UNDERSTANDING THE ROLE OF CEREBROVASCULAR HEALTH IN COGNITIVE AGING: A MULTI-MODAL NONINVASIVE HUMAN NEUROIMAGING APPROACH

BY

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DISSERTATION

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ABSTRACT

The brain's vasculature undergoes age-related physiological and anatomical changes similarly to the rest of the cardiovascular system. However, the health of the cerebrovasculature may be related to cognitive ability. Thus, it is critical to determine the effect of cerebrovascular health on cognition and the mediators of cerebrovascular health across the lifespan.

Since aerobic fitness is known to alleviate both cognitive and volumetric losses in the brain, it is important to investigate some of the possible mechanisms underlying these beneficial changes. In one experiment, we investigated the role that cardiorespiratory fitness (CRF) plays in determining the relationship between aging and cerebral blood flow (CBF) in a group of older adults (ages 55-85). Using arterial spin labeling (ASL) to quantify CBF, we found that blood flow in the gray matter was positively correlated with CRF and negatively correlated with age (Zimmerman et al., 2014). Subsequent analyses revealed that CRF fully mediated the effects of age on CBF in the gray matter, but not in the white matter.

Whether this same effect holds true for younger adults is unknown. In the next study, using a large sample of resting cerebral blood flow measured with arterial spin labelling in younger adults, we demonstrate that the relationship between cardiorespiratory fitness and cerebral blood flow is negative. Although the relationship is weak, the observation demonstrates that the interpretation of resting cerebral blood flow as a measure of cerebrovascular health should be made with caution.

In order to gain an improved understanding of how cerebrovascular health impacts cognitive aging and relates to CRF, ASL was used in a third study to investigate both the resting and activation CBF in healthy older adults ranging in age from 56-88. To this end, we analyzed measures of both baseline CBF and changes in CBF during activation from a visual task. We found that the change in CBF in the visual cortex to a reversing checkerboard stimulus, but not the baseline CBF, was associated with neuropsychological measures of executive function. While baseline CBF was correlated with age and CRF, the change in CBF was correlated with the participants' pulse pressure. These results indicate that the measures of baseline CBF and activation-related CBF are separable measures of vascular health in older age that relate differentially to measures of physiology and cognition.

Because reactivity measures are dynamic and contain a temporal component, we were interested in whether improved temporal resolution could reveal differences in the time course of cerebrovascular reactivity across age or arterial compliance. In this final study, multi-distance, frequency-domain near infrared spectroscopy (NIRS) was used to measure changes in oxy- and deoxy-hemoglobin concentrations induced by breath holding in the right prefrontal cortex concurrently with ASL in a magnetic resonance imaging (MRI) scanner. Studying participants ranging in age from 55-87, we found that the superior temporal resolution of NIRS allowed us to observe differences in the speed of the oxy-hemoglobin response to a period of breath holding between our older and younger participants and between participants split by arterial compliance, where older individuals and participants with stiffer arteries tended to have a delayed hemodynamic response. This finding highlights the usefulness of utilizing a multi-modal neuroimaging approach for the investigation of time-sensitive aspects of cerebrovascular health.

Overall, this set of experiments highlights the complexity of measures of cerebrovascular health both across the lifespan and in their relationships to cognition. We demonstrated that in older adults, resting CBF was related to CRF, and CRF mediated age-related declines in CBF. Interestingly, the relationship between CBF and CRF reversed in a sample of younger adults. Further analysis revealed that resting CBF did not predict cognitive decline in a sample of older adults. In contrast, the level of task-related change in CBF did positively relate to executive functioning. Pursuing measures of reactivity further, we found that adding NIRS measures provided temporal resolution that allowed us to see differences in the timing of cerebrovascular reactivity across age and arterial compliance in the brain. These timing differences may be complementary to the amplitude differences found through ASL, and future research will continue to resolve these separable components of cerebrovascular health.

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CHAPTER 1

GENERAL INTRODUCTION

The Challenges of Societal Aging

Human civilization has been steadily increasing its population since the plague in the middle ages. H. von Foerster and his colleagues, P.M. Mora and L.W. Amiot (1960) famously and controversially published a tongue-in-cheek article modeling world population growth entitled "Doomsday: Friday, 13 November, A.D. 2026" after fitting a simple growth equation with historical data and finding, to his dismay, that the global population exploded to infinity at a finite time not so far in the future. Since then, world fertility rates have dropped, but the world population is still climbing. Besides birth-rate, average lifespan also influences the overall population. Unlike birth rate, average lifespan has been steadily increasing due to advancing healthcare and technology. The result of this is an aging society as a whole, and especially as the baby-boomer generation enters into their golden years, the diseases and challenges of aging have become more important to address. In 2010, a little over 500 million people were aged 65 years or older, which represented about 8 percent of the world population. That number is expected to grow substantially in the coming decades to nearly 1.5 billion by 2050, about 16 percent of the expected population. (World Population Prospects: the 2010 revision, United Nations)

Aging costs society. Chronic and debilitating diseases of aging such as Alzheimer's disease and other dementias are particularly costly. The global costs of dementia have grown by 35.4% from 2010 to 2015, from $604 billion to $818 billion, representing over 1% of the global GDP (World Alzheimer Report, 2015). These economic challenges are matched by emotional ones, and recently focus has shifted away from "lifespan" to "healthspan," the length of time a person is healthy – not just alive. Thus, it has become a scientific priority to understand aging and successfully treat and prevent deleterious age-related conditions.

Cognitive aging

Cognitive aging is typically split into two categories: "normal" cognitive decline and disease-related cognitive decline. It is important to note that there is debate regarding the line between these two types of age-related cognitive decline. On one hand, it is argued that certain diseases have specific etiologies, courses, and biomarkers that clearly differentiate them from other types of decline. However, on the other hand, cognitive decline, disease-related or not, is caused by some biological dysfunction and many of those malfunctioning biological mechanisms may be implicated in eventual disease states. The differentiation between disease-related and non-disease-related cognitive decline has had the unfortunate outcome of the field sometimes using "healthy" cognitive aging as a synonym for "normal." This misnomer is dangerous. All cognitive decline that is caused by biological decline should be considered unhealthy and a target for prevention and treatment.

The work in this thesis is primarily concerned with normal cognitive aging, although research on dementia is sometimes referenced. Research on normal cognitive aging should be approached with a few caveats in mind, which are useful for interpreting and thinking about aging research. Cognitive aging research on humans can be accomplished using cross-sectional or longitudinal designs. Both of these designs have some inherent weaknesses that should be discussed. Cross-sectional designs can suffer from cohort effects, which can be especially bothersome in aging studies. This is partially because there are large differences between age cohorts that may affect the performance on cognitive tests. Other factors may lead to non-representative cohorts. For instance, most academic institutions most easily recruit students that attend that institution. In addition, elderly subjects who choose to participate in a research study are necessarily mobile enough to make it to the experiment and likely to be engaged and interested in science, which may select for a non-representative portion of that population. Middle aged individuals who can participate in research experiments may be more likely to not have a day job that would otherwise prevent them from coming to the lab during normal work hours.

Longitudinal designs may look more convincing because cognitive performance is followed over the course of aging. However, these designs suffer from their own drawbacks. First, it is difficult to

control for training effects, which will inevitably occur if the participant is frequently tested on similar cognitive tasks. The researcher needs the tasks to be similar, so that they can compare the participant's performance over time, but in many tasks, improvements due to practice effects are expected. Another issue is attrition, and particularly attrition that could be caused by some confounding factor. In aging studies, it may be more likely for an older participant who is suffering from cognitive decline to drop out of the study, thus artificially inflating the performance of the group as a whole.

Normal cognitive aging is typically characterized by declines in "fluid intelligence" in contrast to stable or even increasing "crystallized intelligence." Originally conceived of by Cattell and refined by Horn (1965), fluid intelligence is composed of many cognitive domains that deal with flexibly manipulating information in novel situations without relying on previously acquired knowledge, whereas crystallized intelligence represents the ability to utilize experience and knowledge. In terms of cognitive processes, fluid intelligence relies heavily on executive functioning and on processing speed, and unsurprisingly, process based theories of cognitive aging have been primarily focused on these (e.g. Executive functioning, especially inhibition: Hasher & Zacks, 1988; Processing speed: Salthouse, 1996).

Typically, in the context of aging, data is presented cross-sectionally and shows fairly linear declines in domains such as inductive reasoning, spatial orientation, and processing speed, but stable or increasing performance in domains such as numeric and verbal ability, which rely more heavily on crystallized intelligence (e.g. Park et al., 2002). These striking declines in fluid cognitive abilities, which show fairly stable cognitive decline beginning in the twenties are enough to motivate any graduate student into immediate action. However, longitudinal data are typically less striking, and tell a somewhat different story. For instance, longitudinal data from the Seattle Longitudinal Study agree with the cross-sectional conclusion that processing speed declines stably from early adulthood, but most other cognitive domains peak in middle age and then decline in old age (Hedden and Gabrieli, 2004; Schaie, 1996). These differences may be partially explained by the disadvantages inherent to each of these methods discussed above, and the truth likely lies somewhere in between. Whatever the average trajectory is, recent research has demonstrated a telling and hopeful fact: that cognitive aging is highly variable between and plastic

within individuals (Lindenberger, 2014). Understanding that variability well is the first step toward improving cognitive outcomes in old age.

The effect of aerobic exercise on cognitive aging

One of the most promising factors that explains variability in cognitive aging is one's involvement in regular exercise. This is especially evident when examining the effect of exercise on previously sedentary older adults. Evidence from both observational and interventional studies point to the efficacy of physical activity on improving or maintaining cognitive performance in old age (for a review see Kirk-Sanchez & McGough, 2014).

Observational studies

A few large observational studies have found that individuals with greater levels of physical activity, even light and moderate physical activity such as walking, have lower risk for cognitive decline (Honolulu-Asia Aging Study, Abbott et al., 2004; Taaffe et al, 2008; Nurse's Health Study, Weuve et al., 2004). The optimal exercise intensity, duration, and type is still not completely clear, but some studies do suggest a dose response effect, where those who engage in the highest levels of physical activity have the greatest reduction of risk of dementia and cognitive decline (Hamer & Chida, 2009; van Gelder et al., 2004; Yaffe et al., 2001).

Some observational studies have begun to dig deeper into the details of the fitness habits that are most beneficial. Some longitudinal studies have found significant effects of the intensity and the variety of physical activities on cognitive function (Angevaren et al., 2007, Podewils et al., 2005). To summarize, from both cross-sectional and longitudinal observational studies, there seems to be consistent evidence for the protective effects against cognitive decline through greater physical activity.

Intervention studies

A landmark meta-analytic study conducted by Colcombe and Kramer (2003) found robust, yet selective effects of aerobic fitness interventions on cognition in older adults. They observed the greatest effects of aerobic exercise for executive-control processes, although significant effects were also seen for spatial tasks and processing speed. More recent meta-analytic studies have found smaller effects or have failed to observe effects exercise interventions on cognition, which may be due to differences in method and inclusion of studies, but may also be due to "decline effects", which often occur for scientific claims due to publication bias. Smith et al. (2010) observed modest improvements across many cognitive domains including attention, processing speed, executive functioning, and memory. Although the observed effect sizes from this meta-analysis were quite a bit smaller than those found by Colcombe and Kramer, they also allowed samples of younger adults in the study. Snowden et al. (2011) used an expert, multi-disciplinary panel to rate the quality and effectiveness of exercise intervention studies to determine if there was sufficient evidence to make public health recommendations for physical activity and exercise interventions. Unlike the conclusions of the prior meta-analyses, they determined that after judging the quality of the evidence from the existing invention studies, there was insufficient evidence for such a recommendation. In addition, van Uffelen et al. (2008) failed to demonstrate that physical exercise interventions had an overall positive effect on cognitive performance. The authors highlighted the difficulty in pooling studies with large variability in study populations, exercise protocols, and cognitive measurements on the interpretation of results.

On the one hand, similarly designed, high quality trials are necessary to reach a consensus regarding the effects of exercise interventions on cognition. On the other hand, there is tremendous interest in determining the details in interventions that make some more successful than others, including the type of exercise, optimal intensity, duration or frequency of exercise, and variability between different cohorts that might affect the outcome. The existing observation and interventional studies should guide hypotheses about future interventional studies (e.g. incorporating multiple types of activities, or restricting cohorts to specific types of groups, such as sedentary older adults).

How fitness may improve cognition

Current research is building off of the foundation of support for exercise improving cognition and seeking to understand the mechanisms that lead to the improvements. Many mechanisms have been proposed and have support both from human and animal research (see Hillman et al., 2008 for a review). MRI studies in humans have revealed that aerobic fitness is related to larger brain volumes in select regions (Colcombe et al., 2003; Erikson et al., 2009) (although, the regions that are negatively affected by age are not necessarily those same regions that are supported by fitness (Fletcher et al., 2016)), more intact white matter tracts (Colcombe et al., 2003; Colcombe et al., 2006; Burzynska et al., 2014), and differences in functional connectivity (Voss et al., 2010). The molecular and physiological mechanisms thought to contribute to these differences include increased neurogenesis (van Praag et al., 2005), neurotropic factors (Erickson et al., 2011), which influence signaling and synaptic connectivity (Eadie, Redila & Christie, 2005; Hu et al., 2009), and through improved vascular health (Desjardins, 2015), which is the focus of this thesis.

Effect of normal aging on cerebrovascular function

There is good reason to hypothesize that the beneficial effects of physical fitness on cognitive aging are mediated in part through improvements to the cerebrovascular system. This is because the health of the cerebrovasculature, which is so important to the functioning of the metabolically greedy brain, normally declines with age, and it is know that vascular health in general normally improves with cardiorespiratory fitness.

Despite its low proportion of overall body weight (~2%), at rest the brain utilizes about 20% of the oxygen and nutrients in the cardiovascular system. The high consumption of energy is necessary to maintain normal functioning of the brain, and mostly contributes to maintaining membrane potentials and reversing the ion influxes responsible for synaptic potentials and action potentials (Attwell & Laughlin, 2001). Lack of oxygen can damage neurons and impair function. To sustain or optimize neuronal function, functional hyperaemia increases blood flow to the brain where it is necessary. This is typically

thought of in terms of neurovascular coupling, where neuronal activity directs increases of blood flow to metabolically active areas of the brain, but can also occur globally due to increases of CO_2 in the blood or, to a lesser degree, decreases in O_2 (Querido & Sheel, 2007). Therefore, the health of the cerebrovascular system could be considered from two different but interacting perspectives: the resting capabilities of the vascular system to provide blood and nutrients to the brain and the reactive capabilities of the vascular system to respond to dynamic changes in the brain's requirements for blood.

Normal Resting Blood Flow

There are no major energy stores within in the brain, so the high level of metabolism that the brain engages in must be matched by constantly maintained cerebral blood flow (CBF). Resting levels of CBF decline over the lifespan beginning at or even before middle age (Sonntag et al., 2007). These changes in blood flow are due to a few factors including the density of the microvasculature, alterations in the vascular structure, and possibly to chronic decreases in reactivity (Sonntag et al., 2007). The changes in CBF are most likely partially due to decreased metabolic demand, which also declines with age in certain areas of the brain, but there is at least some evidence that age-related decreases in CBF are independent from regional brain atrophy (Chen, Rosas, & Salat, 2011).

Endothelial dysfunction due to oxidative stress seems to be a crucial player in explaining some of the declining vascular health. Reactive oxygen species disrupt endothelial function especially by decreasing nitric oxide bioavailability, which has been shown to relate to lower basal CBF (Pialoux et al., 2009). However, there is evidence for a wide range of mechanisms for vascular aging including increased vascular inflammation, increased arterial stiffness, endothelial cell senescence, and endothelial apoptosis and microvascular rarefaction (Ungvari et al., 2010).

Normal age-related decline in CBF is not sufficient to cause major ischemic injury. However, it is possible that particularly metabolically active areas, or brain regions that are furthest from their supplying arteries, dip below their threshold for hypoperfusion. Especially during times of increased metabolic need,

small levels of hypoperfusion may prevent the supply of adequate oxygen and nutrients from reaching the impoverished region resulting in impaired neuronal processing or even in cellular damage.

Neurovascular Reactivity

Besides supporting the resting metabolic needs of the brain, special physiological mechanisms are in place to support dynamic needs of the brain tissue by locally manipulating CBF. This functional hyperemia is critical for maintaining required levels of blood flow. In fact, when induced by neuronal activation, functional hyperemia typically overshoots the metabolically needs of an area of tissue. The reasons for this overshoot are still not completely understood, but one compelling hypothesis with some support is that the overall overshoot of oxygen is required to maintain the baseline tissue oxygenation at the most distal locations from the supplying blood vessels (Devor et al., 2011).

The control of functional hyperemia is multi-faceted. Increases in blood flow due to neuronal activation are controlled primarily through the neurovascular unit, a structurally and functionally related grouping of neurons, astrocytes, and vascular cells. Neurotransmitters and other signaling factors from the neurons directly activate arteriole smooth muscle and astrocytes, which further manipulate the vessel tone (Hamel, 2006). In addition to neuronally mediated hyperemia, the neurovascular system also responds chemically to CO_2 levels. Therefore, along with neural activity, breathing CO_2 enriched air or breath holding can also subsequently increase blood flow, and has even been used to calibrate blood oxygen level-dependent signals from neuronal activation (Pillai & Mikulis, 2015). Just like resting blood flow, neurovascular coupling has been demonstrated to decline with age (Panczel et al., 1999; Safanova et al., 2004; Galvin et al., 2010).

Cerebrovascular Reserve

Since it is known that the cerebrovascular system can dynamically react to changing needs of blood flow, it is possible that the mechanisms of neurovascular reactivity are utilized to support chronic hypoperfusion through vasodilation. This hypothesis fits nicely into the idea of cerebrovascular reserve

(CVR), which represents the range of cerebral perfusion variation from the resting baseline (Davenport et al., 2012). The concept of cerebrovascular reserve could be expanded to be thought of in a similar way to "cognitive reserve," which describes a resiliency to cognitive decline through the utilization of other brain networks or cognitive strategies (Stern, 2009). In the same vein, the cerebrovascular system may be able to rely on some aspects of its own health to compensate for other aspects of its deterioration.

Aerobic exercise and cerebrovascular function

Aerobic exercise benefits the cerebrovascular system by improving almost all of the same aspects that aging vitiates. It enhances endothelial function and angiogenesis, and it decreases arterial stiffness, oxidative stress, and vascular inflammation (Davenport et al., 2012). Exercise upregulates endothelial NOS expression, and thus an increase in vasodilation (Endres et al., 2003). That increased vasodilation could then lead to increases in resting CBF. Some have postulated that increased blood flow influences the endothelial cells through increases in vascular shear stress (Davenport et al., 2012). It is worth noting that this hypothesis relies on the assumption of increased cerebral blood flow during exercise. Interestingly, changes in cerebral blood flow during exercise are heavily influenced by the amount of CO_2 in the blood. Typically, blood flow does increase with light to moderate exercise, but during vigorous exercise, hyperventilation dramatically decreases CO_2 levels, which in turn reduces cerebral blood flow back to baseline levels (Querido & Sheel, 2007). The implication of this is that if transient increases in shear stress due to increased blood flow do mediate the effects of exercise on cerebrovascular health, greater gains could paradoxically be achieved through less intense aerobic exercise. It is also clear that improvements to the cerebrovascular system interact with the other potential mechanisms by which exercise benefits the brain and cognition. For example, brain-derived neurotrophic factor (BDNF) is partially regulated by NO, which is enhanced by exercise (Cheng et al., 2003).

