Guize, L. (1993). Longitudinal associations between plasma viscosity and cardiovascular risk factors in a middle-aged French population. *Atherosclerosis*, *104*(1-2), 173–182.

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica*, *82*(4), 239–259.

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

Brodaty, H., Heffernan, M., Kochan, N. A., Draper, B., Trollor, J. N., Reppermund, S., … Sachdev, P. S. (2013). Mild cognitive impairment in a community sample: the Sydney Memory and Ageing Study. *Alzheimer's & Dementia: The Journal of the Alzheimer's*

Association, *9*(3), 310–317.e1.

Brown, W. R., Moody, D. M., Challa, V. R., Thore, C. R., & Anstrom, J. A. (2002). Venous collagenosis and arteriolar tortuosity in leukoaraiosis. *Journal of the Neurological Sciences*, *203-204*, 159–163.

Brown, W. R., & Thore, C. R. (2011). Review: Cerebral microvascular pathology in ageing and neurodegeneration. *Neuropathology and Applied Neurobiology*, Vol. 37, pp. 56–74. https://doi.org/10.1111/j.1365-2990.2010.01139.x

Bucur, B., Madden, D. J., Spaniol, J., Provenzale, J. M., Cabeza, R., White, L. E., & Huettel, S. A. (2008). Age-related slowing of memory retrieval: contributions of perceptual speed and cerebral white matter integrity. *Neurobiology of Aging*, *29*(7), 1070–1079.

Burstein, B., Bank, L., & Jarvik, L. F. (1980). Sex differences in cognitive functioning: evidence, determinants, implications. *Human Development*, *23*(5), 289–313.

Bush, A., Chai, Y., Choi, S. Y., Vaclavu, L., Holland, S., Nederveen, A., … Wood, J. (2018). Pseudo continuous arterial spin labeling quantification in anemic subjects with hyperemic cerebral blood flow. *Magnetic Resonance Imaging*, *47*, 137–146.

Buxton, R. B. (2010). Interpreting oxygenation-based neuroimaging signals: the importance and the challenge of understanding brain oxygen metabolism. *Frontiers in Neuroenergetics*, *2*, 8.

Castonguay, N., Lussier, M., Bugaiska, A., Lord, C., & Bherer, L. (2015). Executive functions in men and postmenopausal women. *Journal of Clinical and Experimental Neuropsychology*, *37*(2), 193–208.

Chen, J. J., Rosas, H. D., & Salat, D. H. (2011). Age-associated reductions in cerebral blood flow are independent from regional atrophy. *NeuroImage*, *55*(2), 468–478.

Chen, J. J., Rosas, H. D., & Salat, D. H. (2013). The relationship between cortical blood flow and sub-cortical white-matter health across the adult age span. *PloS One*, *8*(2), e56733.

Chuang, S.-Y., Cheng, H.-M., Bai, C.-H., Yeh, W.-T., Chen, J.-R., & Pan, W.-H. (2016). Blood Pressure, Carotid Flow Pulsatility, and the Risk of Stroke: A Community-Based Study. *Stroke; a Journal of Cerebral Circulation*, *47*(9), 2262–2268.

Cignarella, A., Kratz, M., & Bolego, C. (2010). Emerging role of estrogen in the control of cardiometabolic disease. *Trends in Pharmacological Sciences*, *31*(4), 183–189.

Cook, I. A., Leuchter, A. F., Morgan, M. L., Dunkin, J. J., Witte, E., David, S., … Rosenberg, S. (2004). Longitudinal progression of subclinical structural brain disease in normal aging. *The American Journal of Geriatric Psychiatry: Official Journal of the American Association for Geriatric Psychiatry*, *12*(2), 190–200.

Corrigan, F. E., 3rd, Al Mheid, I., Eapen, D. J., Hayek, S. S., Sher, S., Martin, G. S., & Quyyumi, A. A. (2015). Low testosterone in men predicts impaired arterial elasticity and microvascular function. *International Journal of Cardiology*, *194*, 94–99.

Coutinho, T. (2014). Arterial stiffness and its clinical implications in women. *The Canadian Journal of Cardiology*, *30*(7), 756–764.

Coverdale, N. S., Gati, J. S., Opalevych, O., Perrotta, A., & Shoemaker, J. K. (2014). Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *Journal of Applied Physiology*, *117*(10), 1090–1096.

Cui, C., Sekikawa, A., Kuller, L. H., Lopez, O. L., Newman, A. B., Kuipers, A. L., & Mackey, R. H. (2018). Aortic Stiffness is Associated with Increased Risk of Incident Dementia in Older Adults. *Journal of Alzheimer's Disease: JAD*, *66*(1), 297–306.

Cust, M. P., Gangar, K. F., Hillard, T. C., & Whitehead, M. I. (1990). A risk-benefit assessment of estrogen therapy in postmenopausal women. *Drug Safety: An International Journal of Medical Toxicology and Drug Experience*, *5*(5), 345–358.

D'Esposito, M., Deouell, L. Y., & Gazzaley, A. (2003). Alterations in the BOLD fMRI signal with ageing and disease: a challenge for neuroimaging. *Nature Reviews. Neuroscience*, *4*(11), 863–872.

Davis, S. M., Ackerman, R. H., Correia, J. A., Alpert, N. M., Chang, J., Buonanno, F., … Taveras, J. M. (1983). Cerebral blood flow and cerebrovascular CO2 reactivity in stroke-age normal controls. *Neurology*, Vol. 33, pp. 391–391. https://doi.org/10.1212/wnl.33.4.391

Davis, T. L., Kwong, K. K., Weisskoff, R. M., & Rosen, B. R. (1998). Calibrated functional MRI: mapping the dynamics of oxidative metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(4), 1834–1839.

Dawson, J., & Wyss, A. (2017). Chicken or the Egg? Hyperuricemia, Insulin Resistance, and Hypertension. *Hypertension*, Vol. 70, pp. 698–699. https://doi.org/10.1161/hypertensionaha.117.09685

de Haan, W., Mott, K., van Straaten, E. C. W., Scheltens, P., & Stam, C. J. (2012). Activity dependent degeneration explains hub vulnerability in Alzheimer's disease. *PLoS Computational Biology*, *8*(8), e1002582.

de la Colina, A. N., Badji, A., Sabra, D., Karakuzu, A., Joubert, S., Bherer, L., … Girouard, H. (2019). Augmentation index is a predictor of cerebral blood flow across global grey matter in the elderly. *JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM*, *39*, 134–135. SAGE PUBLICATIONS INC 2455 TELLER RD, THOUSAND OAKS, CA 91320 USA.

de la Torre, J. (2018). The Vascular Hypothesis of Alzheimer's Disease: A Key to Preclinical

Prediction of Dementia Using Neuroimaging. *Journal of Alzheimer's Disease: JAD*, *63*(1), 35–52.

de la Torre, J. C. (1994). Impaired brain microcirculation may trigger Alzheimer's disease. *Neuroscience and Biobehavioral Reviews*, *18*(3), 397–401.

de la Torre, J. C. (1997). Cerebromicrovascular pathology in Alzheimer's disease compared to normal aging. *Gerontology*, *43*(1-2), 26–43.

de la Torre, J. C. (1999). Critical threshold cerebral hypoperfusion causes Alzheimer's disease? *Acta Neuropathologica*, *98*(1), 1–8.

de la Torre, J. C. (2002). Alzheimer disease as a vascular disorder: nosological evidence. *Stroke; a Journal of Cerebral Circulation*, *33*(4), 1152–1162.

de la Torre, J. C., & Mussivand, T. (1993). Can disturbed brain microcirculation cause Alzheimer's disease? *Neurological Research*, *15*(3), 146–153.

De Vis, J. B., Hendrikse, J., Bhogal, A., Adams, A., Kappelle, L. J., & Petersen, E. T. (2015). Age-related changes in brain hemodynamics; A calibrated MRI study. *Human Brain Mapping*, *36*(10), 3973–3987.

de Ziegler, D., Bessis, R., & Frydman, R. (1991). Vascular resistance of uterine arteries: physiological effects of estradiol and progesterone. *Fertility and Sterility*, *55*(4), 775–779.

Deegan, B. M., Sorond, F. A., Galica, A., Lipsitz, L. A., O'Laighin, G., & Serrador, J. M. (2011). Elderly women regulate brain blood flow better than men do. *Stroke; a Journal of Cerebral Circulation*, *42*(7), 1988–1993.

Desjardins, M. (2015). Vascular correlates of aging in the brain: Evidence from imaging data. *IRBM*, *36*(3), 158–165.

Dijk, J. M., Algra, A., van der Graaf, Y., Grobbee, D. E., Bots, M. L., & SMART study group. (2005). Carotid stiffness and the risk of new vascular events in patients with manifest cardiovascular disease. The SMART study. *European Heart Journal*, *26*(12), 1213–1220.

Diomedi, M., Cupini, L. M., Rizzato, B., Ferrante, F., Giacomini, P., & Silvestrini, M. (2001). Influence of physiologic oscillation of estrogens on cerebral hemodynamics. *Journal of the Neurological Sciences*, *185*(1), 49–53.

Dockery, F., Bulpitt, C. J., Donaldson, M., Fernandez, S., & Rajkumar, C. (2003). The relationship between androgens and arterial stiffness in older men. *Journal of the American Geriatrics Society*, *51*(11), 1627–1632.

DuBose, L. E., Boles Ponto, L. L., Moser, D. J., Harlynn, E., Reierson, L., & Pierce, G. L. (2018). Higher Aortic Stiffness Is Associated With Lower Global Cerebrovascular Reserve

Among Older Humans. *Hypertension*, *72*(2), 476–482.

Duff, S. J., & Hampson, E. (2001). A sex difference on a novel spatial working memory task in humans. *Brain and Cognition*, *47*(3), 470–493.

Dufouil, C., Godin, O., Chalmers, J., Coskun, O., MacMahon, S., Tzourio-Mazoyer, N., … PROGRESS MRI Substudy Investigators. (2009). Severe cerebral white matter hyperintensities predict severe cognitive decline in patients with cerebrovascular disease history. *Stroke; a Journal of Cerebral Circulation*, *40*(6), 2219–2221.

DuPont, J. J., Kenney, R. M., Patel, A. R., & Jaffe, I. Z. (2019a). Sex differences in mechanisms of arterial stiffness. *British Journal of Pharmacology*. https://doi.org/10.1111/bph.14624

DuPont, J. J., Kenney, R. M., Patel, A. R., & Jaffe, I. Z. (2019b). Sex differences in mechanisms of arterial stiffness. *British Journal of Pharmacology*. https://doi.org/10.1111/bph.14624

Edzes, H. T., & Samulski, E. T. (1977). Cross relaxation and spin diffusion in the proton NMR or hydrated collagen. *Nature*, *265*(5594), 521–523.

Elias, M. F., Robbins, M. A., Budge, M. M., Abhayaratna, W. P., Dore, G. A., & Elias, P. K. (2009a). Arterial pulse wave velocity and cognition with advancing age. *Hypertension*, *53*(4), 668–673.

Elias, M. F., Robbins, M. A., Budge, M. M., Abhayaratna, W. P., Dore, G. A., & Elias, P. K. (2009b). Arterial pulse wave velocity and cognition with advancing age. *Hypertension*, *53*(4), 668–673.

Ellekjaer, H., Holmen, J., Indredavik, B., & Terent, A. (1997). Epidemiology of stroke in Innherred, Norway, 1994 to 1996. Incidence and 30-day case-fatality rate. *Stroke; a Journal of Cerebral Circulation*, *28*(11), 2180–2184.

Esposito, G., Van Horn, J. D., Weinberger, D. R., & Berman, K. F. (1996). Gender differences in cerebral blood flow as a function of cognitive state with PET. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *37*(4), 559–564.

Fernando, M. S., Simpson, J. E., Matthews, F., Brayne, C., Lewis, C. E., Barber, R., … MRC Cognitive Function and Ageing Neuropathology Study Group. (2006). White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury. *Stroke; a Journal of Cerebral Circulation*, *37*(6), 1391–1398.

Flück, D., Beaudin, A. E., Steinback, C. D., Kumarpillai, G., Shobha, N., McCreary, C. R., … Poulin, M. J. (2014). Effects of aging on the association between cerebrovascular responses to visual stimulation, hypercapnia and arterial stiffness. *Frontiers in Physiology*, *5*, 49.
Fossum, E., Høieggen, A., Moan, A., Nordby, G., Velund, T. L., & Kjeldsen, S. E. (1997).

Whole blood viscosity, blood pressure and cardiovascular risk factors in healthy blood donors. *Blood Pressure*, *6*(3), 161–165.

Fukuhara, M., Matsumura, K., Ansai, T., Takata, Y., Sonoki, K., Akifusa, S., … Takehara, T. (2006a). Prediction of cognitive function by arterial stiffness in the very elderly. *Circulation Journal: Official Journal of the Japanese Circulation Society*, *70*(6), 756–761.

Fukuhara, M., Matsumura, K., Ansai, T., Takata, Y., Sonoki, K., Akifusa, S., … Takehara, T. (2006b). Prediction of cognitive function by arterial stiffness in the very elderly. *Circulation Journal: Official Journal of the Japanese Circulation Society*, *70*(6), 756–761.

Gale, S. D., Baxter, L., Connor, D. J., Herring, A., & Comer, J. (2007). Sex differences on the Rey Auditory Verbal Learning Test and the Brief Visuospatial Memory Test–Revised in the elderly: Normative data in 172 participants. *Journal of Clinical and Experimental Neuropsychology*, *29*(5), 561–567.

Ganepola, T., Nagy, Z., Ghosh, A., Papadopoulo, T., Alexander, D. C., & Sereno, M. I. (2018). Using diffusion MRI to discriminate areas of cortical grey matter. *NeuroImage*, *182*, 456–468.

Garn, S. M., Smith, N. J., & Clark, D. C. (1975). Lifelong differences in hemoglobin levels between Blacks and Whites. *Journal of the National Medical Association*, *67*(2), 91–96.

Gaser, C. (2016). Structural MRI: Morphometry. In M. Reuter & C. Montag (Eds.), *Neuroeconomics* (pp. 399–409). Berlin, Heidelberg: Springer Berlin Heidelberg.

Gatzka, C. D., Kingwell, B. A., Cameron, J. D., Berry, K. L., Liang, Y.-L., Dewar, E. M., … Dart, A. M. (2001). Gender differences in the timing of arterial wave reflection beyond differences in body height. *Journal of Hypertension*, Vol. 19, pp. 2197–2203. https://doi.org/10.1097/00004872-200112000-00013

Gauthier, C. J., Desjardins-Crépeau, L., Madjar, C., Bherer, L., & Hoge, R. D. (2012a). Absolute quantification of resting oxygen metabolism and metabolic reactivity during functional activation using QUO2 MRI. *NeuroImage*, *63*(3), 1353–1363.

Gauthier, C. J., Desjardins-Crépeau, L., Madjar, C., Bherer, L., & Hoge, R. D. (2012b). Absolute quantification of resting oxygen metabolism and metabolic reactivity during functional activation using QUO2 MRI. *NeuroImage*, *63*(3), 1353–1363.

Gauthier, C. J., & Fan, A. P. (2019). BOLD signal physiology: Models and applications. *NeuroImage*, *187*, 116–127.

Gauthier, C. J., & Hoge, R. D. (2012a). Magnetic resonance imaging of resting OEF and CMRO2 using a generalized calibration model for hypercapnia and hyperoxia. *NeuroImage*, *60*(2), 1212–1225.

Gauthier, C. J., & Hoge, R. D. (2012b). Magnetic resonance imaging of resting OEF and CMRO2 using a generalized calibration model for hypercapnia and hyperoxia. *NeuroImage*, *60*(2), 1212–1225.

Gauthier, C. J., Lefort, M., Mekary, S., Desjardins-Crépeau, L., Skimminge, A., Iversen, P., … Hoge, R. D. (2015a). Hearts and minds: linking vascular rigidity and aerobic fitness with cognitive aging. *Neurobiology of Aging*, *36*(1), 304–314.
Gauthier, C. J., Lefort, M., Mekary, S., Desjardins-Crépeau, L., Skimminge, A., Iversen, P., … Hoge, R. D. (2015b). Hearts and minds: linking vascular rigidity and aerobic fitness with cognitive aging. *Neurobiology of Aging*, *36*(1), 304–314.

Gauthier, C. J., Madjar, C., Desjardins-Crépeau, L., Bellec, P., Bherer, L., & Hoge, R. D. (2013a). Age dependence of hemodynamic response characteristics in human functional magnetic resonance imaging. *Neurobiology of Aging*, *34*(5), 1469–1485.

Gauthier, C. J., Madjar, C., Desjardins-Crépeau, L., Bellec, P., Bherer, L., & Hoge, R. D. (2013b). Age dependence of hemodynamic response characteristics in human functional magnetic resonance imaging. *Neurobiology of Aging*, *34*(5), 1469–1485.

Gauthier, C. J., Madjar, C., Desjardins-Crépeau, L., Bellec, P., Bherer, L., & Hoge, R. D. (2013c). Age dependence of hemodynamic response characteristics in human functional magnetic resonance imaging. *Neurobiology of Aging*, *34*(5), 1469–1485.

Gavish, B., Tillin, T., Hughes, A. D., & Chaturvedi, N. (2017). Associations of ambulatory pulse pressure components with hippocampal volume, white matter hyperintensities and brain infarcts. *Artery Research*, Vol. 20, p. 86. https://doi.org/10.1016/j.artres.2017.10.125

Geary, G. G., Krause, D. N., & Duckles, S. P. (1998). Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries. *The American Journal of Physiology*, *275*(1), H292–H300.

Giezendanner, S., Fisler, M. S., Soravia, L. M., Andreotti, J., Walther, S., Wiest, R., … Federspiel, A. (2016). Microstructure and Cerebral Blood Flow within White Matter of the Human Brain: A TBSS Analysis. *PloS One*, *11*(3), e0150657.

Glover, G. H. (1999). Deconvolution of Impulse Response in Event-Related BOLD fMRI1. *NeuroImage*, *9*(4), 416–429.

Goddard, L., Dritschel, B., & Burton, A. (1998). Gender differences in the dual-task effects on autobiographical memory retrieval during social problem solving. *British Journal of Psychology* , *89*(4), 611–627.

Golay, X., & Petersen, E. T. (2006). Arterial spin labeling: benefits and pitfalls of high magnetic field. *Neuroimaging Clinics of North America*, *16*(2), 259–268, x.

Goldman, H., Skelley, E. B., Sandman, C. A., Kastin, A. J., & Murphy, S. (1976). Hormones

and regional brain blood flow. *Pharmacology, Biochemistry, and Behavior*, *5*(Suppl 1), 165–169.

Golestani, A. M., Wei, L. L., & Jean Chen, J. (2016). Quantitative mapping of cerebrovascular reactivity using resting-state BOLD fMRI: Validation in healthy adults. *NeuroImage*, Vol. 138, pp. 147–163. https://doi.org/10.1016/j.neuroimage.2016.05.025

Gorelick, P. B., Nyenhuis, D., American Society of Hypertension Writing Group, Materson, B. J., Calhoun, D. A., Elliott, W. J., … Townsend, R. R. (2012). Blood pressure and treatment of persons with hypertension as it relates to cognitive outcomes including executive function. *Journal of the American Society of Hypertension: JASH*, *6*(5), 309–315.

Grade, M., Hernandez Tamames, J. A., Pizzini, F. B., Achten, E., Golay, X., & Smits, M. (2015a). A neuroradiologist's guide to arterial spin labeling MRI in clinical practice. *Neuroradiology*, *57*(12), 1181–1202.

Grade, M., Hernandez Tamames, J. A., Pizzini, F. B., Achten, E., Golay, X., & Smits, M. (2015b). A neuroradiologist's guide to arterial spin labeling MRI in clinical practice. *Neuroradiology*, *57*(12), 1181–1202.

Grade, M., Hernandez Tamames, J. A., Pizzini, F. B., Achten, E., Golay, X., & Smits, M. (2015c). A neuroradiologist's guide to arterial spin labeling MRI in clinical practice. *Neuroradiology*, *57*(12), 1181–1202.

Gunning-Dixon, F. M., & Raz, N. (2000). The cognitive correlates of white matter abnormalities in normal aging: a quantitative review. *Neuropsychology*, *14*(2), 224–232.