Benefit of exercise vs. lack of sedentariness

An important incomplete task in this line of research is to differentiate between benefits of exercise vs. a lack of sedentariness. Recent research has emphasized this point by demonstrating that the amount of time spent per day sitting explains a different portion of the variability in health outcomes than the amount of time spent exercising (Owen et al., 2010). In much research, there is a somewhat implicit assumption that "sedentary" simply represents the true baseline of a physical activity scale. In reality, in terms of health, the true baseline may be some moderate amount of physical activity. For instance, it could be that above some threshold, exercise did nothing to improve one's health. However, participating in less physical activity than this threshold could be detrimental to one's health. Most likely, the real situation is not that extreme, but this issue still impacts how research is interpreted.

Often, fitness interventions or comparisons between groups look at changes in health in previously sedentary individuals or compare the effects of fitness against a sedentary group (e.g. Kramer et al., 1999; Ainslie et al., 2008). The results of these studies are sometimes presented in terms of the beneficial effects of fitness, which may be true, but only for this sub-group of sedentary adults. Unfortunately, this sedentary "sub-group" may actually be fairly representative of the population. A review by Harvey, Chastin, & Skelton (2013) pointed out that when measured objectively using accelerometry, 67% of the older population were sedentary for more than 8.5 hours daily.

This fact may justify the recommendation to use exercise as an intervention for age-related cognitive decline. However, if sedentariness and physical activity can be regarded as independent variables, that may help to explain some of the discrepancies in the research on the effects of exercise on cognitive performance.

Linking fitness and cognition through cerebrovascular reserve

Because of the positive effects of exercise on the same aspects of vascular health that aging impairs, and the known beneficial effects of exercise on cognition, it can be hypothesized that at least some of the benefits of fitness on cognition are mediated through vascular mechanisms.

This thesis attempts to extend the understanding of the role of neurovascular health in cognitive aging through a multi-modal, noninvasive human neuroimaging approach. Hopefully this chapter has succeeded in setting the stage for this research. Chapter 2 focuses on how cardiorespiratory fitness, and particularly activity level, mediates the normal, age-related declines in resting blood flow in older adults. This chapter attempts to further connect cerebrovascular health directly to fitness in old age. Chapter 3 examines the same relationship but within a sample of younger adults, and finds, unexpectedly, that the relationship between cardiorespiratory fitness and resting blood flow is actually slightly negative. The purpose of this chapter is to point out the complexity of the measure of cardiorespiratory fitness and to highlight the care one needs when utilizing blood flow as a measure of vascular health. Chapter 4 looks at differences between measures of resting vascular health and vascular reactivity. Different relationship profiles with physiology, age, and cognition are observed between these two vascular health measures, which is discussed in terms of the concept of cerebrovascular reserve. Finally, chapter 5 examines how the temporal resolution of optical imaging may be able to be used in conjunction with magnetic resonance imaging to measure aspects of vascular health, which may be especially relevant to measures of vascular reactivity. The thesis is concluded with a discussion synthesizing the results presented in the previous chapters and making recommendations for future experiments.

CHAPTER 2

CARDIORESPIRATORY FITNESS MEDIATES THE EFFECTS OF AGING ON CEREBRAL BLOOD FLOW[1]

Abstract

The brain's vasculature is likely to be subjected to the same age-related physiological and anatomical changes affecting the rest of the cardiovascular system. Since aerobic fitness is known to alleviate both cognitive and volumetric losses in the brain, it is important to investigate some of the possible mechanisms underlying these beneficial changes. Here we investigated the role that estimated cardiorespiratory fitness (CRF) plays in determining the relationship between aging and cerebral blood flow (CBF) in a group of older adults (ages 55-85). Using arterial spin labeling to quantify CBF, we found that blood flow in the gray matter was positively correlated with CRF and negatively correlated with age. Subsequent analyses revealed that CRF fully mediated the effects of age on CBF in the gray matter, but not in the white matter. Additionally, regional measures of CBF were related to regional measures of brain volume. These findings provide evidence that age-related effects on cerebrovascular health and perfusion in older adults are largely influenced by their CRF levels.

Introduction

Normal aging is accompanied by structural and functional changes that occur throughout all bodily systems, which in turn lead to physiological and psychological changes. These complex and interacting biological changes are at the basis of many of the diseases prevalent in older adults and also

[1] This chapter appeared in its entirety in *Frontiers in Aging Neuroscience* and is cited later in this dissertation as "Zimmerman et al., 2014". Zimmerman, B., Sutton, B.P., Low, K.A., Fletcher, M.A., Tan, C.H., Schneider-Garces, N., Li, Y., Ouyang, C., Maclin, E.L., Gratton, G., Fabiani, M. (2014). Cardiorespiratory fitness mediates the effects of aging on cerebral blood flow. *Frontiers in Aging Neuroscience, 6(59),* 1-13 . doi: 10.3389/fnagi.2014.00059. This article is reprinted with the permission of the publisher and is available to open access.

contribute to age-related decline in cognitive function. It is no surprise that recent evidence has begun to uncover links between the health of the central nervous system, and consequently cognition, and the health of the cardiovascular system in aging populations. For example, Chao et al. (2010) recently demonstrated that baseline measures of brain perfusion using arterial spin labeling (ASL) are predictive of cognitive decline and progression to dementia in older adults.

The cerebral vasculature is subject to the same age-related changes that affect other parts of the cardiovascular system, including compromised endothelial function and arterial stiffening (Izzo, 2005). In fact, there is ample evidence that the health and functioning of the brain's vasculature decreases with age. For example, vascular reactivity and compensatory vasodilation become impaired with age (Safonova et al., 2004; see also Fabiani et al., 2013). Furthermore, mean cerebral blood flow (CBF) declines steadily across the lifespan, with gray-matter blood flow significantly decreasing with age, as shown by Parkes et al. (2004), although this study did not investigate adults older than 67. Decline in CBF is related to an overall decline in brain volume, but research has shown that the decline in CBF is more than just a reflection of these volumetric changes (Chen et al., 2011). Thus, CBF may provide a good general index of changes in the health of the cerebral vasculature, in the same way that ventricular expansion has been used as an index of overall tissue shrinkage in the brain (e.g., Raz & Rodrigue, 2006), since it may reflect the overall state of perfusion and the rate of angiogenesis in the brain.

Fortunately, it may be possible to alleviate the decline in vascular health that accompanies aging. There is evidence that aerobic exercise is associated with improved endothelial function, greater arterial elasticity, and reduced risks for vascular diseases in aging adults (Clarkson et al., 1999; DeSouza et al., 2000). Likewise, animal studies have shown improvements in endothelial function and increases in CBF with increased aerobic exercise (Endres et al., 2003; Gertz et al., 2006). These findings become especially important when cognitive health – the degree to which cognitive functionality is maintained across the lifespan – is considered. Research suggests that increased aerobic fitness can prevent or even reverse cognitive decline (Hillman et al., 2008). For example, high-fit older adults have more preserved gray and white matter in the frontal, parietal, and temporal cortex than low-fit older adults (e.g., Colcombe et al.,

2006; Gordon et al., 2008). These volumetric differences in high-fit older adults are accompanied by increased performance in cognitive tasks compared to age-matched low-fit adults (Kramer et al., 2006). It could be hypothesized that aerobic fitness affects the process of cognitive aging, at least in part, through its remediating effects on the cerebral vasculature and perfusion.

Given this evidence, it is important to determine to what extent age-related declines in CBF (used as an indicator of cerebrovascular health) are related to lower levels of fitness (which is an individual difference variable that could be subject to intervention). Previous research has shown that aerobically-trained males have a reduction in their age-related decrease in blood flow velocity in the middle cerebral artery measured using Doppler sonography (Ainslie et al., 2008). However, this method does not probe perfusion directly. Therefore, in the current study we used arterial spin labeling (ASL), a non-invasive magnetic resonance (MR) technique that is capable of determining measures of CBF more directly (see Borogovac & Asllani (2012) for a recent review on ASL).

ASL utilizes the intrinsic water molecules in the blood as a tracer by tagging them with a saturating or inverting radiofrequency (RF) pulse. As this 'tracer' diffuses within brain tissue there is a reduction of tissue magnetization, and, as a consequence, a reduction in the MR signal. An image is taken during this process at some predetermined transit time. The process is repeated without labeling the water, and the difference between this control image and the tagged image yields a perfusion image. Using this method to derive CBF in aging adults, the present study investigated the relative contributions of age and estimated cardiorespiratory fitness (CRF) on CBF in different regions of the brain, corresponding to areas that have been shown to decline most substantially, both anatomically and functionally, during the normal aging process.

Method

Participants

Fifty-five adults (aged 55-87), who satisfied the inclusion criteria listed below, were recruited from the Champaign-Urbana area. Subjects with serious or chronic medical conditions, a history of major

neurological or psychiatric disease, or a history of drug abuse were excluded from this study. Additionally, subjects were screened and excluded if they showed signs of dementia and depression. Participants needed to score at least 51 on the modified Mini-Mental Status examination (Teng & Chui, 1987) and less than 14 on Beck's Depression Inventory (Beck et al., 1996) in order to participate in the study. Participants who smoked more than half a pack of cigarettes and/or consumed more than 2 drinks per day were also automatically excluded. All participants were right-handed (as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971), had normal or corrected-to-normal vision, were non-smokers, and were native speakers of English. Participants were also assessed for level of education, vocabulary (Shipley, 1940), and intelligence quotient (Kaufman & Kaufman, 2004) (Table 2.1). Participants' blood pressure was taken at three time points across the experiment and averaged to provide measures of systolic and diastolic blood pressure[2]. Pulse pressure was derived by taking the difference between systolic and diastolic blood pressure. All participants were paid for their time in the study and signed informed consent in accordance with the University of Illinois at Urbana-Champaign's Institutional Review Board.

In all, data from 14 participants were discarded from subsequent analysis. Of these, five participants did not have a complete MRI dataset, three participants were excluded due to movement artifacts, and six participants were removed as outliers for having at least one MRI measure that was 3 standard deviations different from the mean. The final sample included 41 older adults (aged 55-85, mean age = 69.2 years, 22 females). 16 of those participants reported being on medication for their blood pressure. The demographic characteristics of this sample are presented in Table 2.1.

[2] About 30% (N=12) of our sample show blood pressure values that are consistent with isolated systolic hypertension (systolic > 140, diastolic < 90). Isolated systolic hypertension appears to be a very common feature in older adults (Basile, 2002). Therefore, we felt that eliminating subjects with this particular syndrome could bias our sample and decided to keep these participants in the study.

MRI acquisition

All MRI data collection occurred at the Biomedical Imaging Center using a 3T Siemens (Erlangen, Germany) Trio scanner using a standard body coil transmission and a twelve-channel head array receive coil.

Subjects were instructed to perform a breath-holding task according to a visual cue, which included six repetitions of alternating periods of breath-holding after expiration (18 seconds) and self-paced breathing (36 seconds) for a total acquisition time of 5 minutes and 24 seconds. Breath holding tasks can be used to induce vasodilation in the brain and thus increase cerebral blood flow and allow for the investigation of vascular reactivity along with resting levels of perfusion. Instructions for the breath-holding experiment were presented using EPRIME (Psychology Software Tools, Pittsburgh) and displayed via back projection (BrainLogics, Psychology Software Tools, Pittsburgh). To minimize motion during the breath-holding period, padding was used to stabilize the subject's head.

Six axial imaging slices passing through the middle of the lateral ventricles and covering part of the frontal cortical areas were acquired with localized pseudo-continuous Transfer Insensitive Labeling Technique (pTILT) ASL sequence (Ouyang & Sutton, 2011), and the labeling slice was placed inferior to the imaging slab with a 10 mm gap. As part of a larger study not discussed here, near-infrared optical data were recorded concurrently from a patch on the forehead during the MR experiment. Therefore, the axial slices were lined up with the optical coverage. The pTILT ASL method is less sensitive to the transit time issues that may confound other ASL methods as a function of age. In fact, this method tags close to the slice of interest, minimizing the differences in transit time that may result from age-related changes in the vasculature.

The imaging and tagging parameters of the localized pTILT ASL sequence were: windowed-sinc 45^{o} RF pulses with 2560 μs-duration, tagging repetition spacing = 30 ms, number of concatenated RF pulse pairs = 100, tagging duration = 3 s, post-labeling delay = 0.5 s, tagging slice thickness = 10 mm, gradient spoiler duration and amplitude = 4000 μs/[±10, ±12, ±14, ±16] mT/m, SE-EPI readout, FOV = 220×220

mm, scan matrix size = 64×64, TR/TE = 4500/44 ms, slice thickness = 6 mm, slice gap = 1.2 mm, 36 control and 36 tag repetitions, scan time of one acquisition = 5 minutes and 24 seconds.

To assist with the registration procedure, two additional brain scans were taken: a high-resolution 2D turbo-spin echo (TSE) acquisition with the imaging slices at the same location as the ASL images, and a high-resolution T1-weighted 3D anatomical image. The T1-weighted brain image was acquired using a 3D MPRAGE (Magnetization Prepared RApid Gradient Echo) protocol (TR = 1900 ms, TI (inversion time) = 900 ms, TE = 2.32 ms, field of view = 230×230x172.8 mm^3 (sagittal), matrix size = 256×256×192, flip angle = 9°, slice thickness = 0.9 mm).

MRI data processing

The pTILT functional data processing was carried out using SPM 8 (Wellcome Department of Cognitive Neurobiology, University College of London, UK) and FSL 4.1.4 (FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl). The fMRI modeling of the BOLD, baseline perfusion, and activation perfusion responses were determined using the general linear model (GLM) with the ASL modeling framework described by Hernandez-Garcia, Jahanian, and Rowe (2010). The unsubtracted pTILT data were first realigned to remove motion artifacts. Four regressors were modeled in the GLM analysis: (1) the breath hold task BOLD response (a canonical hemodynamic response function, HRF); (2) baseline perfusion (a consistent, alternating waveform); (3) activation blood flow (an alternating waveform during the breath-hold task); and (4) a baseline signal (uniform intensity). After regression analysis, gray and white matter masks were formed from segmenting the T1 structural scan using FSL's FAST software (Zhang et al., 2001). The gray and white matter masks were then transformed into the subject's pTILT space using a registration between the control image in pTILT and the MPRAGE from FSL's FLIRT (Jenkinson & Smith, 2001).

Regional measures

In addition to the global gray and white matter masks above, the Harvard-Oxford cortical and subcortical structural atlases provided by FSL were used to isolate activity in more localized areas of the brain. A linear registration between the subject's MPRAGE and the atlas was used to bring regions back into the individual subject's MPRAGE space and then on to the pTILT space. A frontal region was isolated by dilating the frontal pole with a 3x3x3 voxel kernel three times. The parietal region was isolated by combining five parietal areas including the postcentral gyrus, superior parietal lobule, supramarginal gyrus, anterior division, supramarginal gyrus, posterior division, and the angular gyrus. Averaging activity in only these regions provided separate regional measurements for frontal and parietal analysis. Figure 2.1 (top panel) shows a sagittal section with the imaging volume. Figure 2.1 (bottom panel) provides an example of the locations of the frontal and parietal regions for a single participant within the six slices imaged. Because the six axial slices were lined up with the optical recording patch on the forehead, the temporal lobes were not adequately covered for regional analysis.

Motion correction

In several subjects, significant head motion artifacts were observed at particular locations in the time series of the pTILT breath-holding data. In order to reduce the motion influence and increase the reliability of estimation from other time points, the SPM 8 Robust Weighted Least Square (rWLS) toolbox (Diedrichsen & Shadmehr, 2005) was used. The rWLS toolbox reveals images that are impacted by motion or other noise, based on the residual-mean-square estimate, which is calculated by adding up the squared residuals over the whole volume for each individual time point when applying the linear model. As an alternative to deleting data points that have been contaminated by motion, the rWLS toolbox "soft"-excludes those images by weighting each observation with the inverse of its variance. Since image volumes that have been corrupted by motion will have high variance relative to the linear model, the "soft"-exclusion method results in the "bad" images being significantly down-weighted in the subsequent analysis (Diedrichsen & Shadmehr, 2005). Nevertheless, the contrast between breath-holding and baseline

measurements was invalidated by the present of consistent movements during the breath-hold period in some subjects. For this reason we focused instead on the baseline period.

CBF quantification

Baseline perfusion images in mL/100 g/min units were calculated, based on a single compartment model in which no blood exchange is assumed (Ouyang & Sutton, 2011):

$$CBF = \frac{\Delta M}{M_0} \cdot \frac{6000}{\lambda_{blood} \cdot T_{1,blood}} \cdot \exp\left(\frac{w + T_{slc} \cdot (n-1)}{T_{1,blood}}\right) \cdot \exp\left(\frac{TE}{T_{2,blood}}\right)$$

where ΔM is the estimated coefficient of the tag-control difference (i.e., the perfusion-weighted control-tag image); M_0 is the estimated coefficient of the static tissue signal (i.e. the control image); λ_{blood} is the water content of blood (0.9 as used in Chalela et al., 2000); w is the post-labeling delay (0.5 seconds); $T_{1,blood}$ (1680 ms at 3 T) and $T_{2,blood}$ (275 ms at 3T) are the longitudinal and transversal relaxation rates of blood (Stanisz et al., 2005); T_{slc} is the acquisition duration of one single slice, and n is the index of acquired slice; and TE (44 ms) is the echo time of the SE-EPI sequence. Figure 2.2 shows an example of perfusion image in six axial slices from one subject.

Anatomical Volume Estimation

Structural MRI images were processed with FreeSurfer (Dale et al., 1999). FreeSurfer allows for automated segmentation of cortical and sub-cortical volumes (Fischl et al., 2002; 2004a; 2004b; Desikan et al., 2006). Estimates of cortical volumes were obtained using an automated probabilistic labeling procedure based on the Desikan-Killiany anatomical atlas (Fischl et al., 2002; Desikan et al., 2006). Subcortical and cortical volumes were normalized by intracranial volume using a co-variance approach to account for

volumetric differences in head size (Jack et al., 1989; Buckner et al., 2004)[3]. FreeSurfer output was inspected for errors through extensive visual screening performed by three different highly trained individuals, with each person examining all slices for errors. Corrections to FreeSurfer output were performed according to the methods found online (http://surfer.nmr.mgh.harvard.edu /fswiki/FsTutorial/ TroubleshootingData).

Cardiorespiratory fitness

The "gold standard" measure for physical fitness is VO_{2max}, which is a measure of the maximum oxygen consumption in an individual's body obtained during a maximal graded bout of exercise. However older and low-fit individuals may have conditions that prevent them from participating in the stressful exercise routine. Since these individuals are of crucial interest for the examination of the relationship between CBF, fitness, and aging, in the current study cardiorespiratory fitness (CRF) was estimated according to this equation (see Jurca et al. 2005):

CRF = Gender (2.77) – Age (0.10) – Body Mass Index (0.17) – Resting Heart Rate (0.03) + Self-Reported Activity Score + 18.07.

This measure utilizes easily acquired parameters that are highly predictive of VO_{2max} (Jurca et al., 2005). It has been demonstrated to approximate VO_{2max} with good accuracy in a large sample (N>10,000), and recently, Mailey et al. (2010) extended the validity of the CRF estimate specifically to older adults, ranging in ages from 60-80. McAuley et al. (2011) further validated this measure for smaller sample sizes by showing that there was no significant difference between this estimated measure of CRF and the gold-standard, physician-supervised, maximal exercise test in a sample of 86 older adults. Finally, in a survey

[3] Although it is true that intracranial size is related to gender, intra-individual differences in intracranial volume represent more than only gender, so it is important to control for both. Further support for this comes from recent evidence showing that intracranial volume measurements themselves may be biased by gender (Nordenskjöld et al., 2013). Since finding partial correlations with gray-matter volume requires that gender be removed from both, we believe that removing gender after correcting for intracranial volumes was justified.

of over 32,000 individuals ranging in age from 35 to 70 years, Stamatakis and colleagues (2013) found CRF to be a good predictor of cardiovascular (and overall) mortality, comparable to associations between exercise testing CRF and mortality.

Analysis of CRF

The current study was focused on the relationship between mean CBF, age, and CRF. Since motion was correlated with the breath-holding task itself, even after using rWLS to correct for movement, we lacked sufficient power to analyze the activation CBF during the breath-holding intervals, and therefore focused on the baseline CBF, corresponding to rest periods.

Since we sought to determine the impact of CRF independently of known gender differences in VO_{2max} (Hutchinson et al., 1991), we first regressed out gender from each of our variables of interest and then used the residuals for all subsequent comparisons. We used regression analysis to test the simple effects of age and CRF on CBF for each region of the brain.

To test whether CRF mediated the effects of age on CBF, we performed a mediation analysis (see Baron and Kenny, 1986) using multiple regression. This type of mediation analysis consists of three steps. The first two steps are aimed at demonstrating the significant relationships between the proposed mediator (CRF) and the independent variable (age) and between the dependent variable (CBF) and the independent variable (age), which we knew to be true from our previous analysis of bivariate correlations. The final step involves regressing the dependent variable on both the independent variable and mediator. After this step, the unique effect of the independent variable when the mediator is included as a predictor variable is compared to the simple effect of the independent variable alone. If including both the mediator and independent variable into the regression equation eliminates CBF's dependence on the independent variable (age), then the remaining significant variable is said to fully mediate the effects of the other on the dependent variable (Baron & Kenny, 1986). Three separate mediation analyses were conducted, one examining global effects and the other two exploring mediation in parietal gray and white matter, as these were the cases in which we observed all of the prerequisite relationships.

Various formal statistical tests have been devised for the purpose of directly testing the significance of the mediating effect. One reason for this is that the rules that Baron and Kenny provide for determining whether a mediation exists do not account for the possibility of observing a loss in the significance of the relationship between the independent and dependent variables without a significant change in the size of the actual coefficient (Holmbeck, 2002). The Sobel test (Sobel, 1982) analyzes the significance of the change in the predictive ability of the independent variable on the dependent variable before and after a mediator variable is included in the analysis, and thus provides a more formal treatment of the mediation. However, because the Sobel test depends on a normally distributed mediation effect, more recent work has recommended the use of bootstrapping the sampling distribution of the mediation effect to build an empirically based confidence interval (Preacher & Hayes, 2004). In consideration of these recommendations, we report the confidence intervals established through Preacher and Hayes' bootstrapping procedure using 10,000 resamples.

Results

Table 2.2 reports the means and standard deviations of the mean CBF measures by region, for gray and white matter separately. Dependent sample *t*-tests revealed that gray matter CBF was significantly greater than the white matter CBF globally ($t(38) = 22.52$, $p < .001$), as well as in the frontal ($t(38) = 13.19$, $p < .001$) and parietal ($t(38) = 21.71$, $p < .001$) regions that we investigated.

Table 2.3 shows the partial correlations between the measures of CBF, age, CRF, and blood pressure after controlling for gender. Based on previous research, we hypothesized that CRF would be positively correlated with CBF whereas age would be negatively correlated with it, even in our restricted age-range sample of older adults. It should be noted here that there were no significant differences between participants who took medication to control their blood pressure and participants who did not on any of the measures reported in this paper. Given the previously established directionality of blood flow changes with age and our corresponding directional hypotheses, we performed one-tailed significance tests. Figures 2.3 and 4 (top panels) show the relationship between the independent variables (CRF and age) and gray and

white matter mean flow, respectively. As we expected, CRF positively correlated with gray matter mean flow, and age negatively correlated with gray matter mean flow. CRF was also correlated with the white matter mean flow (Figure 2.3, top panel). However, age was only marginally predictive of white matter mean flow $r(38) = -.22$, $p = .08$.

Because many age-related effects on cognition are related to attention control and working memory functions (see Fabiani, 2012), which involve the frontal and parietal regions of the cortex, and because other studies have shown more severe rates of brain deterioration with aging in these regions (Hillman, 2008; Raz & Rodrigue, 2006; Resnick et al., 2003), we examined how age and CRF affected the mean blood flow in these specific regions of the brain using the same linear regression analysis. Figures 2.3 and 2.4 (middle panels) respectively show the relationships with frontal gray and white matter mean flow. In frontal regions, CRF predicted gray and white matter mean flow, similarly to what we found when examining global effects. In contrast, frontal regions did not show a reliable relationship between age and mean CBF in either the gray, $r(38) = -.09$, $p = .28$, or white matter, $r(38) = -.02$, $p = .44$. Figures 2.3 and 2.4 (bottom panels) show the correlations with parietal mean flow. In parietal regions, we observed relationships similar to those reported in the global analysis, with both age and CRF predicting mean blood flow in gray and white matter.

Mediation Analysis

Since CRF and age were significantly correlated ($r(38) = -.42$, $p < .01$), an important question to address is whether CRF mediates the age-related effects of mean CBF. Therefore we performed the mediation analyses as described in the method section for the global gray matter mean flow and the parietal gray and white matter mean flow (i.e., the flow measures that showed relationships with both age and CRF). For both global and parietal gray matter, we found that CRF fully mediated the effects of age on CBF. However, no significant mediation by CRF was evident for the effects of age on parietal white matter flow.

Figure 2.5 (top panel) shows the path diagram of this mediation on global gray matter blood flow. Two-tailed significance tests were performed throughout the mediation analysis. Using the global measures of gray matter CBF, the regression of CBF on age, ignoring CRF, was marginally significant ($b = -.35$,

$t(39) = -2.00$, $p = .05$). Furthermore, the regression of CRF on age, also proved to be significant ($b = -.08$, $t(39) = -2.89$, $p < .01$). Finally CBF was regressed on both age and CRF at the same time. Critically, the unique effect of CRF remained significant ($b = -2.24$, $t(39) = -2.39$, $p < .05$) whereas the original effect of age completely disappeared ($b = -.17$, $t(39) = -.92$, $p = .36$). When using the bootstrap estimate, the mediation effect varied between -.37 and -.03 with 95% confidence. Because the 95% confidence interval did not include the 0 value, the result indicated a significant mediation effect.

A similar analysis was performed using the parietal measures of gray matter CBF (Figure 2.5, middle panel). The regression of these regional CBF measures on age was significant, ($b = -.38$, $t(39) = -2.17$, $p < .05$). When CBF was regressed on age and CRF at the same time, the effect of CRF remained significant ($b = 2.27$, $t(39) = 2.39$, $p < .05$), whereas the original effect of age went completely away ($b = -.20$, $t(39) = -1.08$, $p = .29$). Similar to the global analysis, the bootstrapping procedure put the mediation effect between -.45 and -.01 at 95% confidence, indicating a significant mediation effect.

Finally, a third mediation analysis (Figure 2.5, bottom panel) was conducted using the CBF measures from the white matter located in the parietal region. The regression of these CBF measures on age was not significant using two-tailed significance tests ($b = -.22$, $t(39) = -1.78$, $p = .08$). Here, when CBF was regressed on age and CRF together, neither CRF ($b = .903$, $t(39) = 1.29$, $p = .21$) nor age ($b = -.15$ $t(39) = -1.09$, $p = .28$) remained predictive of CBF. Therefore, in the white matter, we did not observe any mediating effects of CRF on age-related differences in CBF.

In our model, we conceptualize CRF as the mediator of the well-known age effect on CBF. An alternative, perhaps less intuitive model, is that age acts a mediator of the CRF effect on CBF. This result would be far less interesting and so further analysis is warranted to demonstrate that CRF is the mediator of an age effect and not the other way around. Over the global gray matter, when testing age as the potential mediator of the effect of CRF on CBF, the mediation effect varied between -.34 and 1.43 with 95% confidence. Because the 95% confidence interval did include the 0 value, the result indicated a non-significant mediation effect. Likewise, in the parietal gray matter, the mediation effect varied between -.27 and 1.39 with 95% confidence, and in the parietal white matter, the mediation effect varied between -.22

and 1.10 with 95% confidence. Again, these results indicated that age was not a significant mediator of the effect of CRF on CBF in the regions we analyzed. This result lends support for our original model, which conceptualized CRF as the mediator.

Analysis of CRF components

Since we demonstrated that CRF acted as a mediator of the age effects on CBF, it was of interest to understand how the individual components that were used to derive the CRF score related to CBF. Because all of our prior analyses controlled for the effects of gender, we continued to partial out gender while reporting correlations for the sake of interpretability (Table 2.4). We observed the strongest correlation between the activity score component and global and parietal gray matter CBF. Interestingly, body mass index (BMI) was negatively correlated with frontal gray matter CBF, but not with the global CBF or parietal CBF. Both of these components had stronger correlations with CBF than age.

In addition, to further corroborate these findings, we carried out a sensitivity analysis, where we subdivided our sample first by gender, and then into younger and older groups within each gender. Within each of these four groups we calculated the mean CRF and global gray matter CBF value, computed the residuals for each subject, and then computed correlations on the bases of these residual scores (thus effectively eliminating the confounds due to gender and age). The same procedure was also applied for the other three components of the CRF score (BMI, pulse rate, and activity score; see Figure 2.6). In this stratified analysis, we hoped to further observe what aspects, if any, contributed to the relationship between CRF and CBF when equating gender and age groups. We found that the residuals CRF scores were still significantly correlated with the residual CBF scores ($r(39) = .28$, $p = .04$). Although neither the residuals of BMI nor those of pulse rate were correlated with the residuals of CBF, the residual activity scores were marginally significantly correlated with the residual CBF scores ($r(39) = .28$, $p = .07$), which corresponds to our the analysis of the components of CRF presented above.

Analysis of anatomical volumes

In order to investigate whether or not localized CBF was predictive of other local brain measures, we analyzed the correlations between the volumes of the superior frontal and inferior parietal cortex and the regional measures of CBF (Table 2.5). These anatomical parcellations were chosen on the basis of their large size and likelihood to overlap with the frontal and parietal regional masks that were used to assess regional CBF in each individual subject. We observed a dissociation between the relationship of regional measures of CBF and differing regions of anatomy: frontal CBF predicted superior frontal brain volume but not inferior parietal volume, and parietal CBF marginally predicted inferior parietal volume but not superior frontal brain volume. These results suggest that local CBF may be related to variations in regional cortical volumes.

Discussion

Our findings provide evidence that the declines in CBF that accompany aging are highly related to CRF. CBF was negatively correlated with systolic blood pressure and pulse pressure, which provides support that CBF is, in fact, measuring perfusion rather than blood flow in the arteries. If CBF was measured in the arteries, we would have expected increases in blood pressure to be *positively* correlated with increased arterial blood flow. Furthermore, the fitness effects on CBF in the present analysis fully mediated the age effects on blood flow in the regions of the brain that showed a significant relationship between age and blood flow. Surprisingly, in frontal regions, no significant effect of aging on CBF was observed over the restricted age-range of our sample of older adults (aged 55-85). This is in contrast to anatomical studies that have reported reliable age-related changes in the frontal areas of the brain, when comparing younger adults (college age) to older adults (for a review of relevant work, see Raz, 2004). The limited age range of our study may account, at least in part, for this negative finding.

Our overall observation of a negative correlation between global CBF and age is consistent with previous reports using a variety of imaging techniques, including ASL (Ainslie et al., 2008; Beason-Held et al., 2007; Parkes et al., 2004). The findings reported here corroborate previous research indicating that improving CRF may provide a route to stave off or even ameliorate the normal age-related declines in CBF,

as well as the global cerebral atrophy that usually accompanies it (Ainslie et al., 2008). In fact, the mediation analyses suggest that a more direct relationship exists between CRF and CBF than between age and CBF. If this is the case, then working to increase CRF into old age may function as a potent method to preserve brain vascular health and stave off brain pathologies.

In our sample, it is clear that self-reported activity level seems to have a strong contribution to the overall impact of CRF on CBF. This result is important for our interpretation, since there is an inherent "age" component in CRF, and that component could have been explaining a large portion of the variance in CBF. The fact that activity level seems to play such a large role in explaining the impact of CRF on CBF meshes well with other research that has demonstrated striking changes in the brains of older adults after cardiorespiratory exercise interventions (see Bherer, Erikson, & Liu-Ambrose, 2013 for a review). However, our analysis shows that the impact of CRF may be more complex than simply reflecting the level of activity, since BMI shows a negative correlation with CBF only in the frontal brain areas that we examined. Complicating our interpretation, some of the variables that contribute to CRF including pulse rate and BMI may have different implications depending on the time of life at which they are measured. For example, typically a higher resting pulse rate is considered to be a risk factor for cardiorespiratory disease (Fox et al., 2007). However, in older individuals a low resting pulse may also be indicative of heart disease (Ufberg & Clark, 2006). Likewise, BMI may be negatively correlated with fitness earlier in life, but as a sample increases in age, many individuals with high BMI will have expired and the correlation between BMI and fitness may reverse, as being underweight may be associated with more health problems (Yan et al., 2004). It is clear that CRF is a complex measure, and the intriguing disparities in how the different components relate to flow in specific brain regions warrants future research. Specifically, given the correlations between many of the variables we analyzed, it is of particular importance to further investigate the relationship between CRF and CBF in aging adults. Longitudinal studies and interventions that improve CRF will help to further elucidate the impact of fitness on the age-related declines in CBF.

Although our analysis of the white matter revealed similar patterns to the gray matter analysis, CBF in the white matter may not be functionally equivalent to CBF in the gray matter. Parkes et al. (2004)

reported a positive trending correlation (r = .34; p = 0.10) between age and white matter CBF. More recently, Lu et al. (2011) also showed that in certain areas of the white matter CBF increased with age. An age-related increase in white matter CBF alongside of decreases in gray matter CBF may appear to be counterintuitive. A potential explanation was proposed by Aslan et al. (2011). In their study, the authors found an inverse correlation between the anisotropy of water diffusion and the blood flow along white matter fiber tracts. This suggests that the deterioration of white matter integrity is positively related to a local rise in CBF. This inverse relationship is most likely due to the structure of the myelinated axons in the white matter. Demyelination of axons could lead to an increased need for greater ion flux and energy for depolarization, since nodes of Ranvier normally minimize this need. In addition, it may also reduce the ability of the structure of the white matter to offset the pressure of perfusion. Both of these factors may lead to increased blood flow (Aslan et al., 2011). Overall, this study points out that the blood flow in the white matter may not be completely determined by neurovascular coupling in the same way that it is in the gray matter. The measures of white matter blood flow we reported likely reflect a combination of blood flow downstream of the gray matter along with the effects of any age-related white matter degradation. Since these two age-related factors impact blood flow in different directions, it is likely that our measures of CBF in the white matter are much less sensitive to age-related changes, and much more difficult to interpret.

The positive impact of fitness on CBF during healthy aging may work in a number of ways, mostly involving improvements in the cardiovascular system at different levels. Maintaining a higher level of fitness has been shown to reduce many cardiovascular risk factors, such as hypertension and diabetes, which may interact with the brain in complex ways (Clarkson et al., 1999; Jennings & Zanstra, 2009). Chronic hypertension has been shown to correlate with long term structural changes in small cerebral vessels (Baumbach & Heistad, 1989), cerebral microbleeds (Wang et al., 2009), brain atrophy (Salerno et al., 1992), and even the permeability of the blood-brain barrier (Yang et al., 1991). Although these examples reflect clear disease states, preclinical physiological changes, such as plaque deposition and insulin resistance, emerge over time. It is possible that accruing these types of changes over time may be involved in age-related declines in brain cognitive function, and that reversing or slowing this process would lead to

protection against related declines. It has also been shown that habitual physical activity is associated with greater endothelial health, including increased endothelium-dependent vasodilation and availability of nitric oxide, which may serve to reduce oxidative stress (Taddei et al., 2000). Lavi et al. (2006) have demonstrated that endothelial dysfunction disrupts vascular reactivity to CO_2, and may disrupt CBF regulation that is dependent on CO_2 levels. This is especially interesting given the age-related impairments in vasodilation to hypercapnia seen by Safonova et al. (2004). Finally, certain chemical factors have also been shown to be upregulated with exercise, and have been implicated in some of the neuroprotective effects of fitness (Kramer et al., 2006). Most research has revolved around brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1), which may improve angiogenesis and neurogenesis (Black et al., 1990; Carro et al., 2001; Garza et al., 2004; Kramer et al., 2006; Neeper et al., 1995). Recent research has also shown correlations between cellular viability (as indexed by N-acetylaspartate) and CRF (Erikson et al., 2012). All these mechanisms may lead either directly or indirectly to increased CBF, and each of them may also lead to improved overall brain health and cognitive function. It has been known for some time that aerobic exercise interventions, which serve to improve CRF, show benefits in both physical and cognitive health in aging subjects (Colcombe et al., 2006; Colcombe & Kramer, 2003). However, what the current study illustrates is how much of the normal physiological declines present in healthy aging is related to decreased fitness. In fact, our findings indicate that a substantial portion of the variance in brain health that was previously attributed to 'aging' in general is mediated by declines in CRF.

The finding that localized measures of CBF are related to localized measures of brain volume supports the effectiveness of ASL as a tool to investigate localized perfusion in the brain, as well as the importance of investigating differences between brain regions. In this particular study, the total global measures of CBF were split up into regions to assess whether CBF differences in these more specific areas were related to local differences in brain volume. In the future, it will be critical to examine even smaller regions to determine how sensitive this measure of CBF truly is to local brain health, beyond global effects. Given the correlative nature of studying CBF in normal aging humans, it is impossible to determine whether changes in volume lead to changes in CBF, or if CBF can be an important mediator of future changes in

brain volume. Further longitudinal research may help elucidate how current regional CBF is involved in predicting subsequent regional changes in brain volume or other measures of brain health.

Methodological considerations

There is an inherent difficulty in studying healthy aging participants. In order to volunteer for and attend our studies, older adults must necessarily have some degree of physical independence and mobility. Many people who may not be fit or genetically protected against vascular disease may die or be incapable of participation before they are old enough to be included in the study. This problem manifests itself in a possible underestimation of the impact of fitness on CBF. Furthermore, because the average life expectancy of females is greater than the life expectancy of males, sampling only from the oldest group of subjects may allow for an oversampling of extra fit males compared to females. Given that baseline CBF may already differ between men and women (Kastrup et al., 1999), it is especially important to control for gender differences when analyzing CBF.

Another potential confound arises while studying CBF with fMRI. Individual voxels likely contain contributions of both some white and gray matter, which could lead to different flow values in separate voxels labelled as gray matter, based solely on differential contributions of white and gray matter. These partial volume effects could lead to misinterpretations of CBF results. However, blood pressure was negatively correlated with CBF, demonstrating that there is a relationship between CBF and at least one physiological measure that is independent from any partial volume effects. This lends support to the claim that the results reported here are not simply artifacts of partial volume effects.