Gunning-Dixon, F. M., & Brickman, A. M. (2009). Aging of cerebral white matter: a review of MRI findings. *Journal of Geriatric …*. Retrieved from https://onlinelibrary.wiley.com/doi/abs/10.1002/gps.2087?casa_token=QlIJGvMyLesAAAAA:XMhyxR8vDd1lwXZ_3p36x_Vi3Vcc956enEyKxEMPU6V5cBUe-MCLyBJQ_HUTTCfKIZ4kLvYj-a0n5fg

Gur, R. C., Gur, R. E., Obrist, W. D., Hungerbuhler, J. P., Younkin, D., Rosen, A. D., … Reivich, M. (1982). Sex and handedness differences in cerebral blood flow during rest and cognitive activity. *Science*, *217*(4560), 659–661.

Guttmann, C. R., Jolesz, F. A., Kikinis, R., Killiany, R. J., Moss, M. B., Sandor, T., & Albert, M. S. (1998). White matter changes with normal aging. *Neurology*, *50*(4), 972–978.

Hajjar, I., Goldstein, F. C., Martin, G. S., & Quyyumi, A. A. (2016). Roles of Arterial Stiffness and Blood Pressure in Hypertension-Associated Cognitive Decline in Healthy Adults. *Hypertension*, *67*(1), 171–175.

Halani, S., Kwinta, J. B., Golestani, A. M., Khatamian, Y. B., & Jean Chen, J. (2015). Comparing cerebrovascular reactivity measured using BOLD and cerebral blood flow MRI:

The effect of basal vascular tension on vasodilatory and vasoconstrictive reactivity. *NeuroImage*, Vol. 110, pp. 110–123. https://doi.org/10.1016/j.neuroimage.2015.01.050

Haller, S., Kövari, E., Herrmann, F. R., Cuvinciuc, V., Tomm, A.-M., Zulian, G. B., … Bouras, C. (2013). Do brain T2/FLAIR white matter hyperintensities correspond to myelin loss in normal aging? A radiologic-neuropathologic correlation study. *Acta Neuropathologica Communications*, *1*, 14.

Halpern, D. F., & LaMay, M. L. (2000). The Smarter Sex: A Critical Review of Sex Differences in Intelligence. *Educational Psychology Review*, *12*(2), 229–246.

Hanon, O., Haulon, S., Lenoir, H., Seux, M.-L., Rigaud, A.-S., Safar, M., … Forette, F. (2005). Relationship between arterial stiffness and cognitive function in elderly subjects with complaints of memory loss. *Stroke; a Journal of Cerebral Circulation*, *36*(10), 2193–2197.

Haratz, S., Weinstein, G., & Molshazki, N. (2015). Impaired cerebral hemodynamics and cognitive performance in patients with atherothrombotic disease. *Journal of*. Retrieved from https://content.iospress.com/articles/journal-of-alzheimers-disease/jad150052

Harman, D. (1990). The aging process: A major risk factor for disease and death. *Free Radical Biology and Medicine*, Vol. 9, p. 13. https://doi.org/10.1016/0891-5849(90)90212-2

Hassler, O. (1965). Vascular changes in senile brains. *Acta Neuropathologica*. Retrieved from https://link.springer.com/article/10.1007/BF00689161

Hatanaka, R., Obara, T., Watabe, D., Ishikawa, T., Kondo, T., Ishikura, K., … Imai, Y. (2011). Association of arterial stiffness with silent cerebrovascular lesions: the Ohasama study. *Cerebrovascular Diseases* , *31*(4), 329–337.

Hayes, A. F. (2017). *Introduction to Mediation, Moderation, and Conditional Process Analysis, Second Edition: A Regression-Based Approach*. Guilford Publications.

Hayes, A. F. (2017). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. Retrieved from https://books.google.com/books?hl=en&lr=&id=8ZM6DwAAQBAJ&oi=fnd&pg=PP1&dq=Hayes+A+F+(2013)+Introduction+to+mediation+moderation+and+conditional+process+analysis+A+regression-based+approach+New+York+NY+Guilford+Press&ots=20DaqH-lYA&sig=hUAeMD1MWZw3naCtSz6aGUFyHgc

Head, D., Bugg, J. M., Goate, A. M., Fagan, A. M., Mintun, M. A., Benzinger, T., … Morris, J. C. (2012). Exercise Engagement as a Moderator of the Effects of APOE Genotype on Amyloid Deposition. *Archives of Neurology*, *69*(5), 636–643.

Henskens, L. H. G., Kroon, A. A., van Oostenbrugge, R. J., Gronenschild, E. H. B. M., Fuss-Lejeune, M. M. J. J., Hofman, P. A. M., … de Leeuw, P. W. (2008). Increased aortic pulse wave velocity is associated with silent cerebral small-vessel disease in hypertensive patients.

Hypertension, *52*(6), 1120–1126.

Herlitz, A., Nilsson, L. G., & Bäckman, L. (1997). Gender differences in episodic memory. *Memory & Cognition*, *25*(6), 801–811.

Herlitz, A., & Rehnman, J. (2008). Sex differences in episodic memory. *Current Directions in Psychological Science*. Retrieved from https://journals.sagepub.com/doi/abs/10.1111/j.1467-8721.2008.00547.x

Herment, A., Kachenoura, N., Lefort, M., Bensalah, M., Dogui, A., Frouin, F., … De Cesare, A. (2010). Automated segmentation of the aorta from phase contrast MR images: validation against expert tracing in healthy volunteers and in patients with a dilated aorta. *Journal of Magnetic Resonance Imaging: JMRI*, *31*(4), 881–888.

Ho, S. C., Woo, J., Sham, A., Chan, S. G., & Yu, A. L. (2001). A 3-year follow-up study of social, lifestyle and health predictors of cognitive impairment in a Chinese older cohort. *International Journal of Epidemiology*, *30*(6), 1389–1396.

Hoge, R. D., Atkinson, J., Gill, B., Crelier, G. R., Marrett, S., & Pike, G. B. (1999). Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model. *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *42*(5), 849–863.

Hyde, J. S., & Linn, M. C. (1988). Gender differences in verbal ability: A meta-analysis. *Psychological Bulletin*, *104*(1), 53.

Ibler, M., & Lage, S. (1984). Influence of Blood-Taking Procedure on the Heart Function of Blood Donors. *Acta Anaesthesiologica Scandinavica*, Vol. 28, pp. 587–590. https://doi.org/10.1111/j.1399-6576.1984.tb02125.x

Ikram, M., Zaccai, K., Poels, M., Verwoert, G., Vernooij, M., Witteman, J., … Breteler, M. (2011). Arterial stiffness is associated with cerebral small vessel disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, *7*(4), S295.

Intzandt, B., Sabra, D., Foster, C., Desjardins-Crepeau, L., Hoge, R. D., Steele, C. J., … Gauthier, C. J. (n.d.-a). *Enhanced fitness relates to reduced cerebrovascular reactivity and perfusion in a sample of very healthy older adults*. https://doi.org/10.1101/444208

Intzandt, B., Sabra, D., Foster, C., Desjardins-Crepeau, L., Hoge, R. D., Steele, C. J., … Gauthier, C. J. (n.d.-b). *Enhanced fitness relates to reduced cerebrovascular reactivity and perfusion in a sample of very healthy older adults*. https://doi.org/10.1101/444208

Intzandt, B., Sabra, D., Foster, C., Desjardins-Crépeau, L., Hoge, R. D., Steele, C. J., … Gauthier, C. J. (2019). Higher cardiovascular fitness level is associated with lower cerebrovascular reactivity and perfusion in healthy older adults. *Journal of Cerebral Blood*

Flow & Metabolism, p. 0271678X1986287. https://doi.org/10.1177/0271678x19862873

Ito, H., Kanno, I., Ibaraki, M., Hatazawa, J., & Miura, S. (2003). Changes in Human Cerebral Blood Flow and Cerebral Blood Volume during Hypercapnia and Hypocapnia Measured by Positron Emission Tomography. *Journal of Cerebral Blood Flow & Metabolism*, Vol. 23, pp. 665–670. https://doi.org/10.1097/01.wcb.0000067721.64998.f5

Ito, S. (2012). Cardiorenal syndrome: an evolutionary point of view. *Hypertension*, *60*(3), 589–595.

Iturria-Medina, Y., Sotero, R. C., Toussaint, P. J., Mateos-Pérez, J. M., Evans, A. C., & Alzheimer's Disease Neuroimaging Initiative. (2016). Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nature Communications*, *7*, 11934.

Iulita, M. F., Noriega de la Colina, A., & Girouard, H. (2018). Arterial stiffness, cognitive impairment and dementia: confounding factor or real risk? *Journal of Neurochemistry*, *144*(5), 527–548.

J. Larner, A. (2012). Mini-Mental Parkinson (MMP) as a Dementia Screening Test: Comparison with the Mini-Mental State Examination (MMSE). *Current Aging Sciencee*, *5*(2), 136–139.

Jaruchart, T., Suwanwela, N. C., Tanaka, H., & Suksom, D. (2016). Arterial stiffness is associated with age-related differences in cerebrovascular conductance. *Experimental Gerontology*, Vol. 73, pp. 59–64. https://doi.org/10.1016/j.exger.2015.11.006

Jefferson, A. L., Cambronero, F. E., Liu, D., Moore, E. E., Neal, J. E., Terry, J. G., … Carr, J. J. (2018). Higher Aortic Stiffness is Related to Lower Cerebral Blood Flow and Preserved Cerebrovascular Reactivity in Older Adults. *Circulation*. https://doi.org/10.1161/CIRCULATIONAHA.118.032410

Jernigan, T. L., Archibald, S. L., Fennema-Notestine, C., Gamst, A. C., Stout, J. C., Bonner, J., & Hesselink, J. R. (2001). Effects of age on tissues and regions of the cerebrum and cerebellum. *Neurobiology of Aging*, *22*(4), 581–594.

Jochemsen, H. M., Muller, M., Bots, M. L., Scheltens, P., Vincken, K. L., Mali, W. P. T. M., … SMART Study Group. (2015). Arterial stiffness and progression of structural brain changes: The SMART-MR study. *Neurology*, *84*(5), 448–455.

Johnson, K. A., Gregas, M., Becker, J. A., Kinnecom, C., Salat, D. H., Moran, E. K., … Greenberg, S. M. (2007). Imaging of amyloid burden and distribution in cerebral amyloid angiopathy. *Annals of Neurology*, *62*(3), 229–234.

Johnson, N. A., Jahng, G.-H., Weiner, M. W., Miller, B. L., Chui, H. C., Jagust, W. J., … Schuff, N. (2005). Pattern of cerebral hypoperfusion in Alzheimer disease and mild cognitive

impairment measured with arterial spin-labeling MR imaging: initial experience. *Radiology*, *234*(3), 851–859.

Karas, R. H., Patterson, B. L., & Mendelsohn, M. E. (1994). Human vascular smooth muscle cells contain functional estrogen receptor. *Circulation*, *89*(5), 1943–1950.

Kastrup, A., Krüger, G., Neumann-Haefelin, T., & Moseley, M. E. (2001). Assessment of cerebrovascular reactivity with functional magnetic resonance imaging: comparison of CO2 and breath holding. *Magnetic Resonance Imaging*, *19*(1), 13–20.

Katulska, K., Wykrętowicz, M., Minczykowski, A., Krauze, T., Milewska, A., Piskorski, J., … Wykrętowicz, A. (2014). Gray matter volume in relation to cardio-vascular stiffness. *Journal of the Neurological Sciences*, *343*(1-2), 100–104.

Kennison, S. M. (2003). Sex differences in cognitive abilities (3rd edition). Diane F. Halpern. Lawrence Erlbaum Associates, Mahwah, NJ, 2000. No. of pages 420. ISBN 0-8058-2792-7. Price: $39.95. *Applied Cognitive Psychology*, Vol. 17, pp. 375–376. https://doi.org/10.1002/acp.883

Kim, J. Y., Yoon, J., Cho, M., Lee, B.-K., Karimi, A., & Shin, S. (2013). Blood characteristics effect on pulse wave velocity. *Clinical Hemorheology and Microcirculation*, *55*(1), 193–203.

Kim, J.-Y., Park, J. B., Kim, D. S., Kim, K. S., Jeong, J. W., Park, J. C., … KAAS investigators. (2014). Gender Difference in Arterial Stiffness in a Multicenter Cross-Sectional Study: The Korean Arterial Aging Study (KAAS). *The Pulse of the Montana State Nurses' Association*, *2*(1-4), 11–17.

Kim, S.-G., & Ogawa, S. (2012). Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *32*(7), 1188–1206.

Kimberly, W. T., Lima, F. O., O'Connor, S., & Furie, K. L. (2013). Sex differences and hemoglobin levels in relation to stroke outcomes. *Neurology*, Vol. 80, pp. 719–724. https://doi.org/10.1212/wnl.0b013e31828250ff

Kramer, J. H., Mungas, D., Reed, B. R., Wetzel, M. E., Burnett, M. M., Miller, B. L., … Chui, H. C. (2007). Longitudinal MRI and cognitive change in healthy elderly. *Neuropsychology*, *21*(4), 412–418.

Krause, D. N., Duckles, S. P., & Pelligrino, D. A. (2006). Influence of sex steroid hormones on cerebrovascular function. *Journal of Applied Physiology*, *101*(4), 1252–1261.

Krejza, J., Mariak, Z., Huba, M., Wolczynski, S., & Lewko, J. (2001). Effect of endogenous estrogen on blood flow through carotid arteries. *Stroke; a Journal of Cerebral Circulation*, *32*(1), 30–36.

Krejza, J., Rudzinski, W., Arkuszewski, M., Onuoha, O., & Melhem, E. R. (2013). Cerebrovascular reactivity across the menstrual cycle in young healthy women. *The Neuroradiology Journal*, *26*(4), 413–419.

Krejza, J., Siemkowicz, J., Sawicka, M., Szylak, A., Kochanowicz, J., Mariak, Z., … Bert, R. (2003). Oscillations of cerebrovascular resistance throughout the menstrual cycle in healthy women. *Ultrasound in Obstetrics & Gynecology: The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *22*(6), 627–632.

Kucharczyk, W., Macdonald, P. M., Stanisz, G. J., & Henkelman, R. M. (1994). Relaxivity and magnetization transfer of white matter lipids at MR imaging: importance of cerebrosides and pH. *Radiology*, *192*(2), 521–529.

Kurlowicz, L., & Wallace, M. (1999). The Mini-Mental State Examination (MMSE). *Journal of Gerontological Nursing*, *25*(5), 8–9.

Kyriazis, J., Tzanakis, I., Stylianou, K., Katsipi, I., Moisiadis, D., Papadaki, A., … Daphnis, E. (2011). Low serum testosterone, arterial stiffness and mortality in male haemodialysis patients. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *26*(9), 2971–2977.

Larner, A. J. (2012). Mini-mental Parkinson (MMP) as a dementia screening test: comparison with the Mini-Mental State Examination (MMSE). *Current Aging Science*, *5*(2), 136–139.

Laurent, S. (2006). European Network for Non-invasive Investigation of Large Arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *European Heart Journal*, *27*, 2588–2605.

Laurent, S., Boutouyrie, P., & Lacolley, P. (2005). Structural and genetic bases of arterial stiffness. *Hypertension*, *45*(6), 1050–1055.

Lee, G., Choi, S., Kim, K., Yun, J., Son, J. S., Jeong, S., … Park, S. M. (2018). Association of Hemoglobin Concentration and Its Change With Cardiovascular and All-Cause Mortality. *Journal of the American Heart Association*. https://doi.org/10.1161/JAHA.117.007723

Lee, S.-T., Jung, K.-H., & Lee, Y.-S. (2007). Decreased vasomotor reactivity in Alzheimer's disease. *Journal of Clinical Neurology* , *3*(1), 18–23.

Leenders, K. L., Perani, D., Lammertsma, A. A., Heather, J. D., Buckingham, P., Healy, M. J., … Herold, S. (1990). Cerebral blood flow, blood volume and oxygen utilization. Normal values and effect of age. *Brain: A Journal of Neurology*, *113 (Pt 1)*, 27–47.

Leoni, R. F., Oliveira, I. A. F., Pontes-Neto, O. M., Santos, A. C., & Leite, J. P. (2017). Cerebral blood flow and vasoreactivity in aging: an arterial spin labeling study. *Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas E Biologicas / Sociedade Brasileira de Biofisica... [et Al.]*, *50*(4), e5670.

Li, X., Wang, D., Auerbach, E. J., Moeller, S., Ugurbil, K., & Metzger, G. J. (2015). Theoretical and experimental evaluation of multi-band EPI for high-resolution whole brain pCASL Imaging. *NeuroImage*, *106*, 170–181.

Lilamand, M., Vidal, J.-S., Plichart, M., De Jong, L. W., Duron, E., & Hanon, O. (2016). Arterial stiffness and medial temporal lobe atrophy in elders with memory disorders. *Journal of Hypertension*, *34*(7), 1331–1337.

Lim, S. L., Gao, Q., Nyunt, M. S. Z., Gong, L., Lunaria, J. B., Lim, M. L., … Ng, T. P. (2016). Vascular Health Indices and Cognitive Domain Function: Singapore Longitudinal Ageing Studies. *Journal of Alzheimer's Disease: JAD*, *50*(1), 27–40.

Liu, C.-C., Liu, C.-C., Kanekiyo, T., Xu, H., & Bu, G. (2013). Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nature Reviews. Neurology*, *9*(2), 106–118.

Liu, T. T., & Wong, E. C. (2005). A signal processing model for arterial spin labeling functional MRI. *NeuroImage*, *24*(1), 207–215.

Lu, H., Xu, F., Rodrigue, K. M., Kennedy, K. M., Cheng, Y., Flicker, B., … Park, D. C. (2011a). Alterations in cerebral metabolic rate and blood supply across the adult lifespan. *Cerebral Cortex* , *21*(6), 1426–1434.

Lu, H., Xu, F., Rodrigue, K. M., Kennedy, K. M., Cheng, Y., Flicker, B., … Park, D. C. (2011b). Alterations in cerebral metabolic rate and blood supply across the adult lifespan. *Cerebral Cortex* , *21*(6), 1426–1434.

Maas, A. H. E. M., A H E, & Appelman, Y. E. A. (2010a). Gender differences in coronary heart disease. *Netherlands Heart Journal*, Vol. 18, pp. 598–603. https://doi.org/10.1007/s12471-010-0841-y

Maas, A. H. E. M., A H E, & Appelman, Y. E. A. (2010b). Gender differences in coronary heart disease. *Netherlands Heart Journal*, Vol. 18, pp. 598–603. https://doi.org/10.1007/s12471-010-0841-y

Madden, D. J., Whiting, W. L., Huettel, S. A., White, L. E., MacFall, J. R., & Provenzale, J. M. (2004). Diffusion tensor imaging of adult age differences in cerebral white matter: relation to response time. *NeuroImage*, *21*(3), 1174–1181.

Magness, R. R., Parker, C. R., Jr, & Rosenfeld, C. R. (1993). Systemic and uterine responses to chronic infusion of estradiol-17 beta. *The American Journal of Physiology*, *265*(5 Pt 1), E690–E698.

Maillard, P., Fletcher, E., Lockhart, S. N., Roach, A. E., Reed, B., Mungas, D., … Carmichael, O. T. (2014). White matter hyperintensities and their penumbra lie along a continuum of injury in the aging brain. *Stroke; a Journal of Cerebral Circulation*, *45*(6), 1721–1726.

Maillard, P., Mitchell, G. F., Himali, J. J., Beiser, A., Fletcher, E., Tsao, C. W., … DeCarli, C. (2017). Aortic Stiffness, Increased White Matter Free Water, and Altered Microstructural Integrity: A Continuum of Injury. *Stroke; a Journal of Cerebral Circulation*, *48*(6), 1567–1573.

Maillard, P., Mitchell, G. F., Himali, J. J., Beiser, A., Tsao, C. W., Pase, M. P., … DeCarli, C. (2016). Effects of Arterial Stiffness on Brain Integrity in Young Adults From the Framingham Heart Study. *Stroke; a Journal of Cerebral Circulation*, *47*(4), 1030–1036.

Mandell, D. M., Han, J. S., Poublanc, J., Crawley, A. P., Stainsby, J. A., Fisher, J. A., & Mikulis, D. J. (2008). Mapping cerebrovascular reactivity using blood oxygen level-dependent MRI in Patients with arterial steno-occlusive disease: comparison with arterial spin labeling MRI. *Stroke; a Journal of Cerebral Circulation*, *39*(7), 2021–2028.