Summary and conclusions

The main findings of our study indicated that CRF is strongly correlated with CBF explored by our 6 slices in the brain, and that the effects of age on CBF are significantly mediated by CRF. It should be emphasized that this effect is observed in an otherwise healthy group of older adults, and refers to

benefits in “normal” aging as opposed to benefits for protection against pathology. By maintaining higher CRF, it appears possible to substantially impact the well-established age-related declines in CBF.

Tables

Table 2.1. Overall demographic characteristics.

	Mean	Standard Deviation	Range
Age (years)	69.15	8.31	55-85
CRF (metabolic equivalents)	6.80	2.25	1.58-11.31
Systolic Blood Pressure	135.41	14.20	105.67-167.33
Diastolic Blood Pressure	79.40	7.62	63.33-96.00
Pulse Pressure	56.01	13.58	34.33-91.33
Education (years – capped at 20)	16.87	2.89	12-20
Modified Mini-Mental Status examination	55.27	1.30	51-57
Beck's Depression Index	3.22	3.55	0-14
Shipley's Vocabulary Test	36.17	2.47	31-40
Kaufman Brief Intelligence Test	117.41	10.92	95-142

Note. N = 41.

Table 2.2. Mean and standard deviation of global and regional blood flow measures.

		Mean	Standard Deviation
Gray Matter CBF (mL/100g tissue/min)	Global	44.79	9.37
	Frontal	37.03	11.76
	Parietal	50.99	9.98
White Matter CBF (mL/100g tissue/min)	Global	23.08	6.45
	Frontal	22.77	9.70
	Parietal	25.41	7.14

Table 2.3. Partial correlations between age, CRF, blood pressure, and CBF, controlling for gender.

	Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
	1. Age	1										
	2. CRF	-.42**	1									
Blood Pressure	3. Systolic	.24	-.21	1								
	4. Diastolic	-.25	-.10	.35*	1							
	5. Pulse	.39*	-.17	.85**	-.20	1						
Gray Matter CBF	6. Global	-.31	.44**	-.27	-.06	-.25	1					
	7. Frontal	-.09	.37*	-.34*	-.14	-.28	.83**	1				
	8. Parietal	-.33*	.45**	-.16	-.03	-.15	.86**	.55**	1			
White Matter CBF	9. Global	-.22	.32*	-.39*	-.10	-.35*	.75**	.70**	.59**	1		
	10. Frontal	-.02	.30	-.39*	-.16	-.31	.62**	.80**	.36*	.78**	1	
	11. Parietal	-.27	.29	-.30	-.05	-.29	.60**	.44**	.62**	.86**	.49**	1

Note. Significance tests between groups (2-tailed): $df = 38$, * $p < .05$, ** $p < .01$

Table 2.4. Partial correlations between the individual components used to derive CRF and measures of blood flow, while controlling for gender.

		Age	BMI	Average Pulse	Activity Score
CRF Components	**Age**	1.00			
	BMI	-0.35*	1.00		
	Average Pulse	-0.01	0.16	1.00	
	Activity Score	-0.10	-0.01	-0.31	1.00
Gray Matter CBF	**Global**	-0.31	-0.05	-0.02	0.37*
	Frontal	-0.09	-0.34*	0.04	0.24
	Parietal	-0.33*	0.10	-0.03	0.48**
White Matter CBF	**Global**	-0.22	-0.13	0.03	0.22
	Frontal	-0.02	-0.30	0.10	0.24
	Parietal	-0.27	-0.07	0.05	0.19

Note. Significance tests between groups (2-tailed): $df = 38$, * $p < .05$, ** $p < .01$.

Table 2.5. Partial correlations between CBF and normalized superior frontal and inferior parietal anatomical volumes, controlling for gender.

		Superior Frontal Volume	Inferior Parietal Volume
Gray Matter CBF	Global	.14	.09
	Frontal	.32*	-.00
	Parietal	.10	.25+
White Matter CBF	Global	.28*	.10
	Frontal	.42**	.09
	Parietal	.12	.19

Note. Significance tests between groups (1-tailed): *df* = 37, $^{+}$p < .10 * p < .05, ** p < .01

Figures

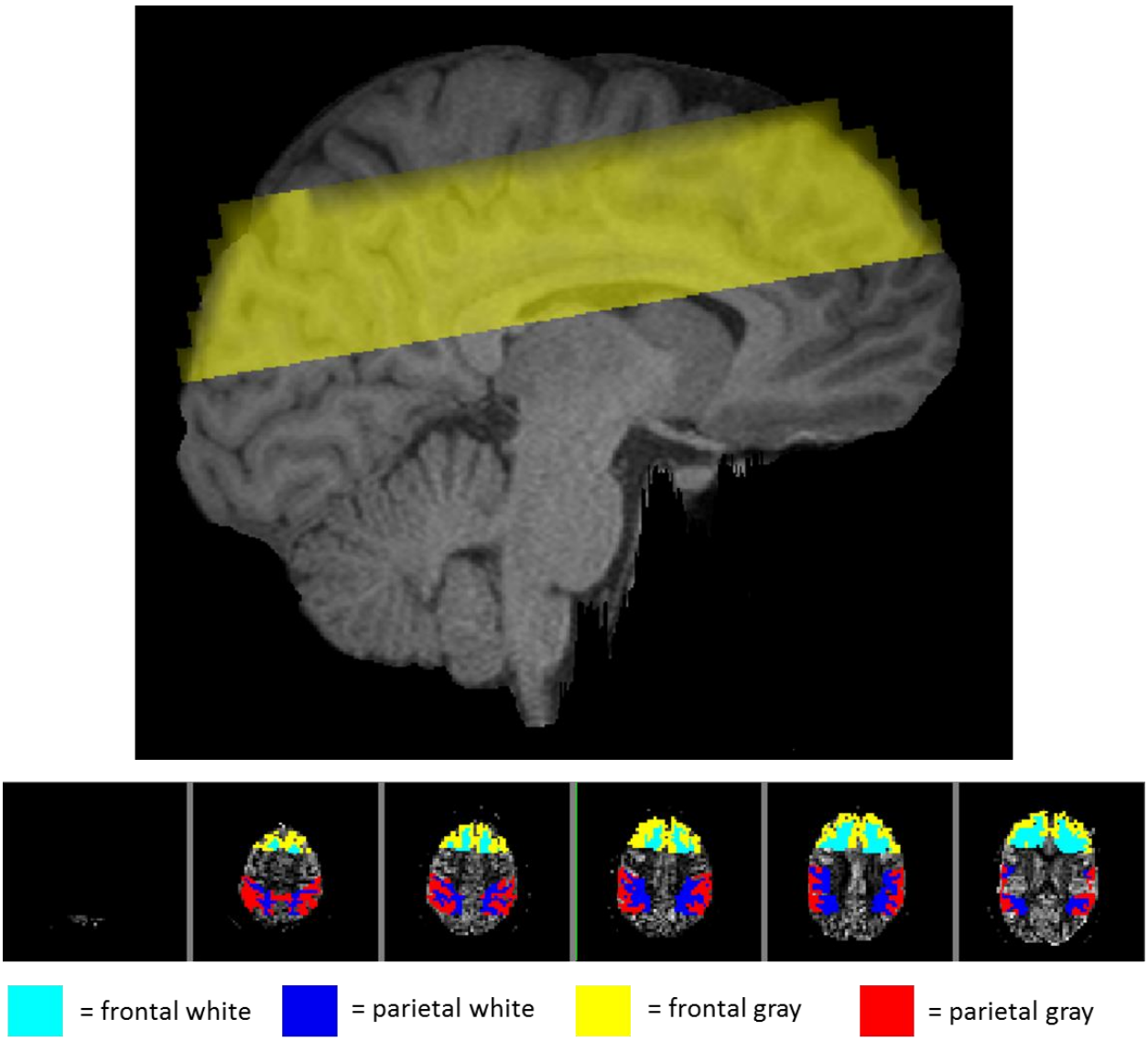

Figure 2.1. Top panel: sagittal view of the location of the imaging volume taken in the study for one representative subject. Bottom panel: axial view of the imaging slices shown from superior (left) to inferior (right) with the frontal and parietal regions highlighted. The teal and blue areas represent the white matter in the frontal and parietal regions, respectively. The yellow and red represent gray matter in the frontal and parietal regions.

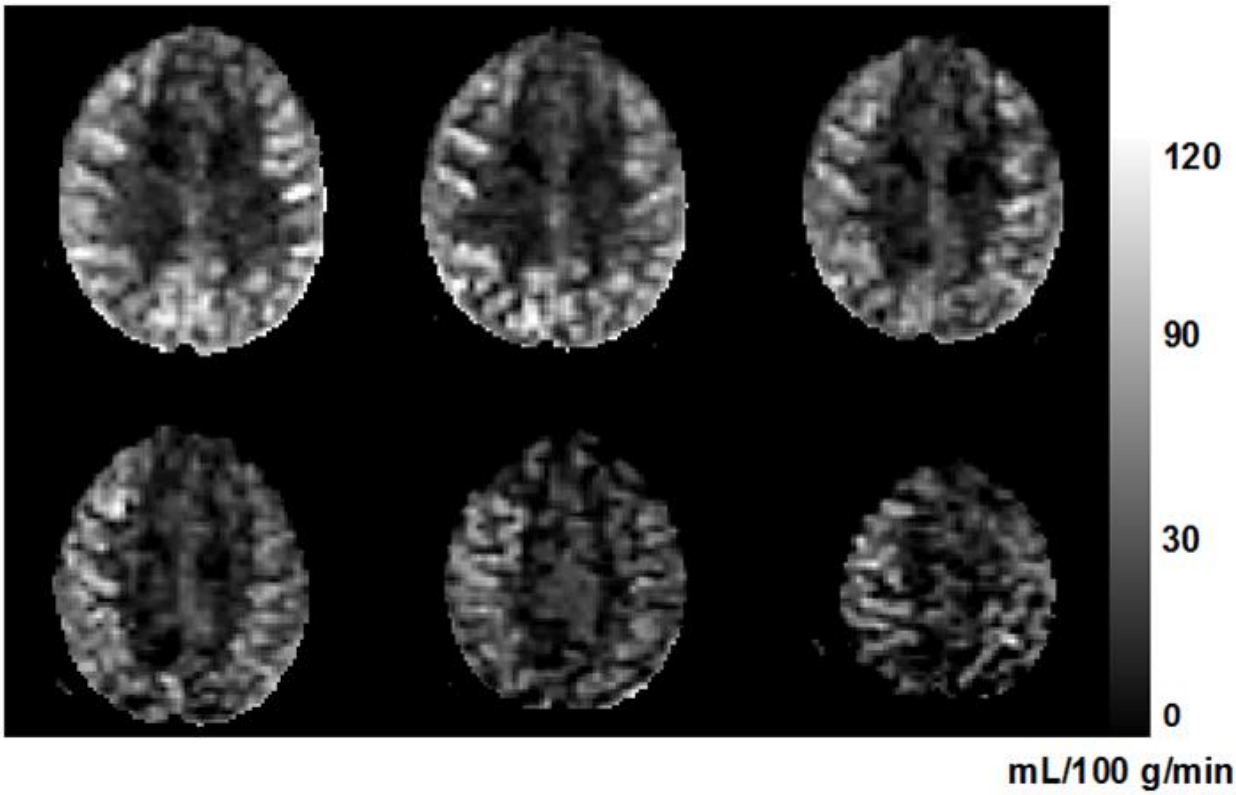

Figure 2.2. An example perfusion image from a 63-year-old subject. Six axial slices are shown from inferior (top left) to superior (bottom right). The higher perfusion in gray matter compared to that in white matter can be clearly seen. The unit of the gray-scale bar is mL/100 g/min.

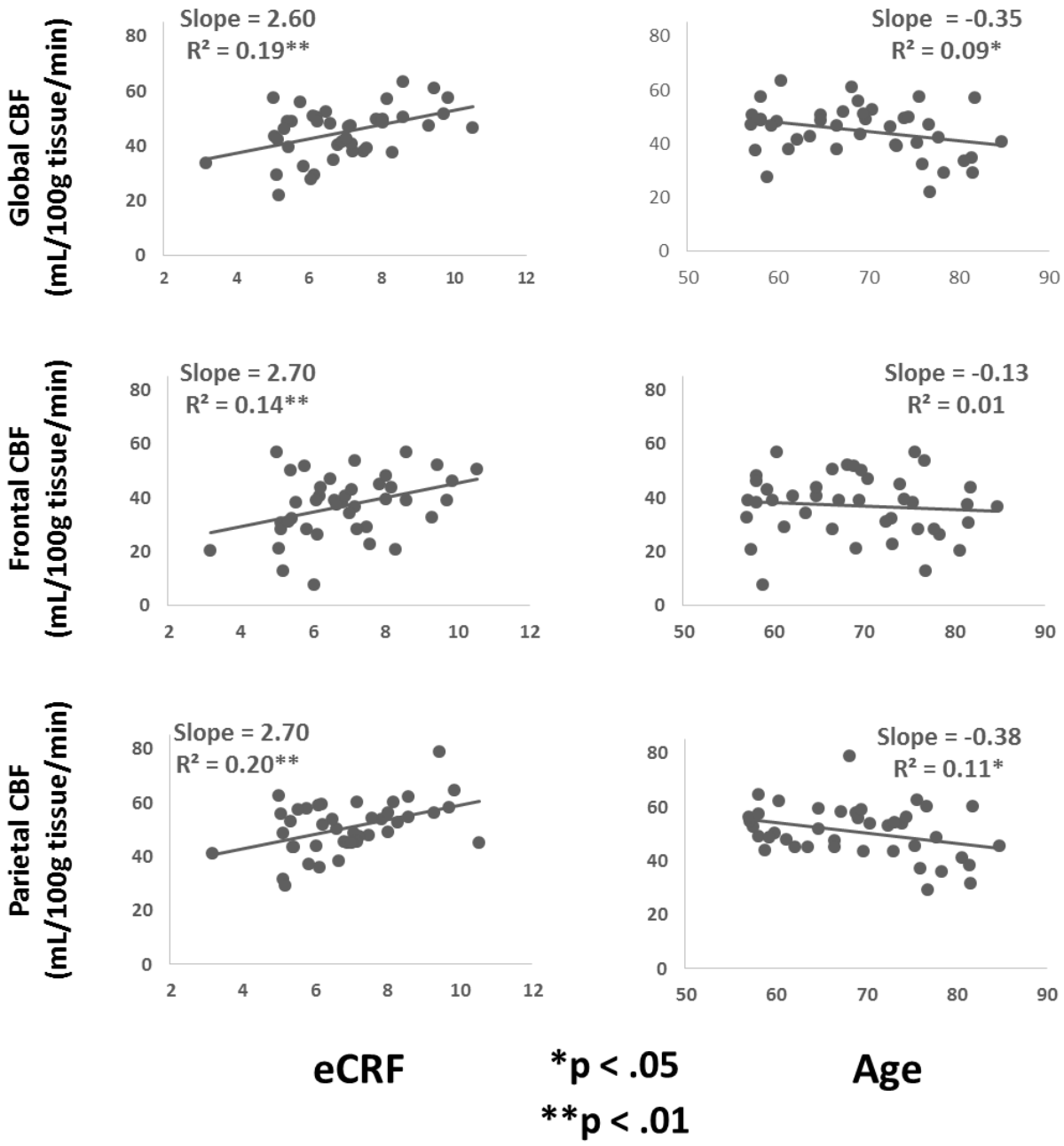

Figure 2.3. Scatter plots depicting the relationships between CBF and CRF (left column) and age (right column) in global (top), frontal (middle), and parietal (bottom) gray matter regions of interest.

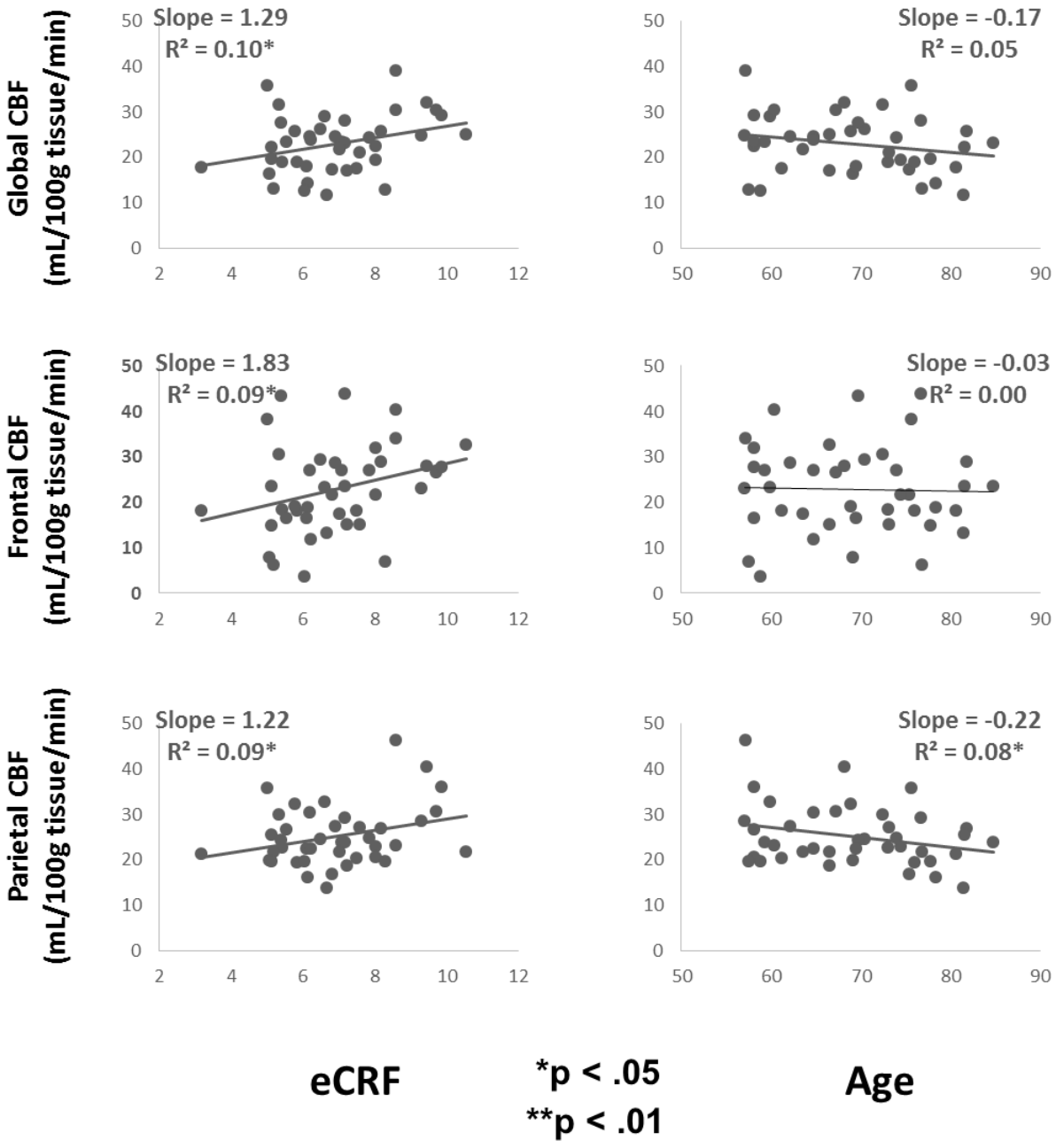

Figure 2.4. Scatter plots depicting the relationships between CBF and CRF (left column) and age (right column) in global (top), frontal (middle), and parietal (bottom) white matter regions of interest.

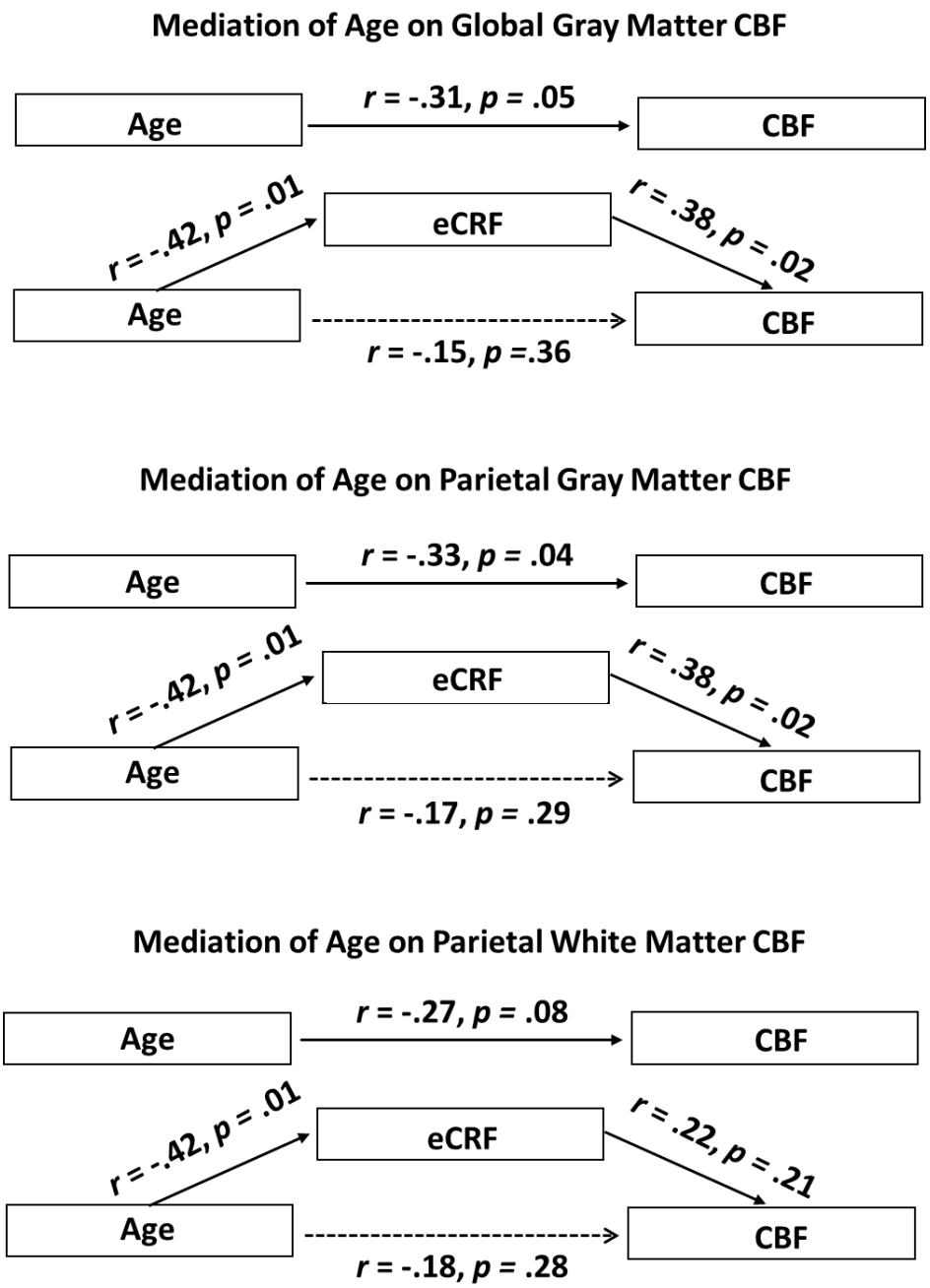

Figure 2.5. Top panel: Schematic representation of the results of the mediation analysis performed with the global gray matter CBF as the dependent variable. Middle panel: Schematic representation of the results of the mediation analysis performed with the parietal gray matter CBF as the dependent variable. Bottom panel: Schematic representation of the results of the mediation analysis performed with the parietal white matter CBF as the dependent variable. CRF significantly mediates the effect of age on CBF in the global gray matter and parietal gray matter, but not in the parietal white matter.

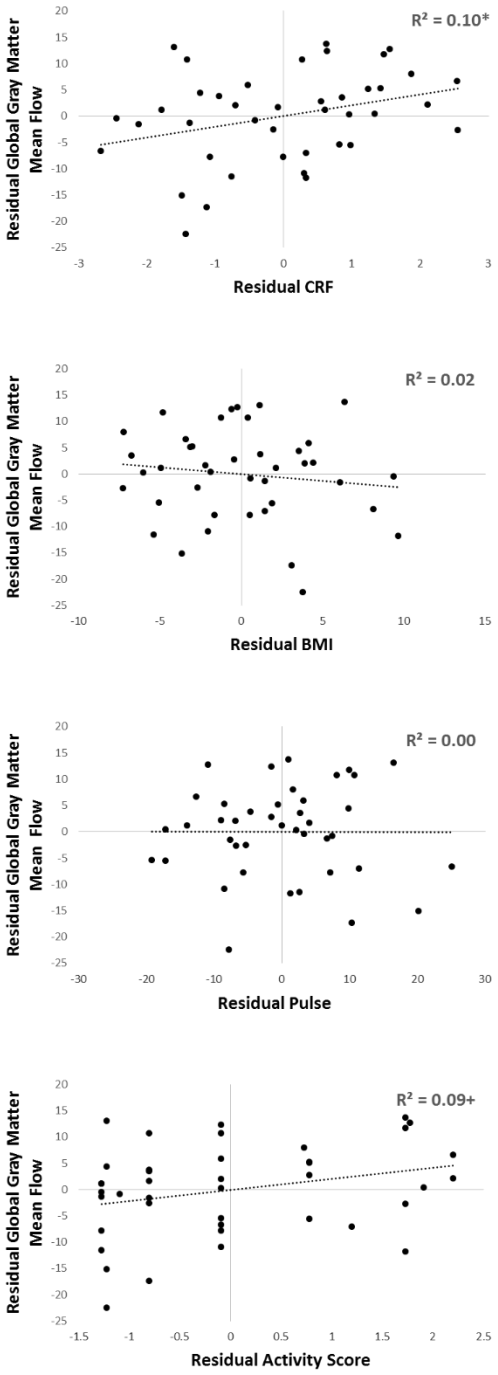

Figure 2.6. Participants were separated by gender, and then into older or younger groups by median split. Within each of those four groups, residuals of CRF, BMI, pulse, activity score, and global gray matter CBF were calculated by subtracting the group mean from each of the observed scores. Shown here are the residuals of CRF (top), and its individual components (BMI, pulse, and activity score; shown below) correlated with the residuals of CBF. $^{+}p < .10$ * $p < .05$.

CHAPTER 3: BASELINE BLOOD FLOW IS ANTI-CORRELATED WITH CARDIORESPIRATORY FITNESS IN A SAMPLE OF YOUNG ADULTS

Abstract

Cerebral blood flow declines with age. However, the process by which resting cerebral blood flow falls and how that decline relates to fitness is incompletely understood. In older samples, the relationship between age and resting cerebral blood flow is mediated by cardiorespiratory fitness, which is positively correlated with cerebral blood flow. Whether this effect holds true for younger adults is unknown. Here, using a large sample of resting cerebral blood flow measured with arterial spin labelling in younger adults, we demonstrate that the relationship between cardiorespiratory fitness and cerebral blood flow is negative. Although the relationship is weak, the observation is theoretically important and should impact how cerebral blood flow measures are interpreted in the future.

Introduction

Aerobic exercise has been shown to reduce the risk of age-related brain diseases and to improve cognition and brain health across the lifespan (Clarkson et al., 1999; DeSouza et al., 2000; Kramer et al., 2006; Colcombe et al., 2006; Hillman et al., 2008). Thus, recent research has been interested in determining the mechanisms by which maintaining and improving cardiorespiratory fitness (CRF) imparts benefits to the brain. One such line of research involves the examination of the cerebral vasculature, in order to determine if the improvement of cerebral vascular health could lead to improved cognition through better perfused brain tissue, healthier endothelial cells and blood-brain barrier, and improved cerebrovascular reactivity (Desjardins, 2015). Resting brain tissue perfusion in particular appears to be a good candidate to look for improvements across the lifespan because it has been shown that resting cerebral blood flow (CBF) decreases about 0.5% annually beginning in early adulthood (Leenders et al., 1990).

However, the relationship between fitness and blood flow is most likely influenced by a variety of factors, including changes to the health of the vascular bed, sympathetic tone, vascular reactivity to CO_2, the partial pressure of CO_2 (P_aCO_2), mean arterial pressure, and other biological factors (Ainslie & Duffin, 2009). Some of these effects, such as a greater proliferation of capillaries through angiogenesis, could lead to increased CBF, while others, such as blunted reactivity to CO_2 (e.g. Thomas et al., 2013) could lead to decreased CBF. There is likely a complex balance between many biological factors which ultimately determine the overall CBF. The relative effects of CRF on each of these biological factors may also change with age, which could lead to different effects of CRF on CBF at different points in the lifespan. Thus, although a positive relationship between CRF and CBF seems intuitive, the situation may actually be more complex. Disregarding this complexity may lead to incorrect assumptions about the desired results regarding changes in resting blood flow due to intervention studies or over the lifespan.

In the last chapter, we saw that age related declines in CBF were mediated by CRF in an older sample (Zimmerman et al., 2014). In addition, Ainslie and colleagues (2008) observed a significant positive relationship between CBF and CRF across the entire lifespan. However, this study examined differences between completely sedentary adults and endurance trained athletes. Thus it remains unclear whether differences in blood flow in younger adults were primarily caused by deficits in the sedentary group or benefits from the endurance trained group. Here, we looked at a large sample size of younger adults using ASL in order to quantify CBF with less rigorous inclusion criteria as part of a larger, multi-faceted study. In addition, our sample includes two time points, which should allow us to observe how CBF changes over time as CRF changes.

Method

Participants

Participants were recruited from the local community as part of a larger cognitive enhancement training intervention study. Although some of the training groups included fitness training as part of the intervention, there were no significant differences in CBF between the groups, or in the change in CBF

between the groups, so the data reported here is collapsed across all of the training groups. The pre-intervention and post-intervention data will be treated as two longitudinal time points. All aspects of the study were approved by the University of Illinois Urbana-Champaign Institutional Review Board, and all participants provided informed consent at the time of enrollment. Participants received monetary compensation for their participation.

Basic demographic and summary statistics are presented in Table 3.1. A total of N = 311 participants from that sample underwent the ASL MRI scan at the first time point. 4 participants were removed due to CBF measures that were greater than 3 standard deviations away from the mean. 3 of these removed participants had negative gray matter CBF and all showed excessive artifact in the images. An additional 10 participants were lost due to missing CRF data or demographic information. N = 177 participants were given the ASL MRI scan at the second time point with 1 participant missing CRF data, and N = 174 overlapping with the first time point sample

All participants were English speaking, right-handed, with normal or corrected-to-normal vision, reported no previous neurological injuries or disorders, and were not pregnant.

Measuring cardiorespiratory fitness

Our measure of CRF, maximal oxygen consumption (VO_{2max}), was acquired using a computerized indirect calorimetry system (ParvoMedics True Max 2400) and a modified Balke protocol (American College of Sports Medicine. ACSM's Guidelines for Exercise Testing and Prescription, 2014) with averages for oxygen uptake (VO_2) and respiratory exchange ratio assessed every 15 s using a mouthpiece. Participants ran at a constant speed on a treadmill, with 2-3% increases in the incline every 2 min until volitional exhaustion. The maximum oxygen consumption in units (ml/kg/min) is reported relative to fat-free mass, which was assessed by DXA scan (Hologic QDR 4500A, Bedford, Massachusetts) at both time points. For all subsequent analyses, the measure of CRF is adjusted to control for gender.

MRI acquisition and analysis

MRI data collection occurred at the Biomedical Imaging Center of the Beckman Institute for Advanced Science and Technology using a 3T Siemens (Erlangen, Germany) Trio whole-body MRI scanner. Six axial imaging slices passing through the whole brain with the bottom slice aligned with the top of the lateral ventricles were acquired with a localized pseudo-continuous Transfer Insensitive Labeling Technique (pTILT) ASL sequence (Ouyang & Sutton, 2011). This sequence is less sensitive to transit time issues that may confound ASL measurements. The method tags close to the slice of interest, minimizing variability in transit time that may result from differences in the vasculature.

The imaging and tagging parameters of the localized pTILT ASL sequence were: windowed-sinc 45^{o} RF pulses with 2560 μs-duration, tagging repetition spacing = 30 ms, number of concatenated RF pulse pairs = 100, tagging duration = 3 s, post-labeling delay = 0.5 s, tagging slice thickness = 10 mm, gradient spoiler duration and amplitude = 4000 μs/[±10, ±12, ±14, ±16] mT/m, SE-EPI readout, FOV = 220×220 mm, scan matrix size = 64×64, TR/TE = 4500/44 ms, slice thickness = 6 mm for an overall voxel size of 3. 4x 3.4 x 6.0mm, 30 control and 30 tag repetitions, scan time of one acquisition = 4.5 minutes.

To assist with the segmentation of brain tissue, a high-resolution T1-weighted 3D anatomical image was also acquired. The T1-weighted brain image was acquired using a 3D MPRAGE (Magnetization Prepared RApid Gradient Echo) protocol (TR = 1900 ms, TI (inversion time) = 900 ms, TE = 2.32 ms, field of view = 230×230x172.8 mm^3 (sagittal), matrix size = 256×256×192, flip angle = 9^{o}, slice thickness = 0.9 mm).

MRI data processing

The pTILT functional data processing was done using FSL (FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl). ASL images were acquired using the asl_file tool in FSL. Gray matter masks were formed from segmenting the T1 structural scan using FSL's FAST software (Zhang et al., 2001). The gray matter masks were then transformed into the subject's own pTILT functional space using a registration between the control image in pTILT and the MPRAGE from FSL's FLIRT (Jenkinson & Smith, 2001).

Arterial territory analysis

A probabilistic arterial territory map was derived by Mutsaerts and colleagues (2015) based on sections from Tatu et al. (1998). This arterial map was co-registered into the participant's native space, and specific irrigation territories for the anterior cerebral artery (ACA), middle cerebral artery (MCA), and posterior cerebral artery (PCA) were used to mask the pTILT functional image. Averages from the gray matter were calculated for each of the three arterial territories. However, because the territory of the PCA was minimally covered in the pTILT slices, it was left out of subsequent analyses.

Results

Cardiorespiratory fitness is anti-correlated with baseline blood flow

Because females typically have greater CBF and lower CRF than males, gender was partialled out of all subsequent analysis. All significance tests are two tailed with $\alpha = .05$. At the first time point, there was a significant negative correlation between CRF and CBF, $r(295) = -.13$, $p = .03$, Figure 1. At the second time point of the study with a substantially smaller sample size, we did not observe a significant relationship between CRF and CBF, $r(174) = -.02$, $p = .77$. However, pooling all of the subjects together across both time points still yielded a significant negative correlation between CRF and CBF, $r(471) = -.11$, $p = .02$.

Change in cardiorespiratory fitness is anti-correlated with change in baseline blood flow

We also observed a significant negative relationship between the change in CRF between the two time points and the change in CBF, $r(172) = -.17$, $p = .03$, Figure 3.2.

Arterial Territory Analysis

Visually, it appeared that the signal intensity in the pTILT images was different between the territories supplied by the ACA, which covers medial gray matter, and the MCA, which covers more lateral gray matter in the cortex (Figure 3.3). To test whether or not this was the case across all subjects, a paired sample t-test was performed between the average gray matter CBF in these arterial territories for both the first ($t(306) = -58.70$, $p < .001$) and second ($t(176) = -48.35$, $p < .001$) time points. In both cases, the CBF was higher in the regions supplied by the MCA. Despite these mean differences in CBF, the CBF was highly correlated between the two regions at the first ($r(306) = .65$, $p < .001$) and second ($r(175) = .65$, $p < .001$) time points.

This strong relationship between the two arterial territories extended to the relationship between the CBF in each of the territories and CRF. Of all of the relationship between the arterial territories and CRF, only the relationship between the changes in ACA CBF and the changes in CRF across the two time points passed our threshold for significance. However, there were trends towards the same negative relationship that was previously observed. At the first time point, the relationships between ACA CBF and CRF ($r(295) = -.11$, $p = .05$) and MCA CBF and CRF ($r(295) = -.11$, $p = .07$) were both negative. In addition, the relationships between the changes in both ACA CBF and CRF ($r(172) = -.15$, $p = .05$) and MCA CBF and CRF ($r(172) = -.14$, $p = .06$) were also negative.

Discussion

Our results show that CRF was weakly, but significantly anti-correlated with CBF in a large sample of young adults. In addition, across two time points, the change in CRF was anti-correlated with the change in CBF. Although these relationships were very weak, their observation makes a theoretically important contribution to research on the effects of aerobic fitness and exercise on cerebral vascular physiology.

Despite seeming to disagree with results presented by Ainslie and colleagues (2008) which showed a positive relationship between CBF and CRF, there are some potential reasons behind the discrepancy. First, there could be differences between the arterial territories that were measured by the

different techniques in the two studies, since different techniques were used to assess CBF. Major arterial territories are known to respond differentially to different exercise conditions. For instance, it is known that during hyperoxic exercise, CBF increases changes much more in the posterior circulation than from the MCA (Braz and Fisher, 2015). Thus it was possible that the relation between resting CBF and fitness was also dependent on arterial territory. Since Ainslie and colleagues (2008) could only measure CBF in the MCA, we attempted to look at more specific regions to see if there were differences in the relationships of CBF and CRF between arterial territories. Indeed, there were differences in the CBF in the territories supplied by the ACA and MCA. However, the resting CBF between these two regions was highly correlated, and there were no apparent differences between the relationships between the CBF in these two regions and CRF. It is unclear whether CBF is actually lower in the territory supplied by the ACA or if the difference was artefactual due to the arterial spin labeling method used.

A more likely reason is that the inclusion criteria between groups in Ainslie's study were at two extremes of the fitness spectrum, encompassing either completely sedentary adults or very athletic adults who had been aerobically trained for a substantial period of time. Our sample likely has much more variance, both in starting fitness levels and in how long participants were physically active. Thus, we may be insensitive to the potential detriments of complete sedentariness, as well as the potential physiological changes to long-term aerobic endurance training.

The complex web of physiological factors that influence the relationship between CRF and CBF and its relationship to age are still not fully understood. One potential cause of a decrease in CBF with CRF could be changes in P_ACO_2 that may be related to aerobic fitness. In fact, Murrell and colleagues (2013) corrected their measures of post-training MCAv at rest in order to account for the lowered post-training $P_{ET}CO_2$, which had resulted in a slightly negative, but insignificant change in MCAv in a group of 10 younger adults. This correction led to an elevation in MCAv, which indicates that multiple physiological changes may be competing in their influence on blood flow. The change in $P_{ET}CO_2$ was clearly impacting the measurement, but after correcting for this variable, other factors were causing MCAv to increase with exercise training. Competing physiological factors like these may be partially

responsible for some of the equivocal findings reported in the literature. Whether these are due to differences in study design (Murrell and colleagues suggest that the anticipation of impending exercise may have been enough to reduce $P_{ET}CO_2$ in aerobically trained individuals) or due to physiological adaptations must be a topic of future study.