Marchal, G., Rioux, P., Petit-Taboué, M. C., Sette, G., Travère, J. M., Le Poec, C., … Baron, J. C. (1992). Regional cerebral oxygen consumption, blood flow, and blood volume in healthy human aging. *Archives of Neurology*, *49*(10), 1013–1020.

Martin, P. J., Evans, D. H., & Naylor, A. R. (1994). Transcranial color-coded sonography of the basal cerebral circulation. Reference data from 115 volunteers. *Stroke; a Journal of Cerebral Circulation*, *25*(2), 390–396.

Matteis, M., Troisi, E., Monaldo, B. C., Caltagirone, C., & Silvestrini, M. (1998a). Age and sex differences in cerebral hemodynamics: a transcranial Doppler study. *Stroke; a Journal of Cerebral Circulation*, *29*(5), 963–967.

Matteis, M., Troisi, E., Monaldo, B. C., Caltagirone, C., & Silvestrini, M. (1998b). Age and sex differences in cerebral hemodynamics: a transcranial Doppler study. *Stroke; a Journal of Cerebral Circulation*, *29*(5), 963–967.

Mawuenyega, K. G., Sigurdson, W., Ovod, V., Munsell, L., Kasten, T., Morris, J. C., … Bateman, R. J. (2010). Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science*, *330*(6012), 1774.

McCarrey, A. C., An, Y., Kitner-Triolo, M. H., Ferrucci, L., & Resnick, S. M. (2016). Sex differences in cognitive trajectories in clinically normal older adults. *Psychology and Aging*, *31*(2), 166–175.

McGowan, J. F., & Duka, T. (2000). Hemispheric lateralisation in a manual–verbal task combination: the role of modality and gender. *Neuropsychologia*, *38*(7), 1018–1027.

McKetton, L., Sobczyk, O., Duffin, J., Poublanc, J., Sam, K., Crawley, A. P., … Mikulis, D. J. (2018a). The aging brain and cerebrovascular reactivity. *NeuroImage*, *181*, 132–141.

McKetton, L., Sobczyk, O., Duffin, J., Poublanc, J., Sam, K., Crawley, A. P., … Mikulis, D. J.

(2018b). The aging brain and cerebrovascular reactivity. *NeuroImage*, *181*, 132–141.

McKetton, L., Sobczyk, O., Duffin, J., Poublanc, J., Sam, K., Crawley, A. P., … Mikulis, D. J. (2018c). The aging brain and cerebrovascular reactivity. *NeuroImage*, *181*, 132–141.

McKetton, L., Sobczyk, O., Duffin, J., Poublanc, J., Sam, K., Crawley, A. P., … Mikulis, D. J. (2018d). The aging brain and cerebrovascular reactivity. *NeuroImage*, *181*, 132–141.

Meinz, E. J., & Salthouse, T. A. (1998). Is age kinder to females than to males? *Psychonomic Bulletin & Review*, *5*(1), 56–70.

Members, W. G., Lloyd-Jones, D., Adams, R. J., Brown, T. M., Carnethon, M., Dai, S., … Others. (2010). Executive summary: heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation*, *121*(7), 948–954.

Mendelsohn, M. E., & Karas, R. H. (1999). The protective effects of estrogen on the cardiovascular system. *The New England Journal of Medicine*, *340*(23), 1801–1811.

Millasseau, S. C., Stewart, A. D., Patel, S. J., Redwood, S. R., & Chowienczyk, P. J. (2005). Evaluation of carotid-femoral pulse wave velocity: influence of timing algorithm and heart rate. *Hypertension*, *45*(2), 222–226.
Miller, K. L., Bulte, D. P., Devlin, H., Robson, M. D., Wise, R. G., Woolrich, M. W., … Behrens, T. E. J. (2007). Evidence for a vascular contribution to diffusion FMRI at high b value. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(52), 20967–20972.

Minkner, K., Lovblad, K. O., Yilmaz, H., Alimenti, A., Sekoranja, L., Delavelle, J., … Rüfenacht, D. A. (2005). White matter lesions in watershed territories studied with MRI and parenchymography: a comparative study. *Neuroradiology*, *47*(6), 425–430.

Mitchell, G. F. (2008). Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *Journal of Applied Physiology*, *105*(5), 1652–1660.

Mitchell, G. F. (2009). Arterial Stiffness and Wave Reflection: Biomarkers of Cardiovascular Risk. *Artery Research*, *3*(2), 56–64.

Mitchell, G. F. (2014). Arterial Stiffness and Hypertension. *Hypertension*, Vol. 64, pp. 210–214. https://doi.org/10.1161/hypertensionaha.114.03449

Mitchell, G. F., van Buchem, M. A., Sigurdsson, S., Gotal, J. D., Jonsdottir, M. K., Kjartansson, Ó., … Launer, L. J. (2011). Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility--Reykjavik study. *Brain: A Journal of Neurology*, *134*(Pt 11), 3398–3407.

Mitchell, G. F., van Buchem, M. A., Sigurdsson, S., Gotal, J. D., Jonsdottir, M. K.,

Kjartansson, Ó., … Others. (2011). Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility--Reykjavik study. *Brain: A Journal of Neurology*, *134*(11), 3398–3407.

Montagne, A., Nation, D. A., Pa, J., Sweeney, M. D., Toga, A. W., & Zlokovic, B. V. (2016). Brain imaging of neurovascular dysfunction in Alzheimer's disease. *Acta Neuropathologica*, *131*(5), 687–707.

Moody, D. M., Santamore, W. P., & Bell, M. A. (1991). Does tortuosity in cerebral arterioles impair down-autoregulation in hypertensives and elderly normotensives? A hypothesis and computer model. *Clinical Neurosurgery*, *37*, 372–387.

Mori, S., & Zhang, J. (2006). Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron*, *51*(5), 527–539.

Murphy, W. G. (2014). The sex difference in haemoglobin levels in adults — Mechanisms, causes, and consequences. *Blood Reviews*, Vol. 28, pp. 41–47. https://doi.org/10.1016/j.blre.2013.12.003

Murrell, C. J., Cotter, J. D., Thomas, K. N., Lucas, S. J. E., Williams, M. J. A., & Ainslie, P. N. (2013). Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: effect of age and 12-week exercise training. *Age* , *35*(3), 905–920.

Najjar, S. S., Scuteri, A., & Lakatta, E. G. (2005). Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension*, *46*(3), 454–462.

Narkiewicz, K., Kjeldsen, S. E., & Hedner, T. (2006). Hypertension and cardiovascular disease in women: Progress towards better understanding of gender-specific differences? *Blood Pressure*, *15*(2), 68–70.

Nevo, O., Soustiel, J. F., & Thaler, I. (2007). Cerebral blood flow is increased during controlled ovarian stimulation. *American Journal of Physiology. Heart and Circulatory Physiology*, *293*(6), H3265–H3269.

Niccoli, T., & Partridge, L. (2012). Ageing as a risk factor for disease. *Current Biology: CB*, *22*(17), R741–R752.

Nieboer, D., Douw, L., van Dijk, B. W., Heymans, M. W., Stam, C. J., & Twisk, J. W. R. (2016). Relation between carotid stiffness, cognitive performance and brain connectivity in a healthy middle-aged population: an observational neurophysiological cohort study with magnetoencephalography. *BMJ Open*, *6*(12), e013441.

Novak, V. (2012a). Cognition and Hemodynamics. *Current Cardiovascular Risk Reports*, *6*(5), 380–396.

Novak, V. (2012b). Cognition and Hemodynamics. *Current Cardiovascular Risk Reports*,

6(5), 380–396.

Novikov, D. S., Kiselev, V. G., & Jespersen, S. N. (2018). On modeling. *Magnetic Resonance in Medicine*, Vol. 79, pp. 3172–3193. https://doi.org/10.1002/mrm.27101

O'Rourke, M. F., & Hashimoto, J. (2007). Mechanical Factors in Arterial Aging. *Journal of the American College of Cardiology*, Vol. 50, pp. 1–13. https://doi.org/10.1016/j.jacc.2006.12.050

O'Rourke, M. F., & Safar, M. E. (2005a). Relationship Between Aortic Stiffening and Microvascular Disease in Brain and Kidney. *Hypertension*, Vol. 46, pp. 200–204. https://doi.org/10.1161/01.hyp.0000168052.00426.65

O'Rourke, M. F., & Safar, M. E. (2005b). Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. *Hypertension*, *46*(1), 200–204.

Ochi, N., Kohara, K., Tabara, Y., Nagai, T., Kido, T., Uetani, E., … Miki, T. (2010). Association of Central Systolic Blood Pressure With Intracerebral Small Vessel Disease in Japanese. *American Journal of Hypertension*, Vol. 23, pp. 889–894. https://doi.org/10.1038/ajh.2010.60

Ogola, B. O., Zimmerman, M. A., Clark, G. L., Abshire, C. M., Gentry, K. M., Miller, K. S., & Lindsey, S. H. (2018). New insights into arterial stiffening: does sex matter? *American Journal of Physiology. Heart and Circulatory Physiology*, *315*(5), H1073–H1087.

Ohkura, T., Teshima, Y., Isse, K., Matsuda, H., Inoue, T., Sakai, Y., … Yaoi, Y. (1995). Estrogen Increases Cerebral and Cerebellar Blood Flows in Postmenopausal Women. *Menopause* , *2*(1), 13.

Ohmine, T., Miwa, Y., Hiroshi, Y. A. O., Yuzuriha, T., Takashima, Y., Uchino, A., … Sasaguri, T. (2008). Association between Arterial Stiffness and Cerebral White Matter Lesions in Community-Dwelling Elderly Subjects. *Hypertension Research*, Vol. 31, pp. 75–81. https://doi.org/10.1291/hypres.31.75

Okamoto, Y., Yamamoto, T., Kalaria, R. N., Senzaki, H., Maki, T., Hase, Y., … Ihara, M. (2012). Cerebral hypoperfusion accelerates cerebral amyloid angiopathy and promotes cortical microinfarcts. *Acta Neuropathologica*, *123*(3), 381–394.

Oláh, L., Valikovics, A., Bereczki, D., Fülesdi, B., Munkácsy, C., & Csiba, L. (2000). Gender-Related Differences in Acetazolamide-Induced Cerebral Vasodilatory Response: A Transcranial Doppler Study. *Journal of Neuroimaging*, Vol. 10, pp. 151–156. https://doi.org/10.1111/jon2000103151

Orshal, J. M., & Khalil, R. A. (2004). Gender, sex hormones, and vascular tone. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *286*(2), R233–

R249.

Ostrovskaya, M. A., Rojas, M., Kupferman, J. C., Lande, M. B., Paterno, K., Brosgol, Y., & Pavlakis, S. G. (2015). Executive function and cerebrovascular reactivity in pediatric hypertension. *Journal of Child Neurology*, *30*(5), 543–546.

Painter, P. R. (2008). The velocity of the arterial pulse wave: a viscous-fluid shock wave in an elastic tube. *Theoretical Biology & Medical Modelling*, *5*, 15.

Palombo, M., Shemesh, N., Ronen, I., & Valette, J. (2018). Insights into brain microstructure from in vivo DW-MRS. *NeuroImage*, *182*, 97–116.

Pantoni, L. (2002). Pathophysiology of Age-Related Cerebral White Matter Changes. *Cerebrovascular Diseases*, Vol. 13, pp. 7–10. https://doi.org/10.1159/000049143

Pantoni, L., Poggesi, A., & Inzitari, D. (2007). The relation between white-matter lesions and cognition. *Current Opinion in Neurology*, Vol. 20, pp. 390–397. https://doi.org/10.1097/wco.0b013e328172d661

Parkes, L. M., Rashid, W., Chard, D. T., & Tofts, P. S. (2004a). Normal cerebral perfusion measurements using arterial spin labeling: reproducibility, stability, and age and gender effects. *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *51*(4), 736–743.

Parkes, L. M., Rashid, W., Chard, D. T., & Tofts, P. S. (2004b). Normal cerebral perfusion measurements using arterial spin labeling: reproducibility, stability, and age and gender effects. *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *51*(4), 736–743.

Parkes, L. M., Rashid, W., Chard, D. T., & Tofts, P. S. (2004c). Normal cerebral perfusion measurements using arterial spin labeling: reproducibility, stability, and age and gender effects. *Magnetic Resonance in Medicine: Official Journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, *51*(4), 736–743.

Parkhurst, K. L., Lin, H.-F., Devan, A. E., Barnes, J. N., Tarumi, T., & Tanaka, H. (2012). Contribution of blood viscosity in the assessment of flow-mediated dilation and arterial stiffness. *Vascular Medicine* , *17*(4), 231–234.

Pase, M. P. (2012). Modifiable Vascular Markers for Cognitive Decline and Dementia: The Importance of Arterial Aging and Hemodynamic Factors. *Journal of Alzheimer's Disease*, Vol. 32, pp. 653–663. https://doi.org/10.3233/jad-2012-120565

Pase, M. P., Herbert, A., Grima, N. A., Pipingas, A., & O'Rourke, M. F. (2012). Arterial stiffness as a cause of cognitive decline and dementia: a systematic review and meta-analysis. *Internal Medicine Journal*, Vol. 42, pp. 808–815. https://doi.org/10.1111/j.1445-5994.2011.02645.x

Pase, M. P., Himali, J. J., Mitchell, G. F., Beiser, A., Maillard, P., Tsao, C., … Seshadri, S. (2016). Association of Aortic Stiffness With Cognition and Brain Aging in Young and Middle-Aged Adults: The Framingham Third Generation Cohort Study. *Hypertension*, *67*(3), 513–519.

Penny, W. D., Friston, K. J., Ashburner, J. T., Kiebel, S. J., & Nichols, T. E. (2011). *Statistical Parametric Mapping: The Analysis of Functional Brain Images*. Elsevier.

Pepine, C. J., Nichols, W. W., & Pauly, D. F. (2006). Estrogen and different aspects of vascular disease in women and men. *Circulation Research*, *99*(5), 459–461.

Petersen, R. C., Roberts, R. O., Knopman, D. S., Geda, Y. E., Cha, R. H., Pankratz, V. S., … Rocca, W. A. (2010). Prevalence of mild cognitive impairment is higher in men. The Mayo Clinic Study of Aging. *Neurology*, *75*(10), 889–897.

Petit-Taboué, M. C., Landeau, B., Desson, J. F., Desgranges, B., & Baron, J. C. (1998). Effects of healthy aging on the regional cerebral metabolic rate of glucose assessed with statistical parametric mapping. *NeuroImage*, *7*(3), 176–184.

Petrea, R. E., Beiser, A. S., Seshadri, S., Kelly-Hayes, M., Kase, C. S., & Wolf, P. A. (2009). Gender differences in stroke incidence and poststroke disability in the Framingham heart study. *Stroke; a Journal of Cerebral Circulation*, *40*(4), 1032–1037.

Pillai, J. J., & Mikulis, D. J. (2015). Cerebrovascular reactivity mapping: an evolving standard for clinical functional imaging. *AJNR. American Journal of Neuroradiology*, *36*(1), 7–13.

Poels, M. M. F., van Oijen, M., Mattace-Raso, F. U. S., Hofman, A., Koudstaal, P. J., Witteman, J. C. M., & Breteler, M. M. B. (2007a). Arterial Stiffness, Cognitive Decline, and Risk of Dementia. *Stroke*, Vol. 38, pp. 888–892. https://doi.org/10.1161/01.str.0000257998.33768.87

Poels, M. M. F., van Oijen, M., Mattace-Raso, F. U. S., Hofman, A., Koudstaal, P. J., Witteman, J. C. M., & Breteler, M. M. B. (2007b). Arterial stiffness, cognitive decline, and risk of dementia: the Rotterdam study. *Stroke; a Journal of Cerebral Circulation*, *38*(3), 888–892.

Poels, M. M. F., van Oijen, M., Mattace-Raso, F. U. S., Hofman, A., Koudstaal, P. J., Witteman, J. C. M., & Breteler, M. M. B. (2007c). Arterial stiffness, cognitive decline, and risk of dementia: the Rotterdam study. *Stroke; a Journal of Cerebral Circulation*, *38*(3), 888–892.

Qiu, H., Depre, C., Ghosh, K., Resuello, R. G., Natividad, F. F., Rossi, F., … Vatner, S. F. (2007). Mechanism of gender-specific differences in aortic stiffness with aging in nonhuman primates. *Circulation*, *116*(6), 669–676.

Rajkumar, C., Kingwell, B. A., Cameron, J. D., Waddell, T., Mehra, R., Christophidis, N., … Dart, A. M. (1997). Hormonal therapy increases arterial compliance in postmenopausal women. *Journal of the American College of Cardiology*, *30*(2), 350–356.

Raz, N., & Rodrigue, K. M. (2006). Differential aging of the brain: Patterns, cognitive correlates and modifiers. *Neuroscience & Biobehavioral Reviews*, Vol. 30, pp. 730–748. https://doi.org/10.1016/j.neubiorev.2006.07.001

Read, S., Pedersen, N. L., Gatz, M., Berg, S., Vuoksimaa, E., Malmberg, B., … McClearn, G. E. (2006). Sex differences after all those years? Heritability of cognitive abilities in old age. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, *61*(3), P137–P143.

Regnault, V., Thomas, F., Safar, M. E., Osborne-Pellegrin, M., Khalil, R. A., Pannier, B., & Lacolley, P. (2012). Sex difference in cardiovascular risk: role of pulse pressure amplification. *Journal of the American College of Cardiology*, *59*(20), 1771–1777.

Reich, T., & Rusinek, H. (1989a). Cerebral cortical and white matter reactivity to carbon dioxide. *Stroke; a Journal of Cerebral Circulation*, *20*(4), 453–457.

Reich, T., & Rusinek, H. (1989b). Cerebral cortical and white matter reactivity to carbon dioxide. *Stroke; a Journal of Cerebral Circulation*, *20*(4), 453–457.

Resnick, S. M., Maki, P. M., Golski, S., Kraut, M. A., & Zonderman, A. B. (1998). Effects of estrogen replacement therapy on PET cerebral blood flow and neuropsychological performance. *Hormones and Behavior*, *34*(2), 171–182.

Robison, L. S., Gannon, O. J., Salinero, A. E., & Zuloaga, K. L. (2019). Contributions of sex to cerebrovascular function and pathology. *Brain Research*, *1710*, 43–60.

Rodrigue, K. M., Kennedy, K. M., Devous, M. D., Sr, Rieck, J. R., Hebrank, A. C., Diaz-Arrastia, R., … Park, D. C. (2012). β-Amyloid burden in healthy aging: regional distribution and cognitive consequences. *Neurology*, *78*(6), 387–395.

Rodriguez, G., Warkentin, S., Risberg, J., & Rosadini, G. (1988). Sex differences in regional cerebral blood flow. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *8*(6), 783–789.

Rosano, C., Kuller, L. H., Chung, H., Arnold, A. M., Longstreth, W. T., & Newman, A. B. (2005). Subclinical Brain Magnetic Resonance Imaging Abnormalities Predict Physical Functional Decline in High-Functioning Older Adults. *Journal of the American Geriatrics Society*, Vol. 53, pp. 649–654. https://doi.org/10.1111/j.1532-5415.2005.53214.x

Rosano, C., Watson, N., Chang, Y., Newman, A. B., Aizenstein, H. J., Du, Y., … Sutton-Tyrrell, K. (2013). Aortic Pulse Wave Velocity Predicts Focal White Matter Hyperintensities in a Biracial Cohort of Older Adults. *Hypertension*, Vol. 61, pp. 160–165.

https://doi.org/10.1161/hypertensionaha.112.198069

Rossi, P., Francès, Y., Kingwell, B. A., & Ahimastos, A. A. (2011). Gender differences in artery wall biomechanical properties throughout life. *Journal of Hypertension*, *29*(6), 1023–1033.

Rostrup, E., Law, I., Blinkenberg, M., Larsson, H. B., Born, A. P., Holm, S., & Paulson, O. B. (2000). Regional differences in the CBF and BOLD responses to hypercapnia: a combined PET and fMRI study. *NeuroImage*, *11*(2), 87–97.

Sachdev, P. S. (2005). White matter hyperintensities are related to physical disability and poor motor function. *Journal of Neurology, Neurosurgery & Psychiatry*, Vol. 76, pp. 362–367. https://doi.org/10.1136/jnnp.2004.042945

Sarrel, P. M. (1990). Ovarian hormones and the circulation. *Maturitas*, *12*(3), 287–298.