We did not assess P_ACO_2 in this study to know whether it contributed to the negative relationship between cardiorespiratory fitness and cerebral blood flow. Even if P_ACO_2 did not correlate with cardiorespiratory fitness, it is still possible that the level of fitness influences the regulation of cerebral blood flow to P_ACO_2.

Another possibility is that the physiological changes co-occurring with changes in cardiorespiratory fitness that would lead to decreases in cerebral blood flow are transient. In this scheme, the physiological response to exercise (e.g. the blood oxygen-carrying capacity, angiogenesis) might adapt at different rates than other physiological changes, which lead to short-lived fluctuations from the body's normal homeostasis. This could help explain why MCAv has been positively correlated with cardiorespiratory fitness in younger adults in cross-sectional samples (Ainslie 2008), but negatively correlated or not related after training interventions such as this study and from Murrell and colleagues (2013).

In conclusion, we hope that this demonstrates the complexity to the issues surrounding the regulation of resting CBF, and contributes to the ongoing debate regarding equivocal findings in this literature. While CBF may be an important and interesting measure of cardiorespiratory health, especially in old age and when CBF reaches extreme values, researchers must seek to better understand the measure across the lifespan and approach it with due thoughtfulness.

Table

Table 3.1. Demographic Information

	First time point sample with CBF data (N = 307)	**Second time point sample with CBF data (N = 177)**
% Female	50.99	51.41
Mean age (std)	23.83 (5.12)	24.79 (5.60)
Mean education (std)	4.63 (1.15)	4.76 (1.21)

Note. Education was on a 7-point scale where 1 = no high school, 2 = some high school, 3 = high school grad, 4 = some college, 5 = college grad, 6 = some post-grad, 7 = master's or higher.

Figures

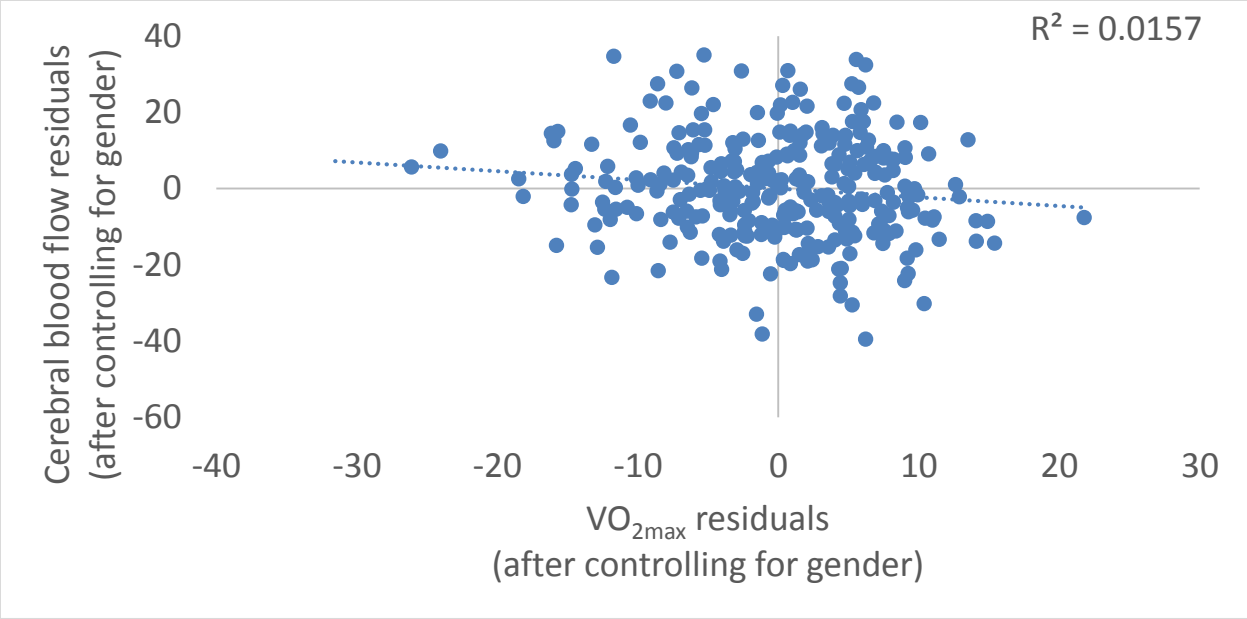

Figure 3.1. VO_{2max} negatively correlates with cerebral blood flow.

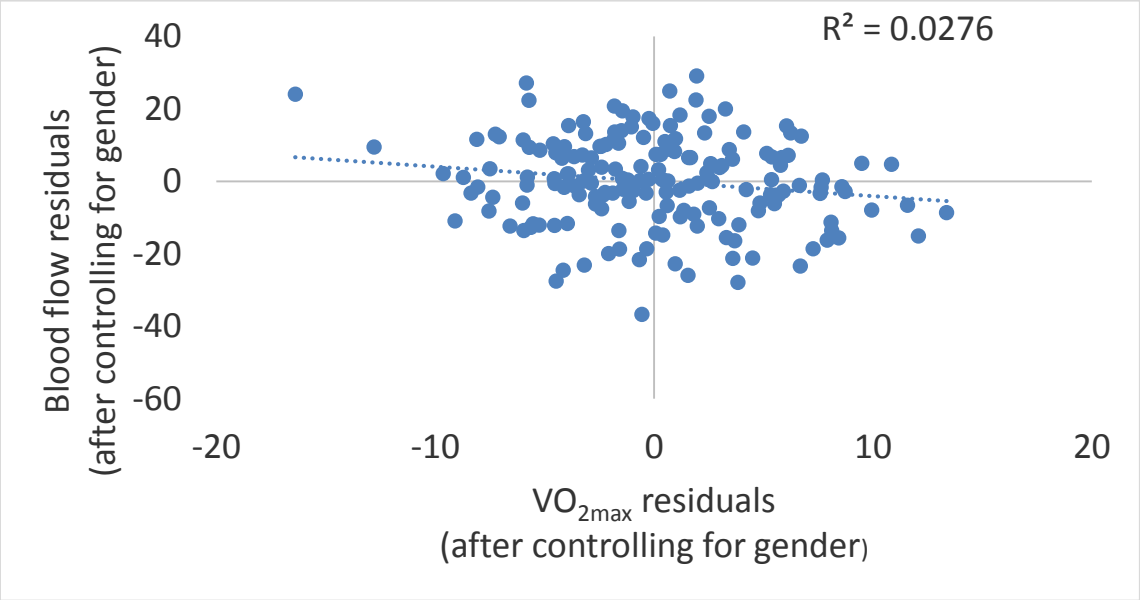

Figure 3.2. Change in VO_{2max} negatively correlates with change in cerebral blood flow.

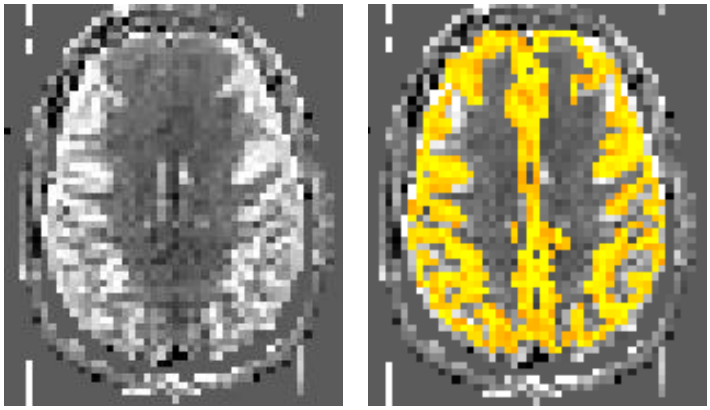

Figure 3.3. An example axial slice of a pTILT image in an individual subject. The left panel shows only the pTILT image, where the brighter areas indicate higher CBF. The right panel shows a gray matter mask superimposed onto the pTILT image. Notably, the most medial and anterior gray matter in the slice, which is supplied by the ACA is much less bright than the lateral gray matter, which is supplied by the MCA.

CHAPTER 4

BASELINE BLOOD FLOW AND ACTIVATION BLOOD FLOW REPRESENT DIFFERENT CEREBROVASCULAR HEALTH PARAMETERS IN AGING

Abstract

Cerebrovascular health is critical for brain function and healthy brain aging. However, details of how different aspects of vascular health impact cognition in aging remain to be ascertained. In order to gain an improved understanding of how cerebrovascular health impacts cognitive aging and relates to cardiorespiratory fitness (CRF), arterial spin labelling (ASL), a functional magnetic resonance technique, was used to study the cerebral blood flow (CBF) in healthy older adults ranging in age from 56-88. Previous research using this technique found that CRF mediated the effects of age on the baseline CBF over a volume through frontal and parietal gray matter, but was unable to demonstrate a relationship between baseline CBF and cognitive function. Here we analyzed measures of both baseline CBF and changes in CBF during activation from a visual task collected on a subset of those same participants one year later in the visual cortex. We found that the change in CBF in the visual cortex to a reversing checkerboard stimulus, but not the baseline CBF, was associated with neuropsychological measures of executive function. While baseline CBF was correlated with age and CRF, the change in CBF was correlated with the participants' pulse pressure. These results indicate that the measures of baseline CBF and activation-related CBF are separable measures of vascular health that relate differentially to measures of physiology and cognition.

Introduction

Understanding how cardiorespiratory fitness ameliorates age-related cognitive decline has become the focus of a growing field of research. It has become clear that aerobic exercise likely influences brain health and cognition through many mediating factors. Exercise benefits many biological

factors that are known to impact cognition including the regulation of metabolism (van Praag, Fleshner, Schwartz, & Mattson, 2014), influencing arousal and affect, which can increase cognitive performance and reduce anxiety (Lambourne & Tomporowski, 2010; Petruzzello & Tate, 1997), affecting sleep (Yang, Ho, Chen, & Chien, 2012; Kredlow et al., 2015; Ferrie et al., 2011), influencing neurotrophic factors (Voss et al., 2013; Huang et al., 2013; Coelho et al., 2013; Szuhany, Bugatti, & Otto, 2014), maintaining immune function across the lifepsan (Simpson et al., 2012), and improving the health of the neurovascular system (Davenport et al., 2012). Likely, many of these factors interact in complex ways leading to the amelioration of age-related cognitive decline in older adults. In this chapter, we focus on characterizing the health of the neurovascular system, leap-frogging off of the earlier chapters, in which we demonstrated that fitness level mediated the age-related declines in cerebral blood flow in a sample of older adults (age 55-85) (Zimmerman et al., 2014). That chapter established an important link between known decreases in cerebral blood flow over the lifespan and level of cardiorespiratory fitness, highlighting how critical physical activity is to cerebrovascular health.

Poor cerebrovascular health has long been implicated in many of the most devastating brain diseases leading to cognitive decline and dementia in old age including strokes, transient ischemic attacks (TIAs), lacunar infarcts, and vascular dementia. In addition, recent research points to declining vascular health playing an important role in Alzheimer's disease (de la Torre, 2004). In addition to these disease states influencing cognition, there is evidence that vascular health plays a role even in normal aging by impacting both brain structure and cognition (Raz et al., 2007). However, a deeper understanding of the details of this connection are still needed.

Improved vascular health can be characterized in many ways, including increased compliance of the arteries, improved integrity of the blood-brain barrier, increased vascularization throughout the brain, and improved reactivity to metabolic needs. Each of these improvements, however, may be subserved by different mechanisms, as well as having different levels of importance in translating to cognitive performance. Some peripheral physiological measures can be used to provide estimates of arterial compliance. Pulse pressure (the difference between systolic and diastolic blood pressure) is often used as

a proxy for arterial compliance. In addition, using an individual's peripheral measures of systolic and diastolic blood pressure along with their heart rate, it is possible to estimate compliance using a Windkessel model. These peripheral measures can be used in conjunction with measures of vascular health from the brain. Arterial spin labelling (ASL) is a non-invasive magnetic resonance (MR) technique that is specifically suited for quantitatively measuring two characteristics of cerebral vascularization, cerebral blood flow (CBF) at rest (baseline CBF) and the change of blood flow due to some event (activation CBF).

In chapter 2, we were able to demonstrate that cardiorespiratory fitness significantly mediated the age-related decline in baseline CBF (Zimmerman et al., 2014), however, we were unable to measure vascular reactivity due to excessive movement artifacts in the ASL data that accompanied the breath-holding task that was used as a vasodilatory stimulus. In the current study, we used ASL to examine baseline CBF alongside changes in blood flow in the visual cortex during passive viewing of checkerboard reversals. Simple visual stimulation tasks can be used to induce vasodilation in the visual cortices and thus increase cerebral blood flow and allow for the investigation of vascular reactivity along with resting levels of perfusion without producing movement artifacts that are difficult to avoid with breath-holding tasks, which are more typically used to induce vasoreactivity. The ability to measure both baseline CBF and activation CBF allowed us to examine possible differences between those two vascular measurements in the way they relate to other factors including age, fitness, and pulse pressure. Particularly importantly, we were able to assess differences between baseline CBF and activation CBF and measures of cognition.

Method

Participants

Research participants (N = 43, aged 56-88), were recruited from the Champaign-Urbana area. These participants were returning for a second year of a broader two-year research experiment. Details regarding the inclusion criteria can be found in chapter 2. Notably, participants with serious medical

conditions, a history of psychiatric illness, depression or dementia were excluded from the study. All participants were right-handed (as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971), had normal or corrected-to-normal vision, and were native speakers of English. Participants were also assessed for level of education, vocabulary (Shipley, 1940), and intelligence quotient (Kaufman & Kaufman, 2004) during the first year of the broader study in order to characterize the demographic. Participants' blood pressure was taken on three separate days (in year 2 of the study) and averaged to provide measures of systolic and diastolic blood pressure. Pulse pressure was derived by taking the difference between systolic and diastolic blood pressure. All participants were paid for their time in the study and signed informed consent in accordance with the University of Illinois at Urbana-Champaign's Institutional Review Board.

Data from 1 participant were discarded from subsequent analysis due to severe image artifacts. The final sample included 42 older adults (aged 56-88, mean age = 71.1 years, 22 females). We did not exclude participants based on usage of blood pressure medication in order to include a more representative sample of older adults. 15 of those participants reported being on medication for their blood pressure. Although about a third of the participants reported taking blood pressure medication, there were no significant differences between those participants on or off medication in age, fitness, or blood flow. The demographic and neuropsychological characteristics of this sample are presented in Table 4.1.

Measuring cognitive function

In the second year follow up, participants were administered a battery of neuropsychological tests, which included the Verbal Fluency Test (letter PRW; Benton, Hamsher, & Sivan, 1994), two versions of the operation-span task (OSPAN, words and letters; Unsworth, Heitz, Schrock, & Engle, 2005), and the Trail Making Tests A and B (Corrigan & Hinkeldey, 1987), to measure working memory and executive function. In addition, scores for forward and backward digit span were derived from the mMMS. In order to derive scores relating to executive functioning skills, we also took the difference between the trail making tasks, and the difference between forward and backward digit span. We were

unable to collect the full neuropsychological battery for 4 participants, so for all analyses using neuropsychological data, $N = 38$.

Measuring cardiorespiratory fitness

Typically, physical fitness is measured through VO_{2max}, which represents the maximum oxygen that is consumed during a maximal graded bout of exercise. However, the stressful exercise routine may be dangerous and not optimal for samples of older individuals who may have conditions that could prevent their participation. In such cases, CRF can be estimated according to a linear equation composed of easily acquired parameters that are highly predictive of VO_{2max} (Jurca et al., 2005). This measure is composed of the sum of weighted variables including gender, age, body mass index, resting heart rate, and a physical activity score, as well as a numerical constant. It has been shown to approximate VO_{2max} accurately in a large sample ($N > 10{,}000$) and specifically in older adults within the same age group as our sample (Mailey et al., 2010). Since CRF is systematically larger in men than women (Jurca et al., 2005), we partialled out gender throughout the analyses to avoid potential biases.

Interestingly, the "physical activity score" component of the equation is a simple 5 point scale that asks questions about the specific amount of time spent doing certain activities each week. Because of the objective nature of time spent doing certain activities, it is a relatively simple task to convert scores from other questionnaires about physical activity that also ask the subject to quantify the time spent doing various activities. In fact, our group previously took this approach using activity scores derived from the Physical Activity Scale for the Elderly (PASE) (Washburn, Smith, Jette, Janney, 1993), which was applied to the equation presented by Jurca and colleagues post-hoc in order to get an estimate of VO_2 max (Chapter 2; Zimmerman et al., 2014). The current experiment builds off of the results from that report, so we have continued to present an estimated CRF measure that uses the PASE-derived activity scores. However, it is worth noting that the CRF values estimated using self-reported physical activity scores directly from Jurca and colleagues are not perfectly correlated with CRF values estimated using self-reported activity scores that are derived from the PASE, $r(38) = .88$. We are of the impression that the

PASE provides a more accurate estimate of activity level, due to the greater detail and smaller time periods regarded by the questions. We were unable to collect activity scores for 2 participants, so for all analyses using CRF, $N = 40$.

MRI acquisition and analysis

All MRI data collection occurred at the Biomedical Imaging Center of the Beckman Institute for Advanced Science and Technology using a 3T Siemens (Erlangen, Germany) Trio scanner using a standard body coil transmission and a twelve-channel head array receive coil.

Participants were instructed to look at a central fixation cross while exposed to alternating periods of a circular, reversing checkerboard (31.2s, visual angle = 9.04) and rest (30s each) for a total acquisition time of 5.1 minutes. The reversing checkerboard was presented using E-PRIME (Psychology Software Tools, Pittsburgh) and displayed via back projection (BrainLogics, Psychology Software Tools, Pittsburgh). To minimize motion during the task, padding was used to stabilize the subject's head.

Four coronal imaging slices passing through the occipital cortex (see Figure 4.1 for an example) were acquired with a localized pseudo-continuous Transfer Insensitive Labeling Technique (pTILT) ASL sequence (Ouyang & Sutton, 2011). This sequence was used because it is less sensitive to the transit time issues that may confound other ASL methods as a function of age. The method tags close to the slice of interest, minimizing the differences in transit time that may result from age-related changes in the vasculature, and the tagging plane can be oriented to different geometries according to the imaging volumes. In this case, the labeling slice was placed anterior to the imaging slab with a 13 mm gap.

The imaging and tagging parameters of the localized pTILT ASL sequence were: windowed-sinc 45^{o} RF pulses with 2560 μs-duration, tagging repetition spacing = 30 ms, number of concatenated RF pulse pairs = 100, tagging duration = 3 s, post-labeling delay = 0.5 s, tagging slice thickness = 10 mm, gradient spoiler duration and amplitude = 4000 μs/[±10, ±12, ±14, ±16] mT/m, SE-EPI readout, FOV = 220×220 mm, scan matrix size = 64×64, TR/TE = 5000/44 ms, slice thickness = 4 mm, slice gap = 0.8 mm, 30 control and 30 tag repetitions, scan time of one acquisition = 5 minutes.

To assist with the registration procedure, two additional brain scans were taken: a high-resolution 2D turbo-spin echo (TSE) acquisition with the imaging slices at the same location as the ASL images, and a high-resolution T1-weighted 3D anatomical image. The T1-weighted brain image was acquired using a 3D MPRAGE (Magnetization Prepared RApid Gradient Echo) protocol (TR = 1900 ms, TI (inversion time) = 900 ms, TE = 2.32 ms, field of view = 230×230x172.8 mm^3 (sagittal), matrix size = 256×256×192, flip angle = 9^o, slice thickness = 0.9 mm).

MRI data processing

The pTILT functional data processing was done using SPM 8 (Wellcome Department of Cognitive Neurobiology, University College of London, UK) and FSL 4.1.4 (FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl). In ASL experiments, it is useful to estimate the contribution of the blood oxygenation level dependent (BOLD) contrast, which otherwise contaminates the quantitative blood flow estimates (Liu & Wong, 2005). The fMRI modeling of the BOLD, baseline CBF, and activation CBF responses were determined using the general linear model (GLM) with the ASL modeling framework described by Hernandez-Garcia, Jahanian, and Rowe (2010). Four regressors were modeled in the GLM analysis: (1) the visual task BOLD response (a canonical hemodynamic response function, HRF); (2) baseline CBF (a consistent, alternating waveform); (3) activation CBF (an alternating waveform during the visual task); and (4) a baseline signal (uniform intensity). After regression analysis, gray and white matter masks were derived by segmenting the T1 structural scan using FSL's FAST software (Zhang et al., 2001), since we are primarily concerned with changes in blood flow to the gray matter of the cortex. The gray and white matter masks were then transformed into the subject's own pTILT functional space using a registration between the control image in pTILT and the MPRAGE from FSL's FLIRT (Jenkinson & Smith, 2001).

Motion correction

Because ASL is a low signal-to-noise technique, artifacts due to motion can substantially reduce the reliability of estimation. The unsubtracted pTILT data were first realigned to remove motion artifacts. In order to reduce any motion influence and increase the reliability of estimation from time points where motion was not present, the SPM 8 Robust Weighted Least Square (rWLS) toolbox (Diedrichsen and Shadmehr, 2005) was also used. The rWLS toolbox is able to find images that are impacted by motion or other noise, based on the residual-mean-square estimate, which is calculated by adding up the squared residuals over the whole volume for each individual time point when applying the linear model. Rather than establishing a threshold to delete data points that have been contaminated by motion, the rWLS toolbox "soft-excludes" those images by weighting each observation with the inverse of its variance.

CBF quantification

From the earlier experiment and with improved guidelines, our quantification of CBF was changed. Baseline perfusion images in mL/100 g/min units were calculated, based on a single compartment model in which no blood exchange is assumed (Ouyang & Sutton, 2011) according to recent modelling guidelines put forward by the International Society for Magnetic Resonance in Medicine (ISMRM) perfusion study group and the European consortium for ASL in dementia (Alsop et al., 2014) :

$$CBF = \frac{\Delta M}{M_0} \cdot \frac{6000 \cdot \lambda \cdot e^{\frac{\omega}{T_{1,blood}}}}{T_{1,blood} \cdot (1 - e^{-\frac{\tau}{T_{1,blood}}})}$$

where ΔM is the estimated coefficient of the tag-control difference (i.e., the perfusion-weighted control-tag image); M_0 is the estimated coefficient of the static tissue signal (i.e. the control image); λ is the blood-brain partition coefficient in ml/g (0.9; Herscovitch & Raichle, 1985); ω is the post-labeling delay (0.5 seconds); $T_{1,blood}$ is the longitudinal relaxation rate of blood (1650 ms at 3T; Lu, Clingman, Golay & van Zijl, 2004); τ is the label duration (3 seconds); 6000 is a constant to convert ml/g/s to ml/100g/min.

In the case of arterial spin labeling, the concentration of the magnetically labeled blood (the 'tracer') is assumed to be equal across the entire "compartment" of the brain. Thus the exchange rate of the tracer is related to the density within this compartment. The signal difference amplitude is proportional not only to cerebral blood flow, but also to the signal intensity due to water. When we want to quantify the blood flow in exact units (e.g. mL/100g/min), it is necessary to normalize the signal intensity of the difference image by the signal intensity due to water using a non-flow image.

Because we assume that ΔM reflects only the signal of blood water, it would be ideal to normalize by the M_0 of arterial blood water. However, it is difficult to find an appropriate number of voxels of pure blood in the brain regions being imaged and there may also be systemic signal variations in the image that would not be controlled for by dividing by a signal from only a small region of the brain. Some have approached this problem by selecting voxels of pure CSF, where most of the signal intensity is due only to water. This method does a good job of estimating the signal intensity due to water, but still has the disadvantage of failing to control for signal variations caused by RF coil inhomogeneity, as well as possible differences in transverse relaxation.

Dividing by a proton-density weighted image, and then scaling with an average blood-brain partition coefficient, the ratio between tissue and blood tracer concentrations at equilibrium, offers a solution that provides a decent estimation of the signal intensity of water while also controlling for the discrepancies in the image due to artifacts that were discussed above. Ideally, the blood-brain partition coefficient would also be an image, since water density differs in tissue types, but this requires additional images and has not been adequately optimized and evaluated to have been adopted by the ASL community (Alsop et al., 2014). The error in quantification due to using a constant blood-brain partition coefficient across the brain is expected to be <10% (Alsop et al., 2014).

The easiest implementation is to ignore the impact of signal differences between tissue types in the control image, and to use the control image from the ASL experiment as the proton-density weighted image. In reality, this image has some T2 weighting because of the echo time needed for the EPI acquisition. Indeed, adopting this approach in Zimmerman et al. (2014), reprinted in chapter 2, we

calculated reasonable blood flow values in accordance with the literature. It is important to note, that this error is in the scaling of a signal to produce the quantification and should not be very important in correlational studies. However, it may become more important when used alongside other quantitative methods, such as measuring vascular occupancy.

Windkessel model

The hemodynamics of the arterial system can be described in terms of resistance (typically attributed mainly to smaller vessels of the microcirculation) and compliance (typically attributed to the larger arteries). By using a subject's average systolic blood pressure, diastolic blood pressure, and heart rate, it is possible to estimate the compliance and resistance of the arterial system at rest through a simple cardiovascular model. We used a Simulink model in MATLAB based on Hoppensteadt & Peskin (2002). This model relates the systemic arterial compliance and resistance to blood pressure in a single equation:

$$C\frac{dP}{dt} = Q - \frac{P}{R}$$

In this model, *C* is the compliance of the systemic arteries, *P* is the arterial pressure, *R* is the resistance of peripheral circulation, and *Q* is the inflow to the systemic arteries. *Q* is a periodic function of time simplified as a triangle function during systole and 0 during diastole, where the period is determined by the heartrate. The model reaches a steady-state where we can estimate compliance and resistance after inputting systolic and diastolic blood pressure and heart rate as parameters. Note, that since we do not know the participants' stroke volume, we used the same typical parameter for all participants (.07 L/ beat) (Hoppensteadt & Peskin, 2002). Thus, the modelled compliance measures are only estimates, and their distribution should be considered with the knowledge that variance in stroke volume exists in our participants that is not included in the modelled measures.

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Results

As expected, dependent sample *t*-tests revealed that gray matter baseline CBF (*M* = 61.77 ml/100g/min, *SD* = 16.34 ml/100g/min) was significantly greater than white matter baseline CBF (*M* =

45.27 ml/100g/min, *SD* = 14.88 ml/100g/min) in the area that we investigated ($t(41) = 6.00$, $p < .0001$). However, although numerically larger, the average activation blood flow was not significantly greater than the baseline blood flow in either gray ($t(41) = -1.60$, $p = 0.12$) or white matter ($t(41) = -1.02$, $p = 0.31$), Figure 4.2).

PCA of neuropsychological tests

PCA with varimax rotation was performed on the group of 38 participants using all 7 neuropsychological tests that were measured. Using a cut-off criterion of Eigenvalue > 1 (Kaiser's criterion), we retained 2 orthogonal components. Together these components accounted for 64.87% of the total variance of the data. The rotated loading matrix is shown in Table x. The component scores for the individual participants were used in subsequent correlational analysis. Although all the tasks utilize executive functions, factor 1 seems to be more representative of working memory capacity, whereas factor 2 seems to be more representative of speed of response.

Correlational Analysis

Table 4.3 shows the partial correlations (controlling for gender) between the measures of gray matter baseline CBF, gray matter % activation CBF, age, CRF, pulse pressure, estimated compliance and cognitive factors. Based on previous research, we hypothesized that CRF would be positively correlated with CBF whereas age would be negatively correlated with it, even in our restricted age-range of older adults. For consistency, all correlations are presented as two-tailed tests, but given the previously established directionality of blood flow changes with age and our corresponding directional hypothesis, we encourage the reader to consider these particular correlations as unidirectional. As we expected, CRF positively correlated with gray matter baseline CBF, and age marginally negatively correlated with gray matter baseline flow if the hypotheses are considered directional. We believed that this result at least warranted further analysis described below. In this study, baseline CBF did not significantly correlate with pulse pressure or either of the cognitive factors. The percent change in blood flow associated with task

activation was not correlated with age or CRF. However, changes in CBF associated with activation negatively correlated with pulse pressure and positively correlated with a cognitive factor representative of working memory ability.

In order to further investigate the predictive ability of baseline and activation blood flow on cognitive ability in older adults, we looked at the correlations between individual cognitive tasks and our blood flow measurements (Table 4.4). To make the tasks more dependent on executive functioning, we used the differences between the trail making B and A tasks, which should control for reaction time, and the difference between backward and forward digit span to reduce the span component of the measurement and emphasize the manipulation of information in the backward span task. Results from this analysis suggested that certain cognitive tasks were particularly sensitive to activation related changes in CBF, but no significant relationship between baseline CBF and any cognitive task in our battery was found.[4]

CRF mediates the relationship between age and baseline CBF

We set out to address whether CRF mediates the effect of age on baseline CBF in the visual cortex in order to replicate the mediation results presented in chapter 2. Therefore, we performed the bootstrapping analyses to test for this indirect effect as described in the method section for the gray matter mean flow in the slices imaged through the visual cortex.

Figure 4.3 shows the path diagram of this analysis on the gray matter mean blood flow. Two-tailed significance tests were performed throughout the analysis. Baseline CBF was regressed on both age and CRF at the same time to determine the indirect effect. Using the bootstrap estimate, the indirect effect varied between -.65 and -.01 at a 95% level of confidence. Because the 95% confidence interval did not include the 0 value, the result indicated a significant indirect effect of age on baseline CBF through CRF.

[4] Controlling for age, in addition to gender, did not reduce the significance of any of the previous significant relationships between activation blood flow and cognition.

Modeling compliance and resistance with a Windkessel model

It is possible to model the compliance and resistance of the arteries based on knowledge of the pulse rate, systolic, and diastolic blood pressure. We used a Windkessel model (Frank, 1899; Hoppensteadt & Peskin, 2002) to get predicted values for arterial compliance and resistance to see if those would be better predictors of cerebral blood flow than from the pulse pressure alone, which is only partially related to arterial compliance. The modelled arterial resistance showed no correlation with baseline flow or % activation. However, the modelled compliance was significantly correlated with the % CBF activation, $r(40) = .39$, $p = .01$ (Figure 4.4). This was a slightly better predictor of % CBF activation than pulse pressure.

Discussion

The results presented in this paper suggest that the ASL measurements of baseline blood flow and activation blood flow reflect different components of cerebrovascular health. Although baseline blood flow significantly decreases with age, a relationship that is mediated in part by cardiorespiratory fitness, it does not significantly correlate with cognitive function in this sample of healthy older adults. In contrast, the change in flow related to neural activation to a simple visual stimulus is predictive of cognition, despite not being significantly related to age and cardiorespiratory fitness.

These findings build on the literature and add insight especially to research on the role of vascular reactivity in cognitive performance. Although we did not observe an age relationship in this sample, vascular reactivity has been shown to decrease with age (Sonntag, Eckman, Ingraham, & Riddle, 2007), and to correlate with cognition (Brown et al., 2010; Tarumi et al., 2015). The remodelling of the structure of cerebral arteries to counteract persistent hypertension, as well as the disruption of multiple signaling pathways, are thought to subsequently damage cerebral autoregulation (Sonntag et al., 2007). In our results, pulse pressure significantly and negatively correlates with activation-related changes in blood flow. In addition, when we modelled compliance with a simplistic Windkessel model based on our participants' blood pressure and heart rate, the measure of estimated compliance was even more strongly correlated with the change in blood flow due to activation than pulse pressure. This supports the proposed

mechanism by which impaired control of blood pressure could lead to impaired vascular reactivity. Some evidence even shows that when cerebrovascular reactivity to CO_2 is controlled for as a covariate, fitness-related group differences in cognition in a middle-aged sample are significantly diminished (Tarumi et al., 2015), which suggests that improved cerebrovascular autoregulation is one of the critical mechanisms by which fitness improves and maintains cognitive function. Cerebral autoregulation is critical to support the brain's fluctuating needs for oxygen, glucose and other nutrients and factors, and clearance of waste. Thus declining cerebral autoregulation is a good candidate for explaining some of the decreasing cognitive function that accompanies aging.

Given the past literature, some of the findings here require further speculation. First, why does baseline blood flow *not* predict cognition in healthy samples? Baseline blood flow clearly declines with age, which has been demonstrated numerous times with a variety of methods (Stoquart-ElSankari et al., 2007; Chen, Rosas & Salat, 2011; Zemcov, Barclay, & Blass, 1984). In both this chapter, and in chapter 2, we demonstrated that declining cardiorespiratory fitness with age plays a role in the declining blood flow. Given this, it is intuitive to think that baseline blood flow would have an even greater ability to predict cognitive decline with age, since there is an abundance of research demonstrating the positive effects of fitness on cognition (see Hillman, Erickson, & Kramer, 2008, for a review). Moreover, increases in resting CBF have been shown to correlate with improved measures of cognition in aging (Mozolic, Hayasaka, & Laurienti, 2010; Heo et al., 2010) and baseline blood flow has been shown to relate to cognition in age-related diseases, such as Alzheimer's disease, and is even predictive of transferring from mild cognitive impairment to dementia (Chao et al., 2010).

One possible explanation of our results is that baseline blood flow is more regionally dependent, whereas the capacity for vascular reactivity is less variable across the brain. In that case, low vascular reactivity in the visual cortex would be fairly equivalent to low vascular reactivity everywhere else in the brain, including areas that were more sensitive to executive functioning tasks. In contrast, low baseline blood flow in the visual cortex may say little about the baseline blood flow in other areas of the brain. Thus cognition may be sensitive to reactivity in the visual cortex, but baseline blood flow may not be.

Zimmerman et al. (2014) previously demonstrated the baseline blood flow was regionally specific to gray matter volume. Gray matter volume in the visual cortex is known to be more robust to aging than in other areas of the brain that play a larger role in executive function. This idea is loosely supported by the relatively weak relationship between age and baseline blood flow in the visual cortex presented in this chapter compared to the stronger relationship between age and baseline blood flow measured through the parietal and frontal cortices presented previously in chapter 2. If poor cerebral autoregulation is a more generalized phenomenon, then that is a possible reason that the change in blood flow to activation is more predictive of cognition. However, this still does not fully explain why baseline blood flow is related to age and fitness in this sample, while activation related blood flow does not.

Another interpretation that does not exclude the previous one is that declining baseline blood flow may represent a general physiological degradation of the cerebrovascular system, but that the brain (and thus cognition) does not suffer from this declining flow until some important threshold of hypoperfusion is reached. In fact, it may be possible that reaching this threshold is prevented in part by utilizing the mechanisms of vascular reactivity to support the declining baseline blood flow. According to this view, the brain has some level of vascular reserve, which is able to compensate for a reduction in the number of functioning capillaries or reduced velocity through the capillaries by vasodilating the arterioles acting as gateways to the capillary beds. Then in cases of neural activity, those same arterioles would simply have to vasodilate even more to increase blood flow to the appropriate levels. At some point, the vasodilation mechanism would hit a limit, and could not compensate properly in cases of neural activity or other physiological events that cause vasodilation. Eventually, the vascular reserve would be used up altogether, and more damaging hypoperfusion would ensue. Notice that a prediction of this hypothesis is that cerebrovascular reactivity should predict cognition before baseline blood flow does. In addition, this hypothesis predicts that once one observes relationships between baseline blood flow and cognition, an

individual's brain is likely to be suffering from damage from improper perfusion even at rest with more severe cognitive deficits likely to follow.

Of course, this interpretation begs the question of why then, in this sample, baseline blood flow is predicted by age and fitness as expected, but the change in blood flow due to activation is not. In fact, other groups have shown relationships between changes in blood flow related to activation and both fitness and age (Brown et al., 2010), so the lack of the relationship here is surprising.

One clue may be the physiological variable of pulse pressure. In our sample, cardiorespiratory fitness was not significantly related to pulse pressure, which is often used as a proxy for vascular health. In contrast, the percent change in blood flow was significantly related to pulse pressure. Cardiorespiratory fitness may normally contribute both to improved blood pressure and to other beneficial modifications to the vascular system, such as angiogenesis. In this sample, the fact that cardiorespiratory fitness was *not* related to blood pressure may have something to do with the fact that we did not exclude participants based on the use of blood pressure medications, or it may simply be a quirk of a relatively healthy sample of older adults. Studies using older adults can become biased to include those who are especially healthy for their age, since those older adults are more likely to volunteer and be mobile. Whatever the cause, the pattern of relationships that we observe suggests that the physiological mechanism behind the relationship between CRF and baseline blood flow is not related to pulse pressure, whereas pulse pressure is strongly predictive of the extent of vascular reactivity.

Similar logic holds for attempting to understand why age does not predict activation blood flow or pulse pressure. For whatever reason, age is not related to pulse pressure in this sample, even though the relationship between age and pulse pressure is well-established (Westerhof & Westerhof, 2012). Recall that the age range of this study was fairly limited (56-88). It is possible that this age range is too limited to show differences in pulse pressure, especially when including participants on blood pressure medications.

A good candidate for the variable of vascular health that contributes so much to baseline blood flow in older adults is the volume of capillaries and small vessels in the tissue. Angiogenesis and capillary density depends very largely on physical activity level and would be expected to decline with age due to

decreasing activity levels, whether or not it related to blood pressure (Thomas, Dennis, Bandettini, & Berg, 2012). Capillary density in the brain is known to decline with age (Brown & Thore, 2011). However, the amount of capillaries does not necessarily speak to other important aspects of vascular health, such as the health of the endothelial cells and the regulation of blood pressure. Thus, the component of vascular health represented by baseline blood flow may be decoupled from the component of vascular health represented by the change in blood flow to activation.

Future research should seek to explore this possibility of vascular reserve further. One way to explore this interpretation would be to examine the different physiological contributors to blood flow separately. We can think of blood flow as a ratio of volume of blood divided by the transit time. In fact, during vasodilation, increase in the speed of blood through the capillaries due to lowered pressure contributes more to overall increases in blood flow than the volume of the blood in the tissue. (Ito et al., 2001). Since it is possible to measure both the blood volume and blood flow with MRI, it is possible to derive the transit time through the capillaries and examine how fast the blood is moving through the capillaries at rest. This would be an informative measure of how much the cerebral vascular system was relying on compensatory vasodilation, even at rest. If this vascular reserve hypothesis was correct, we would expect that the transit time through the capillaries would be correlated with cognition, but not necessarily with baseline blood flow, since the same baseline blood flow could also be achieved with a slower resting capillary transit time and a greater volume of blood in the capillary beds.

Additional physiological questions will also be important to answer to help understand the role of vascular health in cognition. It would be very useful to investigate blood flow and reactivity regionally to know if it varied across regions, and if those regional variations predicted performance on specific cognitive domains. Finally, it is important to understand whether the relationship between blood flow and fitness are present across the lifespan or if it is only relevant in aging. This is especially important if there is hope to use blood flow measures as measures of success in future intervention programs.