Sasson, E., Doniger, G. M., Pasternak, O., Tarrasch, R., & Assaf, Y. (2013). White matter correlates of cognitive domains in normal aging with diffusion tensor imaging. *Frontiers in Neuroscience*, Vol. 7. https://doi.org/10.3389/fnins.2013.00032
Satterthwaite, T. D., Shinohara, R. T., Wolf, D. H., Hopson, R. D., Elliott, M. A., Vandekar, S. N., … Gur, R. E. (2014). Impact of puberty on the evolution of cerebral perfusion during adolescence. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(23), 8643–8648.

Schmahmann, J. D. (2003a). Vascular syndromes of the thalamus. *Stroke; a Journal of Cerebral Circulation*, *34*(9), 2264–2278.

Schmahmann, J. D. (2003b). Vascular syndromes of the thalamus. *Stroke; a Journal of Cerebral Circulation*, *34*(9), 2264–2278.

Scuteri, A., Brancati, A. M., Gianni, W., Assisi, A., & Volpe, M. (2005). Arterial stiffness is an independent risk factor for cognitive impairment in the elderly: a pilot study. *Journal of Hypertension*, *23*(6), 1211–1216.

Scuteri, A., Nilsson, P. M., Tzourio, C., Redon, J., & Laurent, S. (2011). Microvascular brain damage with aging and hypertension: pathophysiological consideration and clinical implications. *Journal of Hypertension*, *29*(8), 1469–1477.

Segers, P., Rietzschel, E. R., De Buyzere, M. L., Vermeersch, S. J., De Bacquer, D., Van Bortel, L. M., … Verdonck, P. R. (2007a). Noninvasive (Input) Impedance, Pulse Wave Velocity, and Wave Reflection in Healthy Middle-Aged Men and Women. *Hypertension*, Vol. 49, pp. 1248–1255. https://doi.org/10.1161/hypertensionaha.106.085480

Segers, P., Rietzschel, E. R., De Buyzere, M. L., Vermeersch, S. J., De Bacquer, D., Van Bortel, L. M., … Verdonck, P. R. (2007b). Noninvasive (Input) Impedance, Pulse Wave Velocity, and Wave Reflection in Healthy Middle-Aged Men and Women. *Hypertension*, Vol.

49, pp. 1248–1255. https://doi.org/10.1161/hypertensionaha.106.085480

Seo, E. H., Lee, D. Y., Choo, I. H., Kim, S. G., Kim, K. W., Youn, J. C., … Woo, J. I. (2008). Normative study of the Stroop Color and Word Test in an educationally diverse elderly population. *International Journal of Geriatric Psychiatry*, *23*(10), 1020–1027.

Shaw, L. J., Merz, C. N. B., Pepine, C. J., Reis, S. E., Bittner, V., Kelsey, S. F., … Others. (2006). Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part I: gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. *Journal of the American College of Cardiology*, *47*(3 Supplement), S4–S20.

Shaywitz, S. E. (1999). Effect of Estrogen on Brain Activation Patterns in Postmenopausal Women During Working Memory Tasks. *JAMA*, Vol. 281, p. 1197. https://doi.org/10.1001/jama.281.13.1197

Sheifer, S. E., Canos, M. R., Weinfurt, K. P., Arora, U. K., Mendelsohn, F. O., Gersh, B. J., & Weissman, N. J. (2000). Sex differences in coronary artery size assessed by intravascular ultrasound. *American Heart Journal*, *139*(4), 649–653.

Sherwin, B. B. (1996). S42 Estrogen and memory in women. *Maturitas*, Vol. 27, p. 36. https://doi.org/10.1016/s0378-5122(97)80945-0

Sherwin, B. B. (1997). Estrogen effects on cognition in menopausal women. *Neurology*, *48*(5 Suppl 7), S21–S26.

Sherwin, B. B. (2003). Estrogen and cognitive functioning in women. *Endocrine Reviews*, *24*(2), 133–151.

Shrestha, I., Takahashi, T., Nomura, E., Ohtsuki, T., Ohshita, T., Ueno, H., … Matsumoto, M. (2009). Association between central systolic blood pressure, white matter lesions in cerebral MRI and carotid atherosclerosis. *Hypertension Research: Official Journal of the Japanese Society of Hypertension*, *32*(10), 869–874.

Silvennoinen, M. J., Kettunen, M. I., & Kauppinen, R. A. (2003). Effects of hematocrit and oxygen saturation level on blood spin-lattice relaxation. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine*, *49*(3), 568–571.

Silvestrini, M., Pasqualetti, P., Baruffaldi, R., Bartolini, M., Handouk, Y., Matteis, M., … Vernieri, F. (2006a). Cerebrovascular reactivity and cognitive decline in patients with Alzheimer disease. *Stroke; a Journal of Cerebral Circulation*, *37*(4), 1010–1015.

Silvestrini, M., Pasqualetti, P., Baruffaldi, R., Bartolini, M., Handouk, Y., Matteis, M., … Vernieri, F. (2006b). Cerebrovascular reactivity and cognitive decline in patients with Alzheimer disease. *Stroke; a Journal of Cerebral Circulation*, *37*(4), 1010–1015.

Singer, J., Trollor, J. N., Baune, B. T., Sachdev, P. S., & Smith, E. (2014). Arterial stiffness, the brain and cognition: a systematic review. *Ageing Research Reviews*, *15*, 16–27.

Singer, J., Trollor, J. N., Crawford, J., O'Rourke, M. F., Baune, B. T., Brodaty, H., … Smith, E. (2013a). The association between pulse wave velocity and cognitive function: the Sydney Memory and Ageing Study. *PloS One*, *8*(4), e61855.

Singer, J., Trollor, J. N., Crawford, J., O'Rourke, M. F., Baune, B. T., Brodaty, H., … Smith, E. (2013b). The association between pulse wave velocity and cognitive function: the Sydney Memory and Ageing Study. *PloS One*, *8*(4), e61855.

Sizemore, B. C., Barrow, G., Johnson, B. D., Kip, K., von Mering, G. O., Kerensky, R. A., … Pepine, C. J. (2005). Pulse pressure is a stronger predictor of mortality and cardiovascular events than systolic blood pressure in women: A report from the NHLBI-sponsored women's ischemia syndrome evaluation (WISE) study. *Circulation*, *112*, U742–U742. LIPPINCOTT WILLIAMS & WILKINS 530 WALNUT ST, PHILADELPHIA, PA 19106-3261 USA.

Smith, W. C., Lowe, G. D., Lee, A. J., & Tunstall-Pedoe, H. (1992). Rheological determinants of blood pressure in a Scottish adult population. *Journal of Hypertension*, *10*(5), 467–472.

Song, S.-K., Yoshino, J., Le, T. Q., Lin, S.-J., Sun, S.-W., Cross, A. H., & Armstrong, R. C. (2005). Demyelination increases radial diffusivity in corpus callosum of mouse brain. *NeuroImage*, Vol. 26, pp. 132–140. https://doi.org/10.1016/j.neuroimage.2005.01.028

Stojadinović, B., Tenne, T., Zikich, D., Rajković, N., Milošević, N., Lazović, B., & Žikić, D. (2015). Effect of viscosity on the wave propagation: Experimental determination of compression and expansion pulse wave velocity in fluid-fill elastic tube. *Journal of Biomechanics*, *48*(15), 3969–3974.

Suchy, Y. (2015). *Executive Functioning: A Comprehensive Guide for Clinical Practice*. Oxford University Press.
Sugawara, N., Yasui-Furukori, N., Umeda, T., Kaneda, A., Sato, Y., Takahashi, I., … Kaneko, S. (2010). Comparison of ankle-brachial pressure index and pulse wave velocity as markers of cognitive function in a community-dwelling population. *BMC Psychiatry*, *10*, 46.

Sullivan, E. V., Rosenbloom, M., Serventi, K. L., & Pfefferbaum, A. (2004). Effects of age and sex on volumes of the thalamus, pons, and cortex. *Neurobiology of Aging*, Vol. 25, pp. 185–192. https://doi.org/10.1016/s0197-4580(03)00044-7

Sullivan, M. L., Martinez, C. M., Gennis, P., & John Gallagher, E. (1998). The cardiac toxicity of anabolic steroids. *Progress in Cardiovascular Diseases*, Vol. 41, pp. 1–15. https://doi.org/10.1016/s0033-0620(98)80019-4

Suzuki, H., Gao, H., Bai, W., Evangelou, E., Glocker, B., O'Regan, D. P., … Matthews, P. M. (2017). Abnormal brain white matter microstructure is associated with both pre-hypertension

and hypertension. *PLOS ONE*, Vol. 12, p. e0187600. https://doi.org/10.1371/journal.pone.0187600

Tardif, C. L., Gauthier, C. J., Steele, C. J., Bazin, P.-L., Schäfer, A., Schaefer, A., … Villringer, A. (2016). Advanced MRI techniques to improve our understanding of experience-induced neuroplasticity. *NeuroImage*, *131*, 55–72.

Tardif, C. L., Steele, C. J., Lampe, L., Bazin, P.-L., Ragert, P., Villringer, A., & Gauthier, C. J. (2017). Investigation of the confounding effects of vasculature and metabolism on computational anatomy studies. *NeuroImage*, *149*, 233–243.

Tarumi, T., Ayaz Khan, M., Liu, J., Tseng, B. Y., Tseng, B. M., Parker, R., … Zhang, R. (2014). Cerebral hemodynamics in normal aging: central artery stiffness, wave reflection, and pressure pulsatility. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *34*(6), 971–978.

Tarumi, T., de Jong, D. L. K., Zhu, D. C., Tseng, B. Y., Liu, J., Hill, C., … Zhang, R. (2015). Central artery stiffness, baroreflex sensitivity, and brain white matter neuronal fiber integrity in older adults. *NeuroImage*, *110*, 162–170.

Tarumi, T., Gonzales, M. M., Fallow, B., Nualnim, N., Pyron, M., Tanaka, H., & Haley, A. P. (2013a). Central artery stiffness, neuropsychological function, and cerebral perfusion in sedentary and endurance-trained middle-aged adults. *Journal of Hypertension*, *31*(12), 2400.

Tarumi, T., Gonzales, M. M., Fallow, B., Nualnim, N., Pyron, M., Tanaka, H., & Haley, A. P. (2013b). Central artery stiffness, neuropsychological function, and cerebral perfusion in sedentary and endurance-trained middle-aged adults. *Journal of Hypertension*, *31*(12), 2400–2409.

Tarumi, T., Khan, M. A., Liu, J., Tseng, B. Y., Parker, R., Riley, J., … Zhang, R. (2014). Cerebral Hemodynamics in Normal Aging: Central Artery Stiffness, Wave Reflection, and Pressure Pulsatility. *Journal of Cerebral Blood Flow & Metabolism*, Vol. 34, pp. 1255–1255. https://doi.org/10.1038/jcbfm.2014.76

Tarumi, T., Shah, F., Tanaka, H., & Haley, A. P. (2011). Association between central elastic artery stiffness and cerebral perfusion in deep subcortical gray and white matter. *American Journal of Hypertension*, *24*(10), 1108–1113.

Tarumi, T., Thomas, B. P., Wang, C., Zhang, L., Liu, J., Turner, M., … Zhang, R. (2019). Ambulatory pulse pressure, brain neuronal fiber integrity, and cerebral blood flow in older adults. *Journal of Cerebral Blood Flow & Metabolism*, Vol. 39, pp. 926–936. https://doi.org/10.1177/0271678x17745027

Tarumi, T., & Zhang, R. (2018). Cerebral blood flow in normal aging adults: cardiovascular determinants, clinical implications, and aerobic fitness. *Journal of Neurochemistry*, *144*(5), 595–608.

Tegeler, C. H., Crutchfield, K., Katsnelson, M., Kim, J., Tang, R., Passmore Griffin, L., … Evans, G. (2013). Transcranial Doppler velocities in a large, healthy population. *Journal of Neuroimaging: Official Journal of the American Society of Neuroimaging*, *23*(3), 466–472.

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

Thompson, P. D., Cullinane, E. M., Sady, S. P., Chenevert, C., Saritelli, A. L., Sady, M. A., & Herbert, P. N. (1989). Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. *JAMA: The Journal of the American Medical Association*, *261*(8), 1165–1168.

Thompson, P. M., Hayashi, K. M., de Zubicaray, G., Janke, A. L., Rose, S. E., Semple, J., … Toga, A. W. (2003). Dynamics of Gray Matter Loss in Alzheimer's Disease. *The Journal of Neuroscience*, Vol. 23, pp. 994–1005. https://doi.org/10.1523/jneurosci.23-03-00994.2003

Thore, C. R., Anstrom, J. A., Moody, D. M., Challa, V. R., Marion, M. C., & Brown, W. R. (2007). Morphometric analysis of arteriolar tortuosity in human cerebral white matter of preterm, young and aged subjects. *Journal of Neuropathology and Experimental Neurology*, Vol. 66, p. 446. https://doi.org/10.1097/01.jnen.0000268923.72881.08

Tombaugh, T. N., & McIntyre, N. J. (1992). The mini-mental state examination: a comprehensive review. *Journal of the American Geriatrics Society*, *40*(9), 922–935.

Tomoto, T., Sugawara, J., Nogami, Y., Aonuma, K., & Maeda, S. (2015). The influence of central arterial compliance on cerebrovascular hemodynamics: insights from endurance training intervention. *Journal of Applied Physiology*, *119*(5), 445–451.

Tontisirin, N., Muangman, S. L., Suz, P., Pihoker, C., Fisk, D., Moore, A., … Vavilala, M. S. (2007). Early childhood gender differences in anterior and posterior cerebral blood flow velocity and autoregulation. *Pediatrics*, *119*(3), e610–e615.

Towfighi, A., Zheng, L., & Ovbiagele, B. (2009). Sex-specific trends in midlife coronary heart disease risk and prevalence. *Archives of Internal Medicine*, *169*(19), 1762–1766.

Townsend, R. R., Wilkinson, I. B., Schiffrin, E. L., Avolio, A. P., Chirinos, J. A., Cockcroft, J. R., … American Heart Association Council on Hypertension. (2015). Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. *Hypertension*, *66*(3), 698–722.

Tsao, C. W., Seshadri, S., Beiser, A. S., Westwood, A. J., Decarli, C., Au, R., … Mitchell, G. F. (2013). Relations of arterial stiffness and endothelial function to brain aging in the community. *Neurology*, *81*(11), 984–991.

Tun, P. A., & Lachman, M. E. (2008). Age differences in reaction time and attention in a

national telephone sample of adults: education, sex, and task complexity matter. *Developmental Psychology*, *44*(5), 1421–1429.

Vahlquist, B. O., & Others. (1950). The cause of sexual differences in erythrocyte, hemoglobin and serum iron levels in human adults. *Blood, J. Hematol.*, *5*, 874–875.

Van Bortel, L. M., Laurent, S., Boutouyrie, P., Chowienczyk, P., Cruickshank, J. K., De Backer, T., … Weber, T. (2012). Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *Journal of Hypertension*, Vol. 30, pp. 445–448. https://doi.org/10.1097/hjh.0b013e32834fa8b0

Van der Elst, W., Van Boxtel, M. P. J., Van Breukelen, G. J. P., & Jolles, J. (2006). The Stroop color-word test: influence of age, sex, and education; and normative data for a large sample across the adult age range. *Assessment*, *13*(1), 62–79.

Van Der Werf, Y. D., Tisserand, D. J., Visser, P. J., Hofman, P. A., Vuurman, E., Uylings, H. B., & Jolles, J. (2001). Thalamic volume predicts performance on tests of cognitive speed and decreases in healthy aging. A magnetic resonance imaging-based volumetric analysis. *Brain Research. Cognitive Brain Research*, *11*(3), 377–385.

van Gelderen, P., de Zwart, J. A., & Duyn, J. H. (2008). Pittfalls of MRI measurement of white matter perfusion based on arterial spin labeling. *Magnetic Resonance in Medicine*, Vol. 59, pp. 788–795. https://doi.org/10.1002/mrm.21515

Veraart, J., Novikov, D. S., & Fieremans, E. (2018). TE dependent Diffusion Imaging (TEdDI) distinguishes between compartmental T2 relaxation times. *NeuroImage*, *182*, 360–369.

Vernooij, M. W., Ikram, M. A., Vrooman, H. A., Wielopolski, P. A., Krestin, G. P., Hofman, A., … Breteler, M. M. B. (2009). White matter microstructural integrity and cognitive function in a general elderly population. *Archives of General Psychiatry*, *66*(5), 545–553.

Vis, J. B. D., De Vis, J. B., Hendrikse, J., Bhogal, A., Adams, A., Kappelle, L. J., & Petersen, E. T. (2015). Age-related changes in brain hemodynamics; A calibrated MRI study. *Human Brain Mapping*, Vol. 36, pp. 3973–3987. https://doi.org/10.1002/hbm.22891

Vis, J. B. D., De Vis, J. B., Peng, S.-L., Chen, X., Li, Y., Liu, P., … Lu, H. (2018). Arterial-spin-labeling (ASL) perfusion MRI predicts cognitive function in elderly individuals: A 4-year longitudinal study. *Journal of Magnetic Resonance Imaging*, Vol. 48, pp. 449–458. https://doi.org/10.1002/jmri.25938

Vlachopoulos, C., Ioakeimidis, N., Miner, M., Aggelis, A., Pietri, P., Terentes-Printzios, D., … Stefanadis, C. (2014). Testosterone deficiency: a determinant of aortic stiffness in men. *Atherosclerosis*, *233*(1), 278–283.

Vlachopoulos, C., O'Rourke, M., & Nichols, W. W. (2011). *McDonald's Blood Flow in*

Arteries: Theoretical, Experimental and Clinical Principles. CRC Press.

Voyer, D., Voyer, S., & Bryden, M. P. (1995). Magnitude of sex differences in spatial abilities: a meta-analysis and consideration of critical variables. *Psychological Bulletin*, *117*(2), 250–270.

Vriens, E. M., Kraaier, V., Musbach, M., Wieneke, G. H., & van Huffelen, A. C. (1989). Transcranial pulsed doppler measurements of blood velocity in the middle cerebral artery: Reference values at rest and during hyperventilation in healthy volunteers in relation to age and sex. *Ultrasound in Medicine & Biology*, Vol. 15, pp. 1–8. https://doi.org/10.1016/0301-5629(89)90125-7

Wagner, M., Jurcoane, A., Volz, S., Magerkurth, J., Zanella, F. E., Neumann-Haefelin, T., … Hattingen, E. (2012). Age-Related Changes of Cerebral Autoregulation: New Insights with Quantitative T2′-Mapping and Pulsed Arterial Spin-Labeling MR Imaging. *AJNR. American Journal of Neuroradiology*, *33*(11), 2081–2087.

Waldstein, S. R., Rice, S. C., Thayer, J. F., Najjar, S. S., Scuteri, A., & Zonderman, A. B. (2008). Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore Longitudinal Study of Aging. *Hypertension*, *51*(1), 99–104.

Wardlaw, J. M., Valdés Hernández, M. C., & Muñoz-Maniega, S. (2015). What are White Matter Hyperintensities Made of? *Journal of the American Heart Association*, Vol. 4. https://doi.org/10.1161/jaha.114.001140

Wardrop, R. M. (2014). Hypertension Awareness and Control Among Young Adults. *AAP Grand Rounds*, Vol. 32, pp. 29–29. https://doi.org/10.1542/gr.32-3-29

Warnert, E. A. H., Verbree, J., Wise, R. G., & van Osch, M. J. P. (2016). Using High-Field Magnetic Resonance Imaging to Estimate Distensibility of the Middle Cerebral Artery. *Neuro-Degenerative Diseases*, *16*(5-6), 407–410.

Watson, N. L., Sutton-Tyrrell, K., Rosano, C., Boudreau, R. M., Hardy, S. E., Simonsick, E. M., … Newman, A. B. (2011a). Arterial stiffness and cognitive decline in well-functioning older adults. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *66*(12), 1336–1342.

Watson, N. L., Sutton-Tyrrell, K., Rosano, C., Boudreau, R. M., Hardy, S. E., Simonsick, E. M., … Newman, A. B. (2011b). Arterial stiffness and cognitive decline in well-functioning older adults. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *66*(12), 1336–1342.

Weller, R. O., Yow, H.-Y., Preston, S. D., Mazanti, I., & Nicoll, J. A. R. (2002). Cerebrovascular disease is a major factor in the failure of elimination of Abeta from the aging human brain: implications for therapy of Alzheimer's disease. *Annals of the New York Academy of Sciences*, *977*, 162–168.

What are White Matter Hyperintensities Made of? (2016). *Journal of the American Heart Association*, *5*(1), e002006.

Wheeler-Kingshott, C. A. M., & Cercignani, M. (2009). About "axial" and "radial" diffusivities. *Magnetic Resonance in Medicine*, Vol. 61, pp. 1255–1260. https://doi.org/10.1002/mrm.21965

Wildman, R. P., Colvin, A. B., Powell, L. H., Matthews, K. A., Everson-Rose, S. A., Hollenberg, S., … Sutton-Tyrrell, K. (2008). Associations of endogenous sex hormones with the vasculature in menopausal women: the Study of Women's Health Across the Nation (SWAN). *Menopause* , *15*(3), 414–421.

Williams, D. S. (n.d.). Quantitative Perfusion Imaging Using Arterial Spin Labeling. *Magnetic Resonance Imaging*, pp. 149–174. https://doi.org/10.1385/1-59745-010-3:149

Wolff, S. D., & Balaban, R. S. (1989). Magnetization transfer contrast (MTC) and tissue water proton relaxationin vivo. *Magnetic Resonance in Medicine*, Vol. 10, pp. 135–144. https://doi.org/10.1002/mrm.1910100113

Wu, J., Hadoke, P. W. F., Mair, I., Lim, W. G., Miller, E., Denvir, M. A., & Smith, L. B. (2014). Modulation of neointimal lesion formation by endogenous androgens is independent of vascular androgen receptor. *Cardiovascular Research*, *103*(2), 281–290.

Wu, W.-C., Lin, S.-C., Wang, D. J., Chen, K.-L., & Li, Y.-D. (2013). Measurement of Cerebral White Matter Perfusion Using Pseudocontinuous Arterial Spin Labeling 3T Magnetic Resonance Imaging – an Experimental and Theoretical Investigation of Feasibility. *PLoS ONE*, Vol. 8, p. e82679. https://doi.org/10.1371/journal.pone.0082679

Xekardaki, A., Rodriguez, C., Montandon, M.-L., Toma, S., Tombeur, E., Herrmann, F. R., … Haller, S. (2015). Arterial Spin Labeling May Contribute to the Prediction of Cognitive Deterioration in Healthy Elderly Individuals. *Radiology*, Vol. 274, pp. 490–499. https://doi.org/10.1148/radiol.14140680

Xing, C.-Y., Tarumi, T., Liu, J., Zhang, Y., Turner, M., Riley, J., … Zhang, R. (2017). Distribution of cardiac output to the brain across the adult lifespan. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *37*(8), 2848–2856.

Yamaguchi, F., Meyer, J. S., Sakai, F., & Yamamoto, M. (1979). Normal human aging and cerebral vasoconstrictive responses to hypocapnia. *Journal of the Neurological Sciences*, *44*(1), 87–94.

Yezhuvath, U. S., Lewis-Amezcua, K., Varghese, R., Xiao, G., & Lu, H. (2009). On the assessment of cerebrovascular reactivity using hypercapnia BOLD MRI. *NMR in Biomedicine*, *22*(7), 779–786.

Yip, R., Johnson, C., & Dallman, P. R. (1984). Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. *The American Journal of Clinical Nutrition*, *39*(3), 427–436.

Zaidi, Z. F. (2010). Gender Differences in Human Brain: A Review. *The Open Anatomy Journal*, Vol. 2, pp. 37–55. https://doi.org/10.2174/1877609401002010037

Zaninotto, P., Batty, G. D., Allerhand, M., & Deary, I. J. (2018). Cognitive function trajectories and their determinants in older people: 8 years of follow-up in the English Longitudinal Study of Ageing. *Journal of Epidemiology and Community Health*, *72*(8), 685–694.

Zeki Al Hazzouri, A., Newman, A. B., Simonsick, E., Sink, K. M., Sutton Tyrrell, K., Watson, N., … Health ABC Study. (2013). Pulse wave velocity and cognitive decline in elders: the Health, Aging, and Body Composition study. *Stroke; a Journal of Cerebral Circulation*, *44*(2), 388–393.

Zhang, H., Schneider, T., Wheeler-Kingshott, C. A., & Alexander, D. C. (2012). NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. *NeuroImage*, Vol. 61, pp. 1000–1016. https://doi.org/10.1016/j.neuroimage.2012.03.072

Zhang, N., Gordon, M. L., & Goldberg, T. E. (2017). Cerebral blood flow measured by arterial spin labeling MRI at resting state in normal aging and Alzheimer's disease. *Neuroscience & Biobehavioral Reviews*, Vol. 72, pp. 168–175. https://doi.org/10.1016/j.neubiorev.2016.11.023

Zhang, N., Gordon, M. L., Ma, Y., Chi, B., Gomar, J. J., Peng, S., … Goldberg, T. E. (2018). The Age-Related Perfusion Pattern Measured With Arterial Spin Labeling MRI in Healthy Subjects. *Frontiers in Aging Neuroscience*, *10*, 214.

Zhang, Z.-Z., Wang, P., Kong, X.-L., Mao, W.-L., & Cui, M.-Y. (2019). Association of hemoglobin with arterial stiffness evaluated by carotid-femoral pulse wave velocity among Chinese adults. *Chronic Diseases and Translational Medicine*, *5*(2), 122–128.

Zhong, W., Cruickshanks, K. J., Schubert, C. R., Carlsson, C. M., Chappell, R. J., Klein, B. E. K., … Acher, C. W. (2014). Pulse wave velocity and cognitive function in older adults. *Alzheimer Disease and Associated Disorders*, *28*(1), 44–49.

Zhu, Y.-S., Tarumi, T., Tseng, B. Y., Palmer, D. M., Levine, B. D., & Zhang, R. (2013). Cerebral vasomotor reactivity during hypo- and hypercapnia in sedentary elderly and Masters athletes. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *33*(8), 1190–1196.

Zhu, Y.-S., Tseng, B. Y., Shibata, S., Levine, B. D., & Zhang, R. (2011). Increases in cerebrovascular impedance in older adults. *Journal of Applied Physiology*, *111*(2), 376–381.

Supplementary Material

1.1 Higher cardiovascular fitness level is associated with lower cerebral vascular reactivity and perfusion in healthy older adults

Article follows on the next page.

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Higher cardiovascular fitness level is associated with lower cerebrovascular reactivity and perfusion in healthy older adults

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Abstract

Aging is accompanied by vascular and structural changes in the brain, which include decreased grey matter volume (GMV), cerebral blood flow (CBF), and cerebrovascular reactivity (CVR). Enhanced fitness in aging has been related to preservation of GMV and CBF, and in some cases CVR, although there are contradictory relationships reported between CVR and fitness. To gain a better understanding of the complex interplay between fitness and GMV, CBF and CVR, the present study assessed these factors concurrently. Data from 50 participants, aged 55 to 72, were used to derive GMV, CBF, CVR and VO_2peak. Results revealed that lower CVR was associated with higher VO_2peak throughout large areas of the cerebral cortex. Within these regions lower fitness was associated with higher CBF and a faster hemodynamic response to hypercapnia. Overall, our results indicate that the relationships between age, fitness, cerebral health and cerebral hemodynamics are complex, likely involving changes in chemosensitivity and autoregulation in addition to changes in arterial stiffness. Future studies should collect other physiological outcomes in parallel with quantitative imaging, such as measures of chemosensitivity and autoregulation, to further understand the intricate effects of fitness on the aging brain, and how this may bias quantitative measures of cerebral health.

Keywords

Aging, cerebrovascular reactivity, fitness, MRI, perfusion-weighted imaging

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Introduction

Continuous and optimal blood flow is thought to be necessary for structural integrity and normal neuronal activity in the brain.[1] During aging, the vascular system undergoes a cascade of events that negatively affect the integrity of the cerebrovascular system, leading to decreased perfusion. However, it may be possible to reduce these deficits, and in some instances, reverse them as a result of plasticity. Plasticity refers to the capacity of the brain to change its function, hemodynamics and microstructure in response to cognitive or physiological challenges.[2,3] In aging, there is some indication that physical activity may be capable of inducing beneficial plastic changes.[4,5] Notably, aerobic exercise has become a subject of particular interest for

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maintaining and even enhancing cognition and brain integrity.[4,6,7] It is likely that these effects are mediated by changes in cerebrovascular health given the well-established positive influence of exercise on the cardiovascular system in aging.[8,9] It has been demonstrated that more highly fit individuals have enhanced endothelial function[10] and reduced arterial stiffness,[9,11] both of which are impaired in aging.[12–14] Of note, individuals who are more "highly fit" have higher cardiovascular fitness (VO_2peak), which can be quantified in multiple ways. Specifically, VO_2max is considered the gold standard measure.[15] However, reaching a true VO_2max is difficult to attain for many older adults, thus utilizing VO_2peak is a more feasible option.[16,17] These measurements will therefore be referred to as VO_2peak for the remainder.

Given the positive relationship between the vascular system and exercise, there is an increasing body of work investigating the relationship amongst aging, VO_2peak, cerebral structural integrity and hemodynamics. Magnetic resonance imaging (MRI) is the method of choice to study these relationships as it is a versatile technique which can be used to measure several of these parameters, including grey matter volume (GMV), cerebral blood flow (CBF) and cerebrovascular reactivity (CVR). In general, GMV and CBF are positively associated with VO_2peak in cross-sectional[18–20] and longitudinal studies.[21–25] However, many of the existing studies showing this beneficial effect have used GMV as a marker of "structural integrity". This is problematic because GMV has been shown to be mainly qualitative and physiologically non-specific,[26,27] making a mechanistic interpretation of these effects difficult.

More physiologically specific approaches have involved looking at the relationship between CBF and VO_2peak. In cross-sectional studies, it has been demonstrated that there is a positive relationship between VO_2peak and CBF.[20,28,29] This also seems partly supported by intervention studies. For instance, Chapman et al.[21] found that individuals who completed a 12-week aerobic training program demonstrated significant increases in CBF compared to the passive control group. Yet, in a later study Chapman et al.[30] found CBF to be unchanged after the same aerobic training program. While this could be due to an insufficient exercise dose, it is possible that CBF is not a sensitive enough marker of cerebrovascular health in isolation. This could be both due to its relatively limited signal-to-noise ratio[31] and the fact that homeostasis seeks to maintain CBF to ensure adequate perfusion to maintain oxygen and glucose delivery.[32] There are indications that while CBF does steadily decrease across the lifespan,[33] more dynamic aspects of hemodynamics, such as CVR may change earlier than CBF in the course of aging.[34–36]

CVR is measured as the hemodynamic response (in terms of CBF or blood-oxygen-level dependent (BOLD) change for example) to a vasodilatory challenge, such as hypercapnia,[37] breath-holds[38] or acetazolamide.[39] CVR is hypothesized to represent the health of the cerebral vasculature.[40] If it is assumed that CO_2-related local chemosensitivity is consistent across age and disease groups, it could be treated as a vascular vasodilatory capacity biomarker. Furthermore, if CVR is taken to be a marker of vascular health, it can be posited that those with higher VO_2peak levels would have greater CVR, as their vascular system would be more compliant and therefore have an increased ability to respond to a vasodilatory stimulus. Consistent with this hypothesis, it has been demonstrated that CVR is decreased in aging,[34,41] stroke[42] and carotid artery stenosis.[43] Yet, the literature has found conflicting results *within* healthy populations, where some have observed elevated CVR in relation to higher VO_2peak levels,[44–47] while others have found that lower CVR is related to increased VO_2peak,[28] and others have found no difference[40–42] in aging. It is unclear, however, if this is due to differences in measurement method, spatial localization of the measurement or an interesting physiological interplay between multiple hemodynamic aspects of brain health.

In summary, there is an assortment of negative consequences that can occur due to an aging vascular system that causes deterioration of brain microstructure and hemodynamics. Importantly, there is evidence that exercise is capable of mitigating some of these adverse age-related complications. Yet, the fitness literature suggests that the effects of exercise on brain hemodynamics may be complex, so a more comprehensive imaging approach is necessary to understand the interplay between the effects of aging and VO_2peakon cerebral hemodynamics. The present study explores the relationship between aging, VO_2peak, cerebral hemodynamics and GMV using a cross-sectional dataset employing a comprehensive imaging approach in healthy younger and older adults of varying VO_2peak.

Methods

Participants

A total of 28 young adults (seven females, mean age 24 ± 3 years) and 50 older adults (37 females, mean age 63 ± 5 years) participated in this study. Participants were recruited through participant databases at the Centre de recherche de l'Institut universitaire de gériatrie de Montréal and Laboratoire D'Etude de la Santé cognitive des Ainés.

Inclusion criteria were comprised of being between 18 and 40 years for young adults and 55 and 75 years for older adults; approval by a geriatrician to

participate (older adults), non-smoker (for at least five years), no evidence of cognitive impairment as determined through cognitive tests conducted by a neuropsychologist, and ability to complete the peak oxygen uptake test (VO_2peak) and MRI. Exclusion criteria included taking prescription medication that could be vasoactive (e.g. diuretics, calcium channel blockers, statins, thyroid replacement hormones, etc.), presence of cardiac disease, hypertensives, neurological or psychiatric illnesses, diabetes, asthma, thyroid disorders, or excessive drinking (more than two drinks per day). Finally, a neuropsychologist completed the Mini Mental Status Examination, a global cognitive screening tool for dementia; participants with scores less than 26 (out of 30) were excluded.[48]

All procedures were approved by Comité mixte d'éthique de la recherche du Regroupement Neuroimagerie/Québec and were conducted according to the Declaration of Helsinki. All participants provided written informed consent.

MRI acquisition

All acquisitions were completed on a Siemens TIM Trio 3T MRI system (Siemens Medical Solutions, Erlangen, Germany). A 32-channel head coil was used for all brain acquisitions. An anatomical 1 mm^3 MPRAGE acquisition (TR/TE/flip angle = 300 ms/3 ms/90°, 256 × 240 matrix) was acquired for registration and GMV estimation. A fluid attenuation inversion recovery (FLAIR) acquisition with parameters: TR/TE/flip angle 9000 ms/107 ms/120° with echo train length of 15, an inversion time of 2500 ms, 512 × 512 matrix for an in-plane resolution of 0.43 × 0.43 mm and 25 slices of 4.8 mm was used to estimate the presence and severity of white-matter lesions in older adults. A pseudo-continuous arterial spin labeling (pCASL) acquisition,[49] providing simultaneous BOLD contrast using dual-echo readouts (TR/TE1/TE2/flip angle = 2000 ms/10 ms/30 ms/90°, 4 × 4×7 mm voxels, 64 × 64 matrix and 11 slices, post-label delay = 900 ms, tag duration = 1.5 s, and a 100 mm gap) was acquired during a hypercapnia challenge.

Aortic exam

As described in Gauthier et al.,[50] during the MRI session a thoracic aortic exam was also acquired using simultaneous brachial pressure recording (Model 53,000, Welch Allyn, Skaneateles Falls, NY USA) using a 24-element spine matrix coil. Black blood turbo spin echo sagittal oblique images were acquired to visualize aortic arch (TR/TE/flip angle: 700 ms/6.5 ms/180°, 1.4 × 1.4 mm in-plane resolution, 2 slices at 7.0 mm). A perpendicular plane to the ascending and descending aorta was defined from these images. A cine phase-contrast velocity encoded series was collected (TR/TE/flip angle: 28.6 ms/1.99 ms/30°, 1.5 × 1.5 mm × 5.5 mm) during 60 cardiac cycles in three segments, with velocity encoding of 180 cm/s. A series of cine FLASH images were also acquired in the same plane (TR/TE/flip angle: 59 ms, 3.44 ms, 15°; 1.2 × 1.2 mm × 6 mm) over 60 cardiac cycles in eight segments.

Hypercapnia. The hypercapnic manipulation used here has been described previously.[34,50] Briefly, it was completed with a computer-controlled gas system with a consecutive gas delivery circuit (Respiract™ GEN3, Thornhill Research Inc., Toronto Canada).[51] End tidal O_2 was targeted to be 100 mmHg throughout the manipulation, while CO_2 had a target of 45 mmHg during the hypercapnia blocks and 40 mmHg during normocapnia. More specifically, two hypercapnia blocks, of 2 min each in duration, were completed after, and followed by 2 min blocks of breathing room air. Participants breathed through a soft plastic mask (Tegaderm 3M Healthcare, St. Paul MN) that was secured on their face with adhesive tape to ensure that no leaks were present. Participants completed the breathing manipulation once prior to being in the scanner (to ensure comfort and tolerance to procedure), and once during the MRI session.

VO_2peak. Participants completed a maximal oxygen consumption test (VO_2peak) to approximate their VO_2peak, where a greater amount of oxygen consumed indicates enhanced VO_2peak.[52] The test was completed on a stationary cycle ergometer and was monitored throughout by an electrocardiogram under medical supervision to ensure participant safety. Initial workload was set based on the body weight of the individual (1 watt (W)/kg) and then increased incrementally by 15 W every minute until voluntary exhaustion. Oxygen uptake was determined using an automated system that averaged in 30-s increments (Moxus, AEI Technologies, Naperville, IL). The highest oxygen uptake over a 30-s period during the test was considered as the VO_2peak (ml/kg/min).

Data analysis

GMV. T1-weighted MPRAGE images were preprocessed using SPM's Computational Anatomy Toolbox (CAT)12[53–55] to calculate voxel-based morphometry (VBM) after data were segmented into grey matter, white matter and cerebrospinal fluid (CSF). VBM calculates the difference between the volume estimated for tissue from an individual compared to the expected volume of tissue from a template. This provided a

statistical map for each voxel type which is then classified into the structural category with the highest probability, allowing for analysis between participants.

The registration matrix was calculated as part of the VBM pipeline and was then applied to the GMV, CBF and CVR maps (described below) to bring them from native to MNI space. Individual BOLD-CVR, resting CBF and CBF-CVR were produced for each participant. Co-registration of these maps from native to native T1 space was performed using a non-linear rigid registration with ANTS[56] with a b-spline interpolation. CAT12[53–55] was used to register from T1 to standard space using a uniform non-linear registration with 12 degrees of freedom and smoothing of the data employed a Gaussian filter of 8 mm. An average grey matter mask from each age group was also created to restrict voxel-wise analyses to the grey matter.

CVR analysis. Preprocessing of the BOLD-CVR has been described previously and was performed using Neurolens2 (www.neurolens.org).[34] All raw images were preprocessed with motion correction[52] and spatial smoothing with a 6 mm Gaussian kernel. The BOLD signal was extracted from the second echo series with a linear surround addition.[57–59] The BOLD fractional change during hypercapnia was obtained by fitting a general linear model to the BOLD signal and dividing the estimated effect size by the estimated constant term. Glover's[60] parameters for a single-gamma hemodynamic response function were used when fitting the linear models, which also included linear, quadratic and third-order polynomials representing baseline signal and drifts. The BOLD percent change obtained was then divided by the average end-tidal CO_2 change during the hypercapnia manipulation to yield BOLD-CVR. CBF-CVR was calculated in the same way as BOLD-CVR, but the CBF signal was isolated from the first series of echoes using linear surround subtraction.[57]

Resting CBF analysis. Resting CBF was quantified using the first echo of the whole pCASL data time series, using the first 2 min of the time series, before the beginning of the first hypercapnia block. The average of the control images was used for each participant with modeling of the T1-recovery to obtain the fully recovered magnetization (M0) using AFNI, FSL and in-house scripts. CSF masks were created for each older adult participant to use as CSF M0 in the CBF quantification. To do this, 10 voxels were chosen in the same axial slice for all older participants where the lateral ventricles were clearly located, except for four participants where a more superior, or inferior slice was required to clearly identify the ventricles from the pCASL scans. All individual masks were visually inspected to ensure the ROIs were located in the ventricles. For the younger adults, one participant was chosen at random and the same method was used to identify 10 voxels. A single CSF mask was used for all younger participants as this mask was confirmed to be located in the ventricles in all participants upon visual inspection. However, this was not possible with the older adults due to varying anatomical structures. FSL's BASIL[61] toolkit was then utilized to quantify CBF, with the following standard parameters: labelling: cASL/pcASL; bolus duration: constant (1.5 s), post label delay: 0.9 s; calibration image: average of the control images; reference tissue type: CSF; mask: CSF mask for each participant; CSF T1: 4.3 s; TE: 10 ms; T2 : 750 ms; blood T2: 150 ms; arterial transit time: 1.3 s, T1: 1.3 s , T1 blood: 1.65 s, inversion efficiency: 0.85.