There are some limitations regarding the interpretations of the results here. One caveat is that change in blood flow to a visual activation task is not a typical measure of pure vascular reactivity. This

was chosen over breath holding in order to reduce movement artifacts in the ASL data, since the technique is very sensitive to small movements. Future studies may benefit from having participants breathe CO_2 enriched air, which may still be less sensitive to movement than breath holding, but be more definitively interpretable as utilizing a measure of vascular reactivity.

In summary, the results of this study indicate that measures of cerebral blood flow at baseline and in response to neural activation represent two distinct components of vascular health. Although baseline blood significantly related to age and fitness, it did not predict cognition. In contrast, the change in blood flow in the visual cortex to a reversing checkerboard was predictive of cognition and was related to pulse pressure. Future studies should further explore the relationship between cerebral blood flow and cognition in order to understand fully how vascular health impacts normal cognitive decline in order to guide future health interventions.

Tables

Table 4.1.

Demographic Characteristics

	Mean	***SD***	**Range**
Age (years)	71.18	8.07	56.83-88.78
Education (years)	16.94	2.87	12.00-20.00
Systolic Blood Pressure	133.89	14.45	113.50-176.00
Diastolic Blood Pressure	79.75	8.41	63.50-106.50
Pulse Pressure	54.14	10.86	35.50-81.00
CRF	7.00	2.38	2.45-11.45
Modified Mini-Mental Status exam (mMMS)	55.62	1.65	48.00-57.00
Beck's Depression Index (BDI)	2.76	3.22	0.00-14.00
Shipley's Vocabulary Test	36.07	2.48	31.00-40.00
Kaufman Brief Intelligence Test (KBIT-2)	115.48	11.53	94.00-142.00
Verbal	115.81	13.74	92.00-145.00
Non-Verbal	113.36	11.28	91.00-131.00
Forward Digit Span	6.98	1.12	5.00-9.00
Backward Digit Span	5.55	1.42	3.00-8.00
Backward – Forward Digit Span	-1.43	1.13	-4.00-0.00
Operation Span (OSPAN), letters	21.90	17.56	0.00-69.00
Operation Span (OSPAN), words	15.73	6.67	4.00-30.00
Verbal fluency (PRW)	44.81	13.16	19.00-78.00

Table 4.1 (cont.)

Trail Test A	17.90	6.38	9.00-36.00
Trail Test B	28.53	10.91	17.00-56.00
Trail B-A	10.63	7.75	-2.00-35.00

Note. $N = 42$ (22 females). $N = 40$ (Trail Test A, Trail Test B, Trail B-A, OSPAN (letters), OSPAN (words), CRF. Education capped at 20 years (representing a post-graduate degree).

Table 4.2.

Rotated loading matrix for PCA analysis using Varimax rotation

	1	**2**
Operation Span Letters	**.78**	.19
Operation Span Words	**.81**	.08
Backward Digit Span	**.81**	.20
Forward Digit Span	.48	**.54**
Verbal Fluency	.38	**.57**
Trail Making A	-.02	**-.87**
Trail Making B	-.17	**-.87**

Note. Factor 1 is loaded primarily by working memory tasks with an operation component, whereas Factor 2 is loaded primarily by executive tasks that have some speed component.

Table 4.3.

Partial correlations between CBF, age, CRF, pulse pressure, and cognition, controlling for gender.

	Variable	1	2	3	4	5	6
Gray Matter CBF	**1. Baseline Flow**	1					
	2. % Activation	-.09	1				
Physical Factors	**3. Age**	-.26	-.03	1			
	4. CRF	.38*	.04	-.30^{+}	1		
	5. Pulse Pressure	.17	-.34*	.14	-.17	1	
	6. Compliance	-.20	.37*	-.14	.13	-.97**	1
Cognitive Factors	**7. Working Memory**	.09	.34*	-.29^{+}	.26	-.23	.21
	8. Response speed	.22	.01	-.59**	.16	.14	-.12

Note. Significance tests (2-tailed): *df* = 39 (for non-cognitive correlations), *df* = 37 (for correlations with CRF) and *df* = 35 (for correlations with cognitive factors) or *df* = 34 (cognitive factors vs. CRF),$^{+}$ p <.10, * p < .05, ** p < .01

Table 4.4.

Correlations between blood flow and individual cognitive performance tasks.

Cognitive Variable	Correlation (*r*) with baseline CBF	Correlation (*r*) with % activation CBF
OSPAN (words)	.13	.34*
OSPAN (letters)	.08	.40*
Backward Digit Span – Forward Digit Span	-.15	.33*
(-) Trail Making B-A	.16	.07
CFL (PRW)	.26	-.01

Note. Significance tests (2-tailed): $df = 35$, * $p < .05$.

Figures

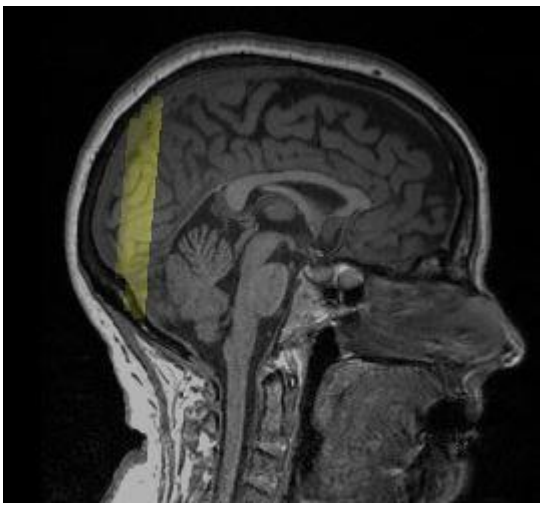


Figure 4.1. Example of the coronal imaging slices through the visual cortex of the brain of a single participant.

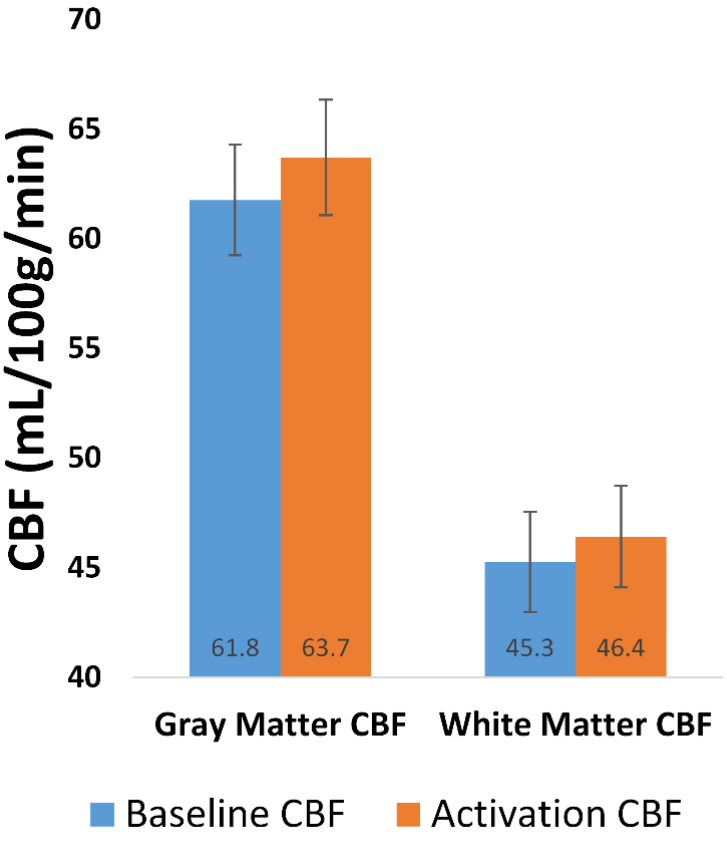


Figure 4.2. Quantitative CBF values during baseline and activation to task in both the gray matter and white matter. Although activation leads to numerically higher blood flow, no significant differences were found between baseline and activation in either gray matter or white matter.

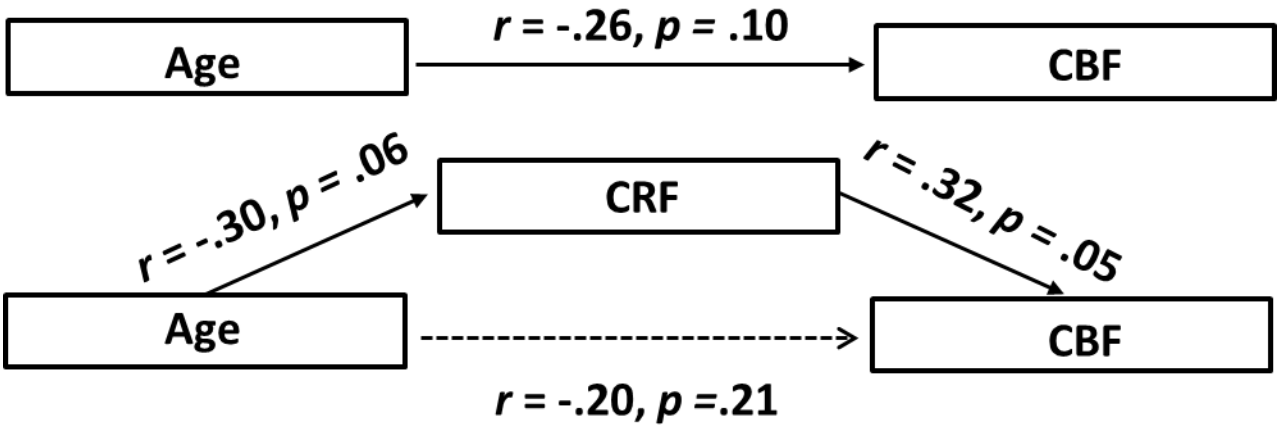


Figure 4.3. Path diagram of the mediation of age on baseline CBF by CRF. A significant indirect effect was found, demonstrating that CRF is an important mediator in the relationship between age and baseline CBF.

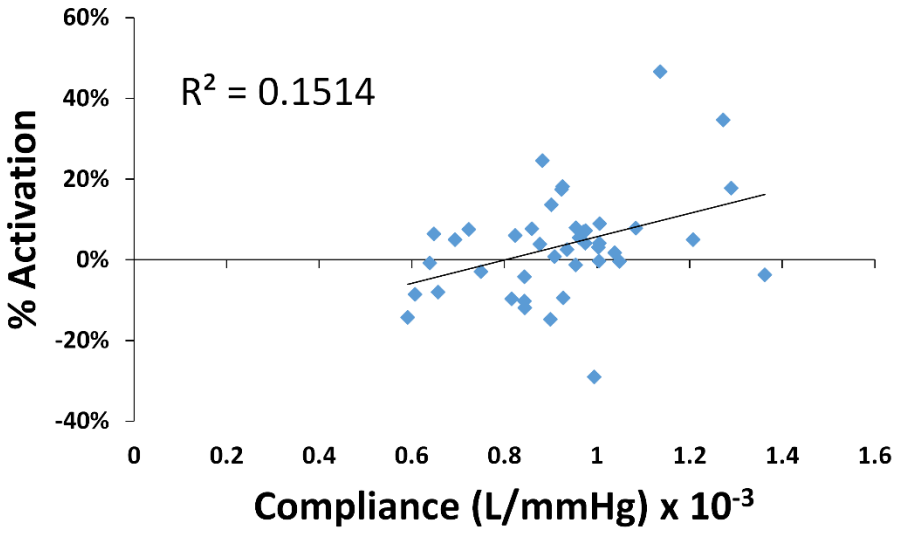


Figure 4.4. The relationship between the modeled compliance (L/mmHg) x 10^{-3} of each participant's arteries and the % CBF activation in the visual cortex. % CBF activation is significantly predicted by compliance.

CHAPTER 5: OPTICAL IMAGING PROVIDES TIMING INFORMATION OF VASCULAR REACTIVITY THAT IS PREDICTED BY AGE AND ARTERIAL COMPLIANCE

Abstract

Deficits in cognition associated with normal aging, such as declines in working memory and executive function/attention control are an increasingly important problem as the population of older adults increases. These cognitive declines may be influenced by changes in cerebrovascular health, i.e., loss of arterial elasticity and vascular reactivity in the brain. Aspects of vascular reactivity, such as the compensatory capacity of cerebral vasculature under hypoxic conditions (hypercapnia), decline with normal aging, and may be related to arterial compliance and cognition. In this study, multi-distance, frequency-domain near infrared spectroscopy (NIRS) was used to measure changes in oxy- and deoxy-hemoglobin concentrations induced by breath holding in the right prefrontal cortex concurrently with arterial spin labelling (ASL) in a magnetic resonance imaging (MRI) scanner. Studying participants ranging in age from 55-87, we found that the superior temporal resolution of NIRS allowed us to observe differences in the oxy-hemoglobin response to a period of breath holding between our older and younger participants and between participants split by arterial compliance, where older individuals and participants with stiffer arteries tended to have a delayed hemodynamic response. This finding highlights the usefulness of utilizing a multi-modal neuroimaging approach for the investigation of time-sensitive aspects of cerebrovascular health.

Introduction

In the last chapter, we saw the importance of differentiating between baseline cerebral blood flow (CBF) and activation CBF. Cerebrovascular reactivity, as measured by % activation CBF to a reversing checkerboard visual stimulus, was seen to be more related to both cognitive performance and to arterial compliance than baseline CBF. It was hypothesized that arterial compliance was directly connected to

cerebrovascular reactivity, and that greater arterial compliance would lead to improved cerebrovascular reactivity, which may allow for the arteries to appropriately serve the needs of the brain tissue that they perfuse. Arterial compliance is essentially the opposite of arterial stiffening, and compliant arteries lead to a sustained and steady blood flow as the arterial walls expand and push back on the blood. The extent and speed that the arterial vasculature increases CBF in response to an increased need of blood by the tissue may partially depend on arterial compliance. Thus both the magnitude, but also the temporal dynamics, of the cerebrovasculature may be useful measures of cerebrovascular health.

Whereas arterial spin labelling (ASL) excels at quantifying blood flow in the tissue and the magnitude of changes in blood flow during a task, it lacks temporal resolution. In fact, in the studies presented here, each image takes about 4.5 s to acquire. Since it is a subtraction technique, each subtraction represents 9 seconds.

In contrast to this, optical imaging can sample the optical properties of the tissue much faster, on the order of tens of milliseconds, making it an ideal method for examining the time course of activity. We were interested in determining if there were differences in the timing of the course of vascular reactivity (beyond the differences in relative amplitude that was presented in chapter 4) based on age and arterial compliance.

One way to approach this question is by using multi-distance, frequency domain near-infrared spectroscopy (NIRS) to look at concentrations of hemoglobin in cerebral tissue. With multiple wavelengths of near infrared light, modulated at a particular radio frequency, and detected from multiple light-source/detector distances, it is possible to estimate absolute concentrations of oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (HHb) in the tissue probed. NIRS has been demonstrated to capably measure cerebrovascular reactivity (Smielewsky et al., 1995).

It is possible to study cerebrovascular reactivity through the robust cerebral autoregulation that occurs during a CO_2 reactivity challenge. Special receptors react to increases in CO_2 in the blood by prompting vasodilation in the vessels, increasing the overall cerebral blood flow. A simple way to increase CO_2 in the blood is through a breath-holding (BH) procedure. Previous research has shown a

diminished hemodynamic response to BH with age (Safonova et al., 2004). In the present study, we investigate whether the timing of vascular reactivity as measured by the hemodynamic response to BH is related to age and compliance.

Method

Participants

The participants for this experiment were the same from chapter 2, recruited as part of a larger two-year study and inclusion criteria was the same as that study. To summarize, participants with serious or chronic medical conditions, a history of major neurological or psychiatric disease, dementia, depression, or a history of drug abuse were excluded from this study. Furthermore, participants were excluded if they showed signs of dementia and depression. All participants were right-handed, had normal or corrected-to-normal vision, and were native speakers of English. All participants were paid for their time in the study and signed informed consent in accordance with the University of Illinois at Urbana-Champaign's Institutional Review Board.

In all, data from 8 participants were discarded from subsequent analysis. Of these, 3 participants did not have complete NIRS data, four participants did not have complete MRI data, and 1 participant was missing both MRI and NIRS data. The final sample included 47 older adults. For analyses utilizing the compliance measure, an additional one subject was lost due to missing data. The demographic characteristics of this sample are presented in Table 5.1.

MRI acquisition

All MRI data collection occurred at the Biomedical Imaging Center using a 3T Siemens (Erlangen, Germany) Trio scanner using a standard body coil transmission and a twelve-channel head array receive coil.

Participants were instructed to perform a breath-holding task according to a visual cue, which included six repetitions of alternating periods of breath-holding after expiration (18 seconds) and self-paced breathing (36 seconds) for a total acquisition time of 5 minutes and 24 seconds. Instructions for the breath-

holding experiment were presented using EPRIME (Psychology Software Tools, Pittsburgh) and displayed via back projection (BrainLogics, Psychology Software Tools, Pittsburgh). To minimize motion during the breath-holding period, padding was used to stabilize the subject's head.

Six axial imaging slices passing through the middle of the lateral ventricles and covering part of the frontal cortical areas were acquired with localized pseudo-continuous Transfer Insensitive Labeling Technique (pTILT) ASL sequence (Ouyang & Sutton, 2011), and the labeling slice was placed inferior to the imaging slab with a 10 mm gap. The pTILT ASL method is less sensitive to the transit time issues that may confound other ASL methods as a function of age. In fact, this method tags close to the slice of interest, minimizing the differences in transit time that may result from age-related changes in the vasculature.

The imaging and tagging parameters of the localized pTILT ASL sequence were: windowed-sinc 45^{o} RF pulses with 2560 μs-duration, tagging repetition spacing = 30 ms, number of concatenated RF pulse pairs = 100, tagging duration = 3 s, post-labeling delay = 0.5 s, tagging slice thickness = 10 mm, gradient spoiler duration and amplitude = 4000 μs/[±10, ±12, ±14, ±16] mT/m, SE-EPI readout, FOV = 220×220 mm, scan matrix size = 64×64, TR/TE = 4500/44 ms, slice thickness = 6 mm, slice gap = 1.2 mm, 36 control and 36 tag repetitions, scan time of one acquisition = 5 minutes and 24 seconds.

To assist with the registration procedure, two additional brain scans were taken: a high-resolution 2D turbo-spin echo (TSE) acquisition with the imaging slices at the same location as the ASL images, and a high-resolution T1-weighted 3D anatomical image. The T1-weighted brain image was acquired using a 3D MPRAGE (Magnetization Prepared RApid Gradient Echo) protocol (TR = 1900 ms, TI (inversion time) = 900 ms, TE = 2.32 ms, field of view = 230×230x172.8 mm^3 (sagittal), matrix size = 256×256×192, flip angle = 9^{o}, slice thickness = 0.9 mm).

MRI data processing

The pTILT functional data processing was carried out using FSL (FMRIB Software Library; http://www.fmrib.ox.ac.uk/fsl). Because the breath holding task caused significant movement artifacts in the MRI scan, we could not use the ASL task related reactivity in chapter 2. However, we were able to look

at the time course of the reactivity in the ASL scans by eliminating the images surrounding the beginning and end of the breath holding procedure, where the most movement was likely to occur. This left 1 tag-control pair during each breath holding period, and 3 tag-control pairs for each of the non-breathholding periods, which we will hereafter refer to as "recovery 1" (Rec1), "recovery 2" (Rec2), and "rest". Figure 5.1 shows a schematic of the images that were discarded from the analysis. In addition