Vascular lesion quantification. The volume of white matter hyperintensities (WMHs) in the brain was estimated in a semi-automatic way as described in Gauthier et al.[50] Briefly, a single trained rater, who was blinded to clinical information, visually identified WMH on the FLAIR images, which were then delineated using Jim image analysis package, version 6.0 (Xinapse Systems Ltd, Northants, UK).

Pulse wave velocity data. The aortic data were analyzed using the ARTFUN software,[62] where pulse wave velocity (PWV) was computed between the ascending and descending aorta using the cine phase contrast images for blood velocity and the cine images for aorta delineation. PWV was calculated as described in Gauthier et al.[50] These data were included as a covariate, so that any relationships that might be present between VO_2peak and the hemodynamic brain outcomes were not due to differences in arterial stiffness in large arteries among the older adults but rather to brain-specific properties.

Voxel-wise analyses. Using FSL's toolbox Randomise,[63] permutation-based threshold-free cluster enhancement (TFCE),[64] using 10,000 permutations, was employed to test for spatial relationships between VO_2peak and structural or hemodynamic outcomes. These analyses were restricted to GM using a group mask of all GM voxels present in all participants (i.e. the intersection of all participants GM segmentation mask in MNI space). A separate group GM mask was created for younger and older adults. Voxel-wise general linear models were used to identify the relationship within GM between: (i) VO_2peak and GMV; (ii) VO_2peak and BOLD-CVR; (iii) VO_2peak and resting CBF; and (iv) VO_2peak and CBF-CVR data for young and older adults. Age and sex were included for both young and older adults as covariates. For the older

adults, we also included sex-specific estimated absolute multivariate risk scoring with the Framingham cardiovascular risk factor, as proposed by Ralph et al.,[65] to estimate general cardiovascular risk and future cardiovascular risk as a confound. Volume of WMH and PWV was also used as potential confounds in the older adults to remove the potential effects of existing WM lesions and central arterial stiffness.

Regions of interest. Voxels that exhibited a significant relationship between BOLD-CVR and VO_2peak were extracted and binarized to be used as regions of interest (ROI) for further analysis. Specifically, this ROI was then used to further investigate if VO_2peak and GMV, resting CBF, or CBF-CVR were related to each other in these regions, in an attempt to disentangle the physiological relationship amongst these factors in aging and VO_2peak. This ROI was then multiplied by each individual's VBM map to create individual ROI masks. The values from each participant for this individual ROI were then extracted for all maps using weighted average with FSLmeants to correct for possible GM atrophy.

Finally, a finite impulse response (FIR) analysis was completed as reported in Gauthier et al.[34] to estimate the temporal course of the BOLD response to hypercapnia in order to identify whether dynamic aspects of the response could be linked to VO_2peak. Briefly, the average time course for BOLD during hypercapnia was determined, where the temporal response was measured starting 15 s prior to and after the end of each hypercapnic block. The beginning of the upward phase of the response, as well as the plateau were identified manually and the linear fit for the values between these two points (slope of the upward response) was identified using a linear regression in the SPSS 20.0 software (IBM, Armonk, New York, USA) for each participant. The BOLD time course was averaged within the BOLD-CVR VO_2peak ROI for both young and older adults, and these values were then extracted. With the exclusive intent of facilitating visualization, the older adult group was then rank ordered according to their VO_2peak. Once rank ordered, they were subdivided into five bins based on VO_2peak to further visualize the relationship between response shape and VO_2peak.

Statistical analysis

Statistical analysis of the behavioural data was completed using SPSS to identify if relationships were present between VO_2peak and demographic data with correlational analyses. A partial correlation was used to identify if there were relationships between VO_2peak and values extracted from the significant CVR-BOLD VO_2peak ROI, while accounting for the covariates described above (e.g. age, sex, PWV, Framingham Risk Factors, white matter hyperintensity volume). As the white matter hyperintensity volumes were found to be non-normal, it was necessary to log transform this data to allow for parametric statistical analyses with the data. All other data were found to be normally distributed. Finally, a partial correlation analysis was also utilized to investigate potential relationships between VO_2peak and the slope of the BOLD upward response where age was included as a covariate. Statistical significance was set to $p \leq 0.05$ for all outcomes and Tukey's post hoc analysis was used where applicable.

Results

Younger versus older adults

A total of 50 older adults and 26 young adults participated in this study, and demographics for both are listed in Table 1.

VO_2peak, brain structure and hemodynamics in GM

The mean values over all GM for hemodynamic parameters in each participant versus VO_2peak for both young and older adults are shown in Figure 1. It was found that younger adults had a significantly higher GMV ($p = 4.43 \times 10^{-15}$), BOLD-CVR ($p = 1.4 \times 10^{-4}$) and resting CBF ($p = 0.015$) in whole GM than older adults. There were no differences between age groups for CBF-CVR in whole GM ($p = 0.315$) (Table 1).

Table 1. Participant demographics separated by age group.

Demographic	Young adults ($n = 26$)	Older adults ($n = 50$)
Sex (M/F)	19/7*	17/33*
Age (years)	23.7 (2.9)*	63.4 (4.9)*
Education (years)	16.7 (1.4)	16.4 (3.6)
VO_2peak (ml/kg/min)	42.7 (7.6)*	29.1 (7.0)*
MMSE (out of 30)	–	28.8 (0.9)
Framingham risk factor score	–	8.8 (2.6)
Log WMH volume	–	0.367 (0.162)
Grey matter volume (mm^3)	0.551 (0.038)*	0.466 (0.034)*
BOLD-CVR (%change/mmHg CO_2)	0.261 (0.094)*	0.176 (0.041)*
Resting CBF (ml/100 g/min)	48.6 (10.7)*	42.4 (9.9)*
CBF-CVR (ml/100 g/min/mmHg CO_2)	5.13 (1.22)	4.63 (2.35)

Note: Independent samples *t*-test was used to identify differences between young and older adults. *Statistically different $p < 0.05$; all values reported are mean (±SD).

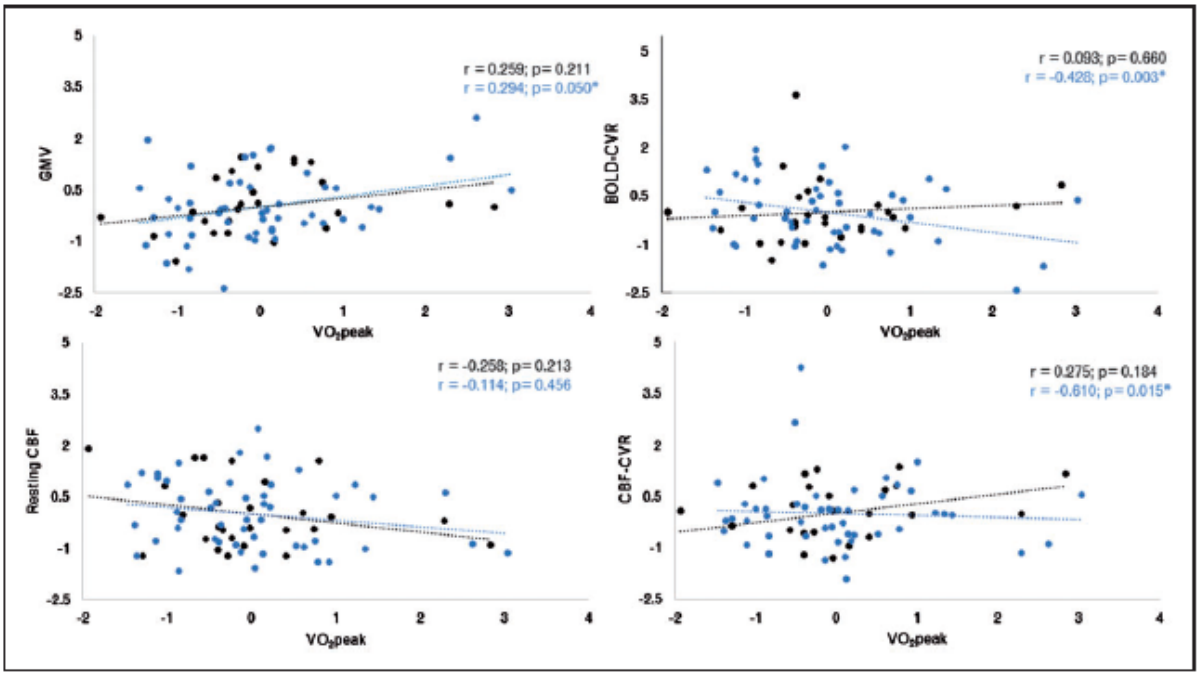

Figure 1. Results from voxel-wise analysis for relationships between structural and hemodynamic outcomes with VO_2peak z-scores (ml/kg/min) for young adults (black dots) and older adults (blue dots). (a) grey matter volume z-score (mm^3); (b) BOLD-CVR (%BOLD/mmHg CO_2) z-score; (c) resting CBF (ml/100g/min) z-score and; (d): CVR CBF (ml/100g/min/mmHg CO_2) z-score. The regression line for each group is plotted in their corresponding colours. *Indicates significant correlation ($p \leq 0.05$).

Young adults. Correlations found no significant relationships between VO_2peak and the mean extracted values for GMV, BOLD-CVR, resting CBF or CBF-CVR in GM for young adults ($p > 0.05$).

Older adults. A partial correlation in older adults including all covariates (e.g. age, sex, Framingham Risk Factor score, WMH and PWV) revealed a significant relationship between VO_2peak and: (i) all GMV ($r = 0.294$; $p = 0.05$); (ii) BOLD-CVR in GM ($r = -0.428$; $p = 0.003$); and (iii) CBF-CVR in GM ($r = -0.361$; $p = 0.015$). No significant correlation was found between VO_2peak and resting CBF in all GM ($p > 0.05$).

VO_2peak, structure and hemodynamics

Voxel-wise analyses within the younger adults did not reveal any significant relationships between VO_2peak and GMV or between VO_2peak and the hemodynamic outcomes ($p > 0.05$) within GM. In older adults, voxel-wise analyses within GM revealed a significant positive relationship between GMV and VO_2peak ($r = 0.320$; $p = 0.025$) and a significant negative association between BOLD-CVR and VO_2peak ($r = -0.392$; $p = 0.005$). The positive relationship between GMV and VO_2peak was present within the superior temporal gyrus (see Figure 2(a)). The negative association between BOLD-CVR and VO_2peak was found in large portions of temporal, parietal cortices and smaller amounts of the frontal lobes (see Figure 2(b)). No relationship was found between VO_2peak and resting CBF or CBF-CVR ($p > 0.05$).

Region of interest analysis. To understand whether the relationship between VO_2peak and other structural or hemodynamic parameters could help to explain the negative association between VO_2peak and BOLD-CVR, other parameters were averaged in the areas significantly negatively related between BOLD-CVR and VO_2peak. Within these ROI, a significant negative relationship was identified between VO_2peak and resting CBF ($r = -0.328$, $p = 0.025$), and VO_2peak and CBF-CVR ($r = -0.322$, $p = 0.029$). The relationships between all structural or hemodynamics outcomes within these ROI with VO_2peak are shown in Figure 3.

FIR. Finally, to identify potential relationships between VO_2peak and BOLD response dynamics, a FIR analysis was run within the ROI derived from the voxel-wise analysis of CVR and VO_2peak. For visualization purposes, the BOLD time course to hypercapnia in these areas for older adults consisted of rank ordering based on VO_2peak, then creating five different bins according to their rank order (see Figure 4(a)).

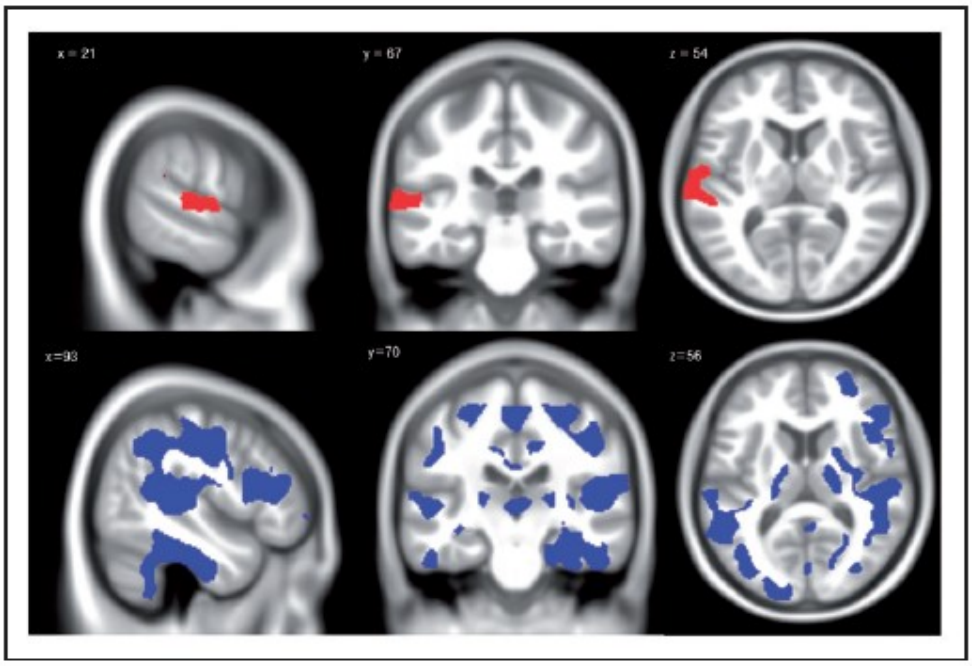

Figure 2. Voxel-wise analyses results in older adults. Significant regions identified with the voxel-wise analysis between VO_2peak z-score and GMV z-score (a). This figure shows areas of the brain where there is a positive association between VO_2peak z-score and GMV z-score (red), indicating that in these areas, those with higher fitness have significantly higher GMV compared to those with lower fitness ($p < 0.05$). Significant regions identified with the voxel-wise analysis between VO_2peak z-score and BOLD-CVR z-score. Areas of the brain where there is a negative association between VO_2peak and BOLD-CVR (blue), indicating that in these areas, those with higher fitness have significantly reduced BOLD-CVR compared to those with lower fitness ($p < 0.05$).

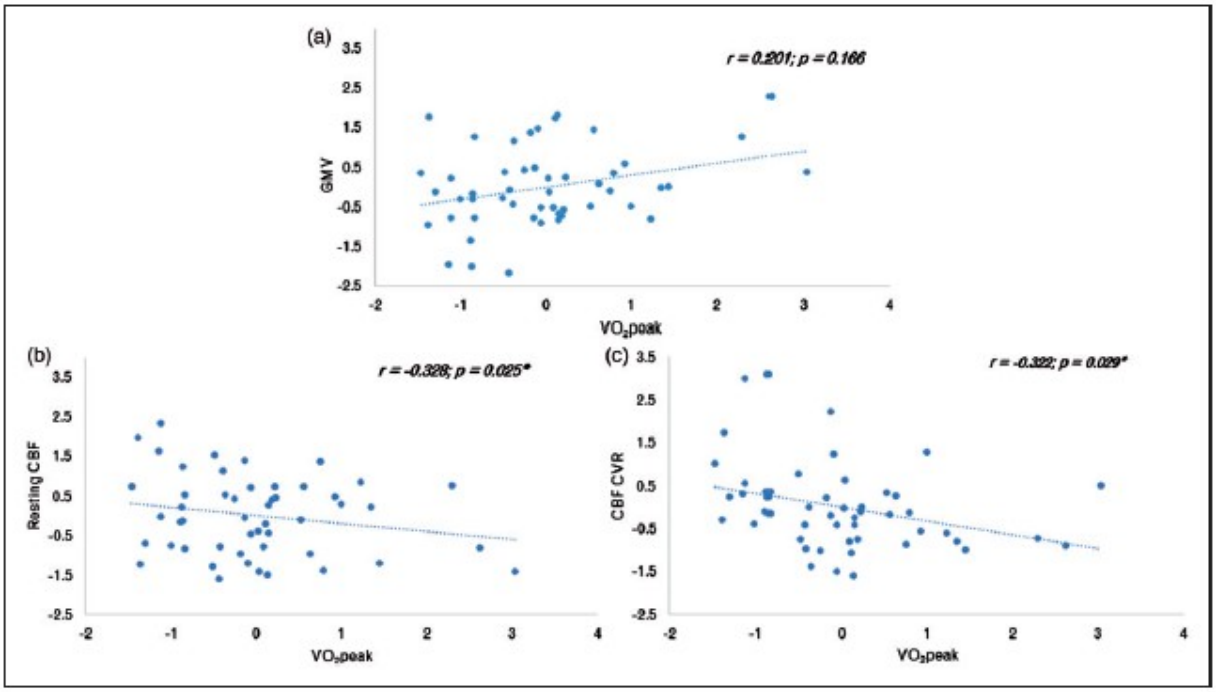

Figure 3. Association between fitness, structure and hemodynamics in the BOLD-CVR vs VO_2peak ROI. Relationships from the CVR VO_2peak z-score ROI in; (a): GMV z-score; (b): Resting CBF z-score; and (c): CBF-CVR z-score. Graphs demonstrate the relationship between each of these parameters and fitness in older adults. *Represents significant correlation ($p < 0.05$).

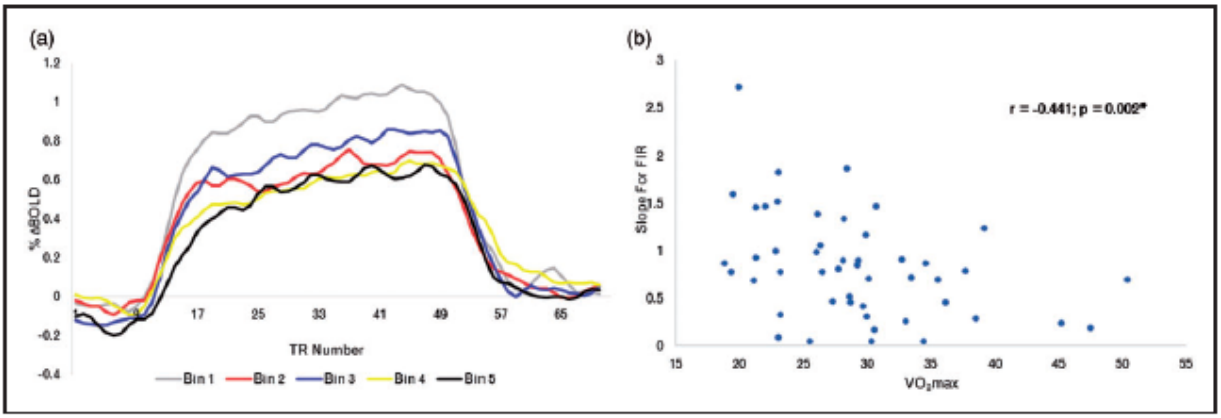

Figure 4. Time course of the BOLD response to hypercapnia. (a) Time course showing the percent BOLD response to hypercapnia in BOLD-CVR VO_2peak ROI. Where the fitness level for older adults was binned into five categories; Bin 1 representing the lowest VO_2peak bin, and increasing until Bin 5, which includes the data from those with the highest binned VO_2peak in this sample. (b) Linear regression of the relationship between the slope of the upward portion of the response and fitness in older adults.

The partial correlation analysis revealed that there was a significant negative correlation between VO_2peak and the slope of the linear regression ($r = -0.441$; $p = 0.002$). This relationship is shown in Figure 4(b).

Discussion

This study investigated the relationship between VO_2peak, GMV and brain hemodynamics in a population of healthy older adults. This group showed the expected pattern of reduced GMV, BOLD-CVR and resting CBF as compared to healthy younger adults. Voxel-wise analyses over all GM demonstrated a significant positive relationship between VO_2peak and GMV and a somewhat surprising significant inverse relationship between BOLD-CVR and VO_2peak in older adults in a number of GM regions throughout the cortex. A more in-depth review of hemodynamics within these regions demonstrated that the relationship between VO_2 and other hemodynamic parameters also exhibited this inverse relationship. Specifically, there was no relationship between VO_2peak and GMV, but a significant negative relationship between VO_2peak and resting CBF, and between VO_2peak and CBF-CVR. To determine whether these relationships between VO_2peak and BOLD-CVR were confined to response amplitude or if it was also present in response dynamics, we performed an analysis of the time course of the BOLD response to hypercapnia. This analysis revealed a slower ramp-up towards a plateau in those with higher VO_2peak, regardless of age as demonstrated in Figure 4(b). Together, these results indicate that the relationship between VO_2peak and hemodynamics in aging is more complex than previously thought and that BOLD-CVR in particular may be biased by physiological mechanisms affected by exercise.

Age-group comparisons

The impact of healthy aging on brain structure and hemodynamics is an active field of research and the age group comparisons performed as part of this study are consistent with these existing results. In comparison to young adults, older adults were found to have lower: GMV,[45,66,67] CVR,[34,35,68–70] and resting CBF.[34,35]

Regional relationships between VO_2peak, structure and hemodynamics

The main result from this study is the finding that BOLD-CVR in GM is negatively correlated with VO_2peak in older adults. Voxel-wise analysis revealed large sections of GM including temporal, parietal and frontal regions were responsible for this negative relationship. Given that higher CVR is typically interpreted as being related to better cerebral health,[40] this reverse relationship was counter-intuitive. Interestingly, to date, only one other published study (in addition to our own work[50]) has also demonstrated a negative relationship between VO_2peak and BOLD-CVR in older adults.[28,50] Specifically, Thomas et al.[28] found that Master athletes had significantly lower BOLD-CVR compared to their sedentary counterparts over most of cerebral cortex, including the parietal and temporal cortices. Notably, most studies investigating VO_2peak and hemodynamics have used transcranial Doppler (TCD) to identify a positive relationship between CVR and VO_2peak.[44–47] To the best of our knowledge, MRI studies have only identified a negative relationship, perhaps reflecting the different vascular compartments and properties imaged with both techniques. TCD images flow velocity in major arteries, while the BOLD signal reflects a mixture of blood flow, blood

volume and oxidative metabolism arising from the parenchyma and veins. Therefore, it is possible that changes in the venous system, such as venous collagenosis, or related to the parenchymal vasculature lead to the BOLD-CVR results measured using MRI.

The voxel-wise analysis also revealed a positive relationship between VO_2peak and GMV within the superior temporal gyrus. This is consistent with other studies pointing to a general association between VO_2peak and GMV,[6] and specifically within the superior temporal gyrus.[71] However, as mentioned previously, GMV should be interpreted with caution as it is qualitative, not physiologically specific, and may be biased by differences in blood volume.[26,27]

Notably, the lack of relationship with other hemodynamic parameters could be attributable to the fact that the present study involved a very healthy group of older adults. Exclusion criteria were numerous, including but not limited to, taking most medication regularly, suffering from chronic diseases, or cardiovascular risk factors. Moreover, the Framingham scores for this group is low, with the average (8.8) just below the score expected solely due to the average age of the group (9), indicating overall absence of cardiovascular risk factors within the group. Furthermore, participants in this study had VO_2peak values that were greater than the 50th percentile for their age and sex, thus demonstrating higher than average VO_2peak levels.[72] Therefore, it is possible that the relationship between VO_2peak and these other hemodynamic parameters is below the detection limit in this healthy group of older adults, especially in the context of the limited SNR provided by ASL and the stringent thresholding required by the numerous multiple comparisons performed in voxel-wise analyses. Our findings suggest that CVR may be one of the first hemodynamic properties to decline in aging and indicate that it may be more sensitive to aging-related changes in the cerebral vasculature, than CBF and GMV, in line with previous published work.[34]

Physiological underpinnings of BOLD-CVR and fitness association

To better understand the physiological underpinnings of this negative relationship between VO_2peak and BOLD-CVR, a more in-depth investigation of the relationship between VO_2peak and other hemodynamic parameters and GMV within these regions was performed. No relationship between GMV and VO_2peak was revealed; however, resting CBF and CBF-CVR had significantly negative associations with VO_2peak, indicating that lower fit individuals had higher CBF and CBF-CVR. These findings are in opposition to those reported in the extant literature by Tarumi et al.,[29] where endurance-trained older adults showed a higher CBF in the occipitoparietal area as compared to their sedentary counterparts. These results are difficult to compare to those of the present study. Firstly, because the coordinates for the areas used in Tarumi et al. are not available, so that any putative overlap between region and ROI used in the present study is impossible to determine. Secondly, the endurance trained group had considerably higher VO_2peak than this cohort. Zimmerman et al.[20] also found higher global, frontal and parietal CBF in those with greater VO_2peak levels. However, 39% of their participants were taking blood pressure medication, thus it could be that medication impacted these results given the vasoactive nature of these molecules. Future studies, with both larger VO_2peak and cardiovascular health ranges, are therefore necessary to determine whether non-linear effects in the link between CBF and VO_2peak can account for these contradictions in the literature.

While hyperperfusion is not typically associated with aging, one area of research that has identified a pattern of hyperperfusion in similar areas is the APoE4 literature.[73–75] Scarmeas et al.[73] found that both young and older individuals with the APoE4 gene demonstrated hyperperfused areas of the brain compared to non-carriers.[74] Furthermore, a longitudinal study in older adults found that areas that were hyperperfused at baseline in carriers as compared to non-carriers, had significantly lower CBF at the eight-year follow-up.[75] Given the similarity in the hyperperfusion pattern, it is possible that part of our results could be explained by putative over-representation of APoE4 carriers in the low VO_2peak participants. However, as we did not measure APoE expression in our participants, we cannot assess whether this is the case. In general, however, one could speculate that these patterns of hyperperfusion in APoE4 carriers and in the less fit individuals included in this study could indicate that there exists a set of physiological compensatory mechanisms which initially seem like preserved hemodynamics, but that are in fact associated with poorer health or greater damage over time.

Chemosensitivity and autoregulation

In addition to the relationship between BOLD-CVR and VO_2peak already discussed (Figure 4(a)), we also identified a relationship between the slope of the upswing of the response to hypercapnia and VO_2peak. The slower BOLD response to hypercapnia in more highly fit older adults could be indicative of a local desensitization to CO_2, or to pre-dilation[76]; however, the latter is unlikely here as lower resting CBF was found to be linked to higher VO_2peak within these same regions. Further studies including additional measurement of autoregulation

and the respiratory response to exercise, for example, could help untangle the physiological underpinning of these response dynamics.

Overall, there are a few rationales that could explain our results of low BOLD-CVR in higher fit individuals. For example, the first, hypothesized by Thomas et al.,[28] is that perhaps higher fit individuals have decreased local sensitivity to CO_2, likely from a lifetime of increased exposure because of increased aerobic activity. This idea is consistent with studies showing that endurance training reduces the ventilatory response at a given workload, indicating a decrease in local chemosensitivity.[77,78] Nitric oxide is the primary mechanism to respond to changing pH levels from CO_2 in an attempt to maintain homeostasis in the brain.[79,80] It is thus possible that higher fit individuals could have lower levels of nitric oxide in response to hypercapnia than lower fit individuals, which in turn would explain the reduced blood flow response to hypercapnia. Moreover, it has also been proposed that cerebral inflammation could lead to increased nitric oxide signaling,[79] thus lower fit individuals could have increased nitric oxide due to the presence of inflammation. This would also be consistent with the higher resting CBF observed in lower fit individuals. The presence of inflammation is, however, unlikely to be the main explanation for our results, given the overall health of this cohort. Lastly, nitric oxide and CO_2 have an effect on cerebral autoregulation[80]; when present, it increases the ability of the cerebral blood vessels to dilate or constrict in response to a sudden change in blood pressure, allowing for sufficient blood to flow. For example, it has been found that those with arterial hypertension, have greater central chemosensitivity than those without.[77,81] Moreover, during exhaustive exercise, cerebral autoregulation was decreased compared to rest,[82] and was observed to be reduced in young master athletes compared to sedentary counterparts.[83] It is therefore possible, given the interplay between CO_2 sensitivity (local vs. central), nitric oxide presence and cerebral autoregulation, that in combination this may account for the reduced BOLD-CVR in higher fit individuals in aging.

The results of this and other studies have shown that quantitative techniques such as MRI measurements of CVR and CBF may be biased by health components not typically taken into account in MRI studies (e.g. local or central chemosensitivity and cerebral autoregulation). This is problematic as it may lead to bias in group comparisons or longitudinal studies. Though we were not able to test these additional parameters in the present study, it highlights the need for comprehensive studies that seek to measure all the components of the complex relationship between cerebral hemodynamics and VO_2peak. These studies are necessary to make these techniques truly quantitative and reveal the physiological changes that occur in aging and disease.

Limitations

Although we found that higher VO_2peak is related to decreased BOLD-CVR in aging, it is difficult to interpret our results in comparison to other studies due to the high level of variability of BOLD-CVR in the aging literature. For example, some report 0.19% BOLD/mmHg in line with our results,[84] yet another study has reported higher levels at 0.28%[85] and lower levels at 0.13%.[86] This indicates that there is physiological variability within individuals and between studies, and potentially technical variability (i.e. type of scanner, delivery of CO_2, amount of CO_2 inhaled). Moreover, given that our CO_2 challenge was 5 mmHg, it could be that our data suffer from worse SNR than what would be expected with a greater amount of CO_2 delivered, such as 10 mmHg. Therefore, more work is necessary to further comprehend inter-individual variability, and to implement more robust study designs with a greater breadth of outcome measures and to implement progressive hypercapnia in addition to block designs.

A limitation of the use of ASL for measuring CBF is that extensive coverage of the entire brain is typically not possible without advanced parallel imaging techniques. Given that the original aim of this study at the time of data collection was more focused on executive functions and the frontal lobes, it was not possible to capture structural and hemodynamics of the hippocampus. Therefore, while the hippocampus is a structure associated with VO_2peak-related changes, we are unable to test for associations between the hippocampus and VO_2peak here. Furthermore, the post-label delay chosen here is suboptimal for older adults, so that lower perfusion measured could be the result of slower transit time, rather than lower perfusion. Moreover, given that blood flow velocity is likely increased during hypercapnia, and it is known that labeling efficiency decreases as blood velocity increases,[87] there is a potential for our CBF data during hypercapnia to be underestimated as we assumed a consistent labeling efficiency which is likely not the case. Thus, future work aiming to disentangle the relationships among aging, cognition and VO_2peak should optimize the acquisition, using a multi-band acquisition approach for example, to capture both the entire cerebral cortex and the hippocampus, and multiple post-label delays to better capture perfusion across age and VO_2peak.

Another limitation to this study is its cross-sectional design, which makes it difficult to draw clear conclusions about the relationships between VO_2peak, aging and brain health. While large longitudinal cohorts exist, none have so far also included measurement of CVR and VO_2peak, likely because these techniques are challenging to implement. On the other hand, ASL acquisitions are becoming more common and future studies could attempt to use cohorts of older adults for studying

the relationship between physical activity, CBF and other measures of vascular health. Dedicated longitudinal studies over several years including VO_2peak, CBF and CVR would however be necessary to truly understand these relationships. Although VO_2max is considered the gold standard of cardiovascular fitness, there are some indications from the literature that a true VO_2max may not be practically attainable in older adults.[17] Thus, we used VO_2peak here. It is also noteworthy that there are inherent limitations to using either as an outcome, as they can be influenced by genetics, pulmonary function, skeletal muscle limitations, cardiac output, to name a few (see Bassett and Howley[88] for in-depth review), which were not measured in this study.

Conclusions

Overall, this paper identified a negative relationship between BOLD-CVR and VO_2peak in a very healthy older adult sample. Within the ROI's that demonstrated a significant relationship, other hemodynamic outcomes also showed negative relationships with VO_2peak. These negative relationships could be the result of changes in CO_2 sensitivity, or autoregulation. In addition, our findings suggest that quantitative measures of CVR and CBF could be biased by unknown physiological changes in these autoregulatory and chemosensitivity properties, and that studies using these markers in aging and disease may underestimate their effects on cerebral hemodynamics. Thus, to further understand and attempt to disentangle the modulatory effect that VO_2peak has on hemodynamics in aging, more comprehensive studies of physiological outcomes are necessary.

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Authors' contributions

Brittany Intzandt provided substantial contribution to this project through data analysis and interpretation; original drafting of manuscript and revised it for essential intellectual content and approved the version to be published.

Dalia Sabra provided substantial contribution to this project through data analysis and interpretation; revised it critically for essential intellectual content and approved the version to be published.

Catherine Foster provided substantial contribution to this project through data analysis and interpretation; revised it critically for essential intellectual content and approved the version to be published.

Laurence Desjardins-Crépeau made a substantial contribution to this project through her acquisition of the data, intellectual feedback and approved the final version to be published.

Richard D Hoge had a substantial contribution to the concept and design of this study; provided feedback on interpretation and approved the final version to be published.

Christopher J Steele had a substantial contribution to the data analysis and interpretation of data; provided critical intellectual feedback and approved the final version to be published.

Louis Bherer made a substantial contribution to the concept and design; interpretation of data; was essential to revisions for intellectual content and approved the version to be published.

Claudine J Gauthier made a substantial contribution to all components of this manuscript including: concept and design; acquisition, analysis and interpretation of data; essential to the drafting and intellectual content; and approved the version to be published.

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References

1. Erecińska M and Silver I. ATP and brain function. *J Cereb Blood Flow Metab* 1989; 9: 2–19.
2. Knaepen K, Goekint M, Heyman EM, et al. Neuroplasticity – exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects. *Sports Med* 2010; 40: 765–801.
3. Kraft E. Cognitive function, physical activity, and aging: possible biological links and implications for multimodal

interventions. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* 2012; 19: 248–263.
4. Erickson K and Kramer A. Aerobic exercise effects on cognitive and neural plasticity in older adults. *Brit J Sport Med* 2009; 43: 22–24.
5. Sexton C, Betts J, Demnitz N, et al. A systematic review of MRI studies examining the relationship between physical fitness and activity and the white matter of the ageing brain. *Neuroimage* 2016; 131: 81–90.
6. Erickson KI, Leckie RL and Weinstein AM. Physical activity, fitness, and gray matter volume. *Neurobiol Aging* 2014; 35: S20–S28.
7. Gomes-Osman J, Cabral DF, Morris TP, et al. Exercise for cognitive brain health in aging: a systematic review for an evaluation of dose. *Neurol Clin Pract* 2018; 8: 257–265.
8. Heckman GA and McKelvie RS. Cardiovascular aging and exercise in healthy older adults. *Clin J Sport Med* 2008; 18: 479–485.
9. Seals DR, Walker AE, Pierce GL, et al. Habitual exercise and vascular ageing. *J Physiology* 2009; 587: 5541–5549.
10. Ashor A, Lara J, Siervo M, et al. Exercise modalities and endothelial function: a systematic review and dose–response meta-analysis of randomized controlled trials. *Sports Med* 2015; 45: 279–296.
11. Monahan K, Tanaka H, Dinenno F, et al. Central arterial compliance is associated with age- and habitual exercise-related differences in cardiovagal baroreflex sensitivity. *Circulation* 2001; 104: 1627–1632.
12. Meyer M, Tanaka H, Palta P, et al. Correlates of segmental pulse wave velocity in older adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Hypertension* 2015; 29: 114–22.
13. Pugh K and Wei J. Clinical implications of physiological changes in the aging heart. *Drugs Aging* 2001; 18: 263–276.
14. Zieman S, Melenovsky V and Kass D. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005; 25: 932–943.
15. Saltin B and Strange S. Maximal oxygen uptake: 'old' and 'new' arguments for a cardiovascular limitation. *Med Sci Sports Exerc* 1992; 24: 30–37.
16. Hollenberg M, Ngo LH, Turner D, et al. Treadmill exercise testing in an epidemiologic study of elderly subjects. *J Gerontol A Biol Sci Med Sci* 1998; 53: B259–267.
17. Huggett DL, Connelly DM and Overend TJ. Maximal aerobic capacity testing of older adults: a critical review. *J Gerontol A Biol Sci Med Sci* 2005; 60: 57–66.
18. Thomas AG, Dennis A, Bandettini PA, et al. The effects of aerobic activity on brain structure. *Front Psychol* 2012; 3: 86.
19. Tseng BY, Uh J, Rossetti HC, et al. Masters athletes exhibit larger regional brain volume and better cognitive performance than sedentary older adults. *J Magn Reson Imaging* 2013; 38: 1169–1176.
20. Zimmerman B, Sutton BP, Low KA, et al. Cardiorespiratory fitness mediates the effects of aging on cerebral blood flow. *Front Aging Neurosci* 2014; 6: 59.
21. Chapman SB, Aslan S, Spence JS, et al. Shorter term aerobic exercise improves brain, cognition, and cardiovascular fitness in aging. *Front Aging Neurosci* 2013; 5: 75.
22. Colcombe S, Erickson K, Raz N, et al. Aerobic fitness reduces brain tissue loss in aging humans. *J Gerontol A Biol Sci Med Sci* 2003; 58: 176–180.
23. Colcombe S, Erickson K, Scalf P, et al. Aerobic exercise training increases brain volume in aging humans. *J Gerontol A Biol Sci Med Sci* 2006; 61: 1166–1170.
24. Erickson K, Raji C, Lopez O, et al. Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study. *Neurology* 2010; 75: 1415–1422.
25. Maass A, Düzel S, Goerke M, et al. Vascular hippocampal plasticity after aerobic exercise in older adults. *Mol Psychiatry* 2015; 20: 585–593.
26. Tardif C, Steele C, Lampe L, et al. Investigation of the confounding effects of vasculature and metabolism on computational anatomy studies. *Neuroimage* 2017; 149: 233–243.
27. Tardif C, Gauthier C, Steele C, et al. Advanced MRI techniques to improve our understanding of experience-induced neuroplasticity. *Neuroimage* 2016; 131: 55–72.
28. Thomas B, Yezhuvath U, Tseng B, et al. Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO_2. *J Magn Reson Imaging* 2013; 38: 1177–1183.
29. Tarumi T, Gonzales MM, Fallow B, et al. Central artery stiffness, neuropsychological function, and cerebral perfusion in sedentary and endurance-trained middle-aged adults. *J Hypertens* 2013; 31: 2400–2409.
30. Chapman SB, Aslan S, Spence JS, et al. Distinct brain and behavioral benefits from cognitive vs. physical training: a randomized trial in aging adults. *Front Hum Neurosci* 2016; 10: 338.
31. Petcharunpaisan S, Ramalho J and Castillo M. Arterial spin labeling in neuroimaging. *World J Radiol* 2010; 2: 384–398.
32. Willie C, Tzeng Y, Fisher J, et al. Integrative regulation of human brain blood flow. *J Physiol* 2014; 592: 841–859.
33. Chen JJ and Pike BG. MRI measurement of the BOLD-specific flow–volume relationship during hypercapnia and hypocapnia in humans. *Neuroimage* 2010; 53: 383–391.
34. Gauthier CJ, Madjar C, Desjardins-Crépeau L, et al. Age dependence of hemodynamic response characteristics in human functional magnetic resonance imaging. *Neurobiol Aging* 2013; 34: 1469–1485.
35. de Vis J, Hendrikse J, Bhogal A, et al. Age-related changes in brain hemodynamics: a calibrated MRI study. *Hum Brain Mapp* 2015; 36: 3973–87.
36. Jefferson A, Cambronero F, Liu D, et al. Higher aortic stiffness is related to lower cerebral blood flow and preserved cerebrovascular reactivity in older adults. *Circulation* 2018; 138: 1951–1962.
37. Liu P, De Vis JB and Lu H. Cerebrovascular reactivity (CVR) MRI with CO_2 challenge: a technical review. *Neuroimage* 2019; 187: 104–115.

38. Bright MG and Murphy K. Reliable quantification of BOLD fMRI cerebrovascular reactivity despite poor breath-hold performance. *Neuroimage* 2013; 83: 559–568.
39. Inoue Y, Tanaka Y, Hata H, et al. Arterial spin-labeling evaluation of cerebrovascular reactivity to acetazolamide in healthy subjects. *Am J Neuroradiol* 2014; 35: 1111–1116.
40. Mandell D, Han J, Poublanc J, et al. Mapping cerebrovascular reactivity using blood oxygen level-dependent MRI in patients with arterial steno-occlusive disease: comparison with arterial spin labeling. *Stroke* 2008; 39: 2021–2028.
41. Lu H, Xu F, Rodrigue KM, et al. Alterations in cerebral metabolic rate and blood supply across the adult lifespan. *Cereb Cortex* 2011; 21: 1426–1434.
42. Krainik A, Hund-Georgiadis M, Zysset S, et al. Regional impairment of cerebrovascular reactivity and BOLD signal in adults after stroke. *Stroke* 2005; 36: 1146–1152.
43. Hartkamp NS, Hendrikse J, van der Worp HB, et al. Time course of vascular reactivity using repeated phase-contrast MR angiography in patients with carotid artery stenosis. *Stroke* 2012; 43: 553–556.
44. Bailey DM, Marley CJ, Brugniaux JV, et al. Elevated aerobic fitness sustained throughout the adult lifespan is associated with improved cerebral hemodynamics. *Stroke* 2013; 44: 3235–3238.
45. Braz I, Flück D, Lip G, et al. Impact of aerobic fitness on cerebral blood flow and cerebral vascular responsiveness to CO2 in young and older men. *Am J Physiol Heart Circ Physiol* 2017; 27: 634–642.
46. Barnes JN, Taylor JL, Kluck BN, et al. Cerebrovascular reactivity is associated with maximal aerobic capacity in healthy older adults. *J Appl Physiol* 2013; 114: 1383–1387.
47. Tarumi T, Gonzales M, Fallow B, et al. Cerebral/peripheral vascular reactivity and neurocognition in middle-age athletes. *Med Sci Sports Exerc* 2015; 47: 2595–2603.
48. Kurlowicz L and Wallace M. The mini-mental state examination (MMSE). *J Gerontol Nurs* 1999; 25: 8–9.
49. Wu W, Fernández-Seara M, Detre JA, et al. A theoretical and experimental investigation of the tagging efficiency of pseudocontinuous arterial spin labeling. *Magn Reson Med* 2007; 58: 1020–1027.
50. Gauthier C, Lefort M, Mekary S, et al. Hearts and minds: linking vascular rigidity and aerobic fitness with cognitive aging. *Neurobiol Aging* 2015; 36: 304–314.
51. Slessarev M, Han J, Mardimae A, et al. Prospective targeting and control of end-tidal CO_2 and O_2 concentrations. *J Physiol* 2007; 15: 1207–1219.
52. Storer T, Davis J and Caiozzo V. Accurate prediction of VO2max in cycle ergometry. *Med Sci Sports Exerc* 1990; 22: 704–712.
53. Gaser C and Robert D. CAT a computational anatomy toolbox for the analysis of structural MRI data. HBM2016. http://www.neuro.uni-jena.de/hbm2016/GaserHBM2016.pdf.
54. Penny W, Friston K, Ashburner J, et al. *Statistical parametric mapping: The analysis of functional brain images* 2011; 92–100.
55. Ashburner J and Friston KJ. Voxel-based morphometry – the methods. *Neuroimage* 2000; 11: 805–821.
56. Avants B, Epstein C, Grossman M, et al. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Med Image Analy* 2008; 12: 26–41.
57. Liu T and Wong E. A signal processing model for arterial spin labeling functional MRI. *Neuroimage* 2005; 24: 207–215.
58. Gauthier CJ and Hoge RD. Magnetic resonance imaging of resting OEF and $CMRO_2$ using a generalized calibration model for hypercapnia and hyperoxia. *Neuroimage* 2012; 60: 1212–1225.
59. Gauthier CJ, Desjardins-Crépeau L, Madjar C, et al. Absolute quantification of resting oxygen metabolism and metabolic reactivity during functional activation using QUO2 MRI. *Neuroimage* 2012; 63: 1353–1363.
60. Glover GH. Deconvolution of impulse response in event-related BOLD fMRI1. *Neuroimage* 1999; 9: 416–429.
61. Chappell M, Groves A, Whitcher B, et al. Variational Bayesian inference for a nonlinear forward model. *IEEE Transac Signal Process* 2009; 57: 223–236.
62. Herment A, Kachenoura N, Lefort M, et al. Automated segmentation of the aorta from phase contrast MR images: validation against expert tracing in healthy volunteers and in patients with a dilated aorta. *J Magn Reson Imaging* 2010; 31: 881–888.
63. Winkler A, Ridgway G, Webster M, et al. Permutation inference for the general linear model. *Neuroimage* 2014; 92: 381–397.
64. Smith SM and Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* 2009; 44: 83–98.
65. Ralph BD, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* 2008; 117: 743–753.
66. Farokhian F, Yang C, Beheshti I, Matsuda H, et al. Age-related gray and white matter changes in normal adult brains. *Aging Dis* 2017; 8: 899–909.
67. Maillet D and Rajah NM. Association between prefrontal activity and volume change in prefrontal and medial temporal lobes in aging and dementia: a review. *Ageing Res Rev* 2013; 12: 479–489.
68. Flück D, Braz I, Keiser S, et al. Age, aerobic fitness, and cerebral perfusion during exercise: role of carbon dioxide. *Am J Physiol Heart Circ Physiol* 2014; 307: H515–H523.
69. Catchlove SJ, Parrish TB, Chen Y, et al. Regional cerebrovascular reactivity and cognitive performance in healthy aging. *J Exp Neurosci* 2018; 12: 1–11.
70. Peng S-L, Chen X, Li Y, et al. Age-related changes in cerebrovascular reactivity and their relationship to cognition: a four-year longitudinal study. *Neuroimage* 2018; 174: 257–262.
71. Zheng G, Ye B, Zheng Y, et al. The effects of exercise on the structure of cognitive related brain regions: a meta-analysis of functional neuroimaging data. *Int J Neurosci* 2019; 129: 406–415.

72. Kaminsky LA, Imboden MT, Arena R, et al. Reference standards for cardiorespiratory fitness measured with cardiopulmonary exercise testing using cycle ergometry: data from the fitness registry and the importance of exercise national database (FRIEND) registry. *Mayo Clin Proc* 2017; 92: 228–233.
73. Thambisetty M, Lori B-H, An Y, et al. APOE epsilon4 genotype and longitudinal changes in cerebral blood flow in normal aging. *Arch Neurol* 2010; 67: 93–98.
74. Scarmeas N, Habeck CG, Stern Y, et al. APOE genotype and cerebral blood flow in healthy young individuals. *JAMA* 2003; 290: 1581–1582.
75. Wierenga CE, Clark LR, Dev SI, et al. Interaction of age and APOE genotype on cerebral blood flow at rest. *J Alzheimers Dis* 2013; 34: 921–935.
76. Halani S, Kwinta JB, Golestani AM, et al. Comparing cerebrovascular reactivity measured using BOLD and cerebral blood flow MRI: the effect of basal vascular tension on vasodilatory and vasoconstrictive reactivity. *Neuroimage* 2015; 110: 110–123.
77. Katayama K, Sato Y, Morotome Y, et al. Ventilatory chemosensitive adaptations to intermittent hypoxic exposure with endurance training and detraining. *J Appl Physiol* 1999; 86: 1805–1811.
78. McConnell A and Semple E. Ventilatory sensitivity to carbon dioxide: the influence of exercise and athleticism. *Med Sci Sports Exerc* 1996; 28: 685–691.
79. Haas J, Brigitte S-H, Biessmann A, et al. Inducible nitric oxide synthase and argininosuccinate synthetase: co-induction in brain tissue of patients with Alzheimer's dementia and following stimulation with beta-amyloid 1-42 in vitro. *Neurosci Lett* 2002; 322: 121–125.
80. White R, Vallance P and Markus H. Effect of inhibition of nitric oxide synthase on dynamic cerebral autoregulation in humans. *Clin Sci* 2000; 99: 555–560.
81. Malenfant S, Brassard P, Paquette M, et al. Compromised cerebrovascular regulation and cerebral oxygenation in pulmonary arterial hypertension. *J Am Heart Assoc* 2017; 6: e006126.
82. Ogoh S, Dalsgaard MK, Yoshiga CC, et al. Dynamic cerebral autoregulation during exhaustive exercise in humans. *Am J Physiol Heart Circ Physiol* 2005; 288: H1461–H1467.
83. Mikkel L-H, Cotter JD, Helge JWW, et al. Cerebral autoregulation dynamics in endurance-trained individuals. *J Appl Physiol* 2011; 110: 1327–1333.
84. Bhogal AA, Vis JB, Siero J, et al. The BOLD cerebrovascular reactivity response to progressive hypercapnia in young and elderly. *Neuroimage* 2016; 139: 94–102.
85. Mcketton L, Sobczyk O, Duffin J, et al. The aging brain and cerebrovascular reactivity. *Neuroimage* 2018; 181: 132–141.
86. Leoni RF, Oliveira IAF, Pontes-Neto O, et al. Cerebral blood flow and vasoreactivity in aging: an arterial spin labeling study. *Braz J Med Biol Res* 2017; 50: e5670.
87. Aslan S, Xu F, Wang PL, Uh J, et al. Estimation of labeling efficiency in pseudocontinuous arterial spin labeling. *Magn Reson Med* 2010; 63: 765–771.
88. Bassett DR and Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 2000; 32: 70–84.

1.2 Scatterplots

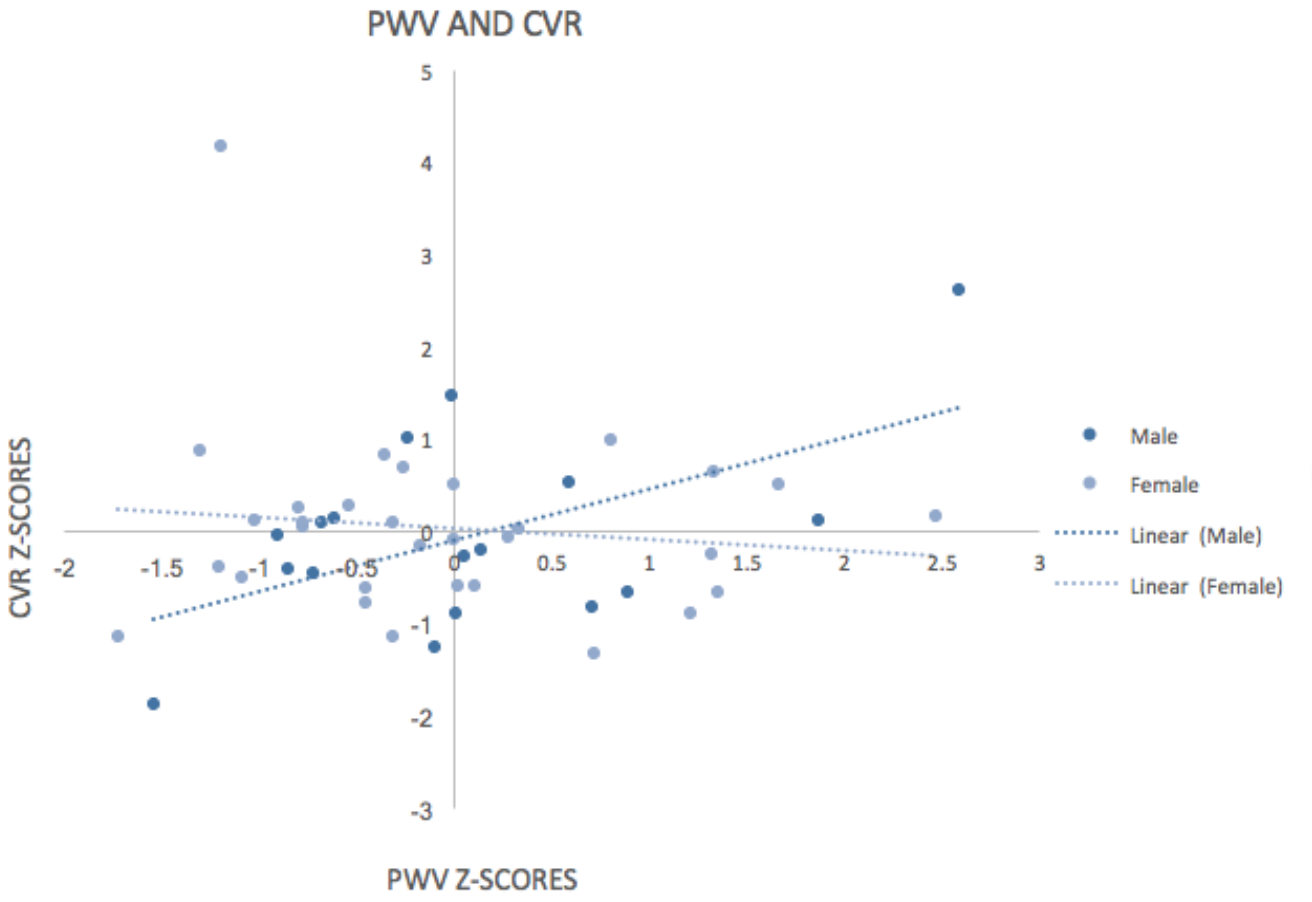

Figure 1: Values reported as Z scores showing a positive relationship between Cerebrovascular reactivity (CVR) and Pulse wave velocity (PWV) among men (r=0.551; p=0.027)

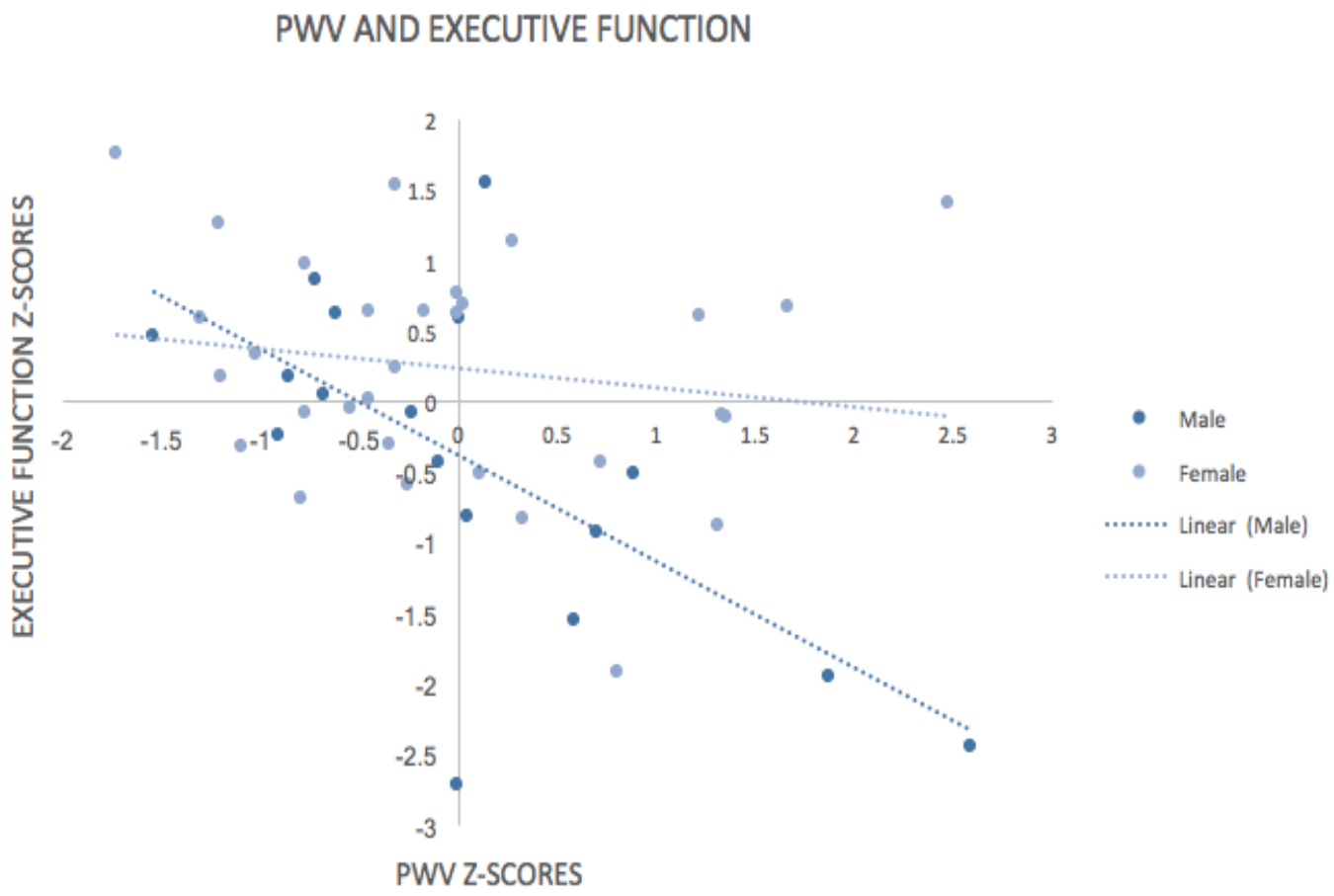

Figure 2: Values reported as Z scores showing a negative relationship between Pulse wave velocity (PWV) and Executive Function among men (r=-0.659; p=0.006)

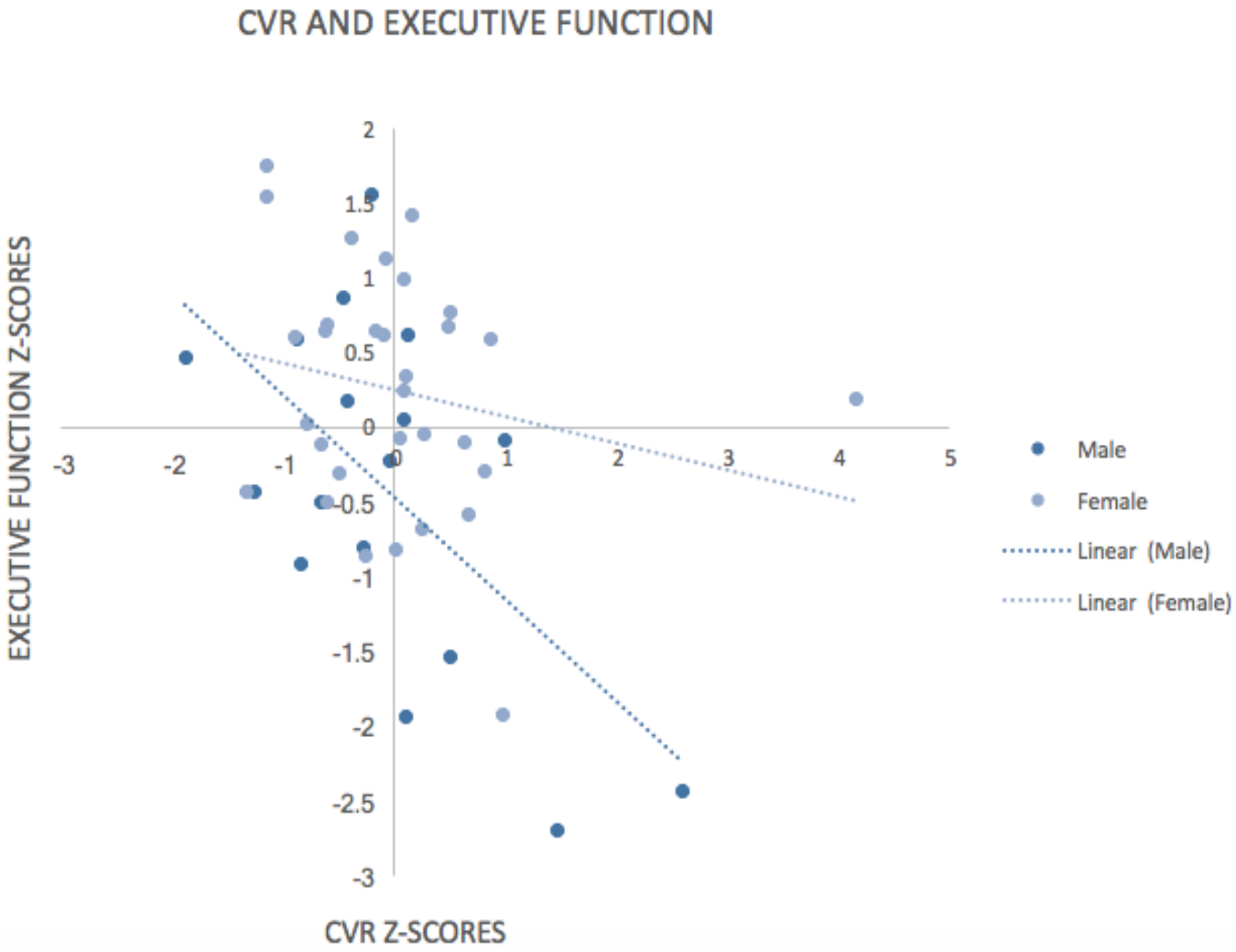

Figure 3: Values reported as Z scores showing a negative relationship between Executive Function and Cerebrovascular reactivity (CVR) among men (r=-0.640; p=0.008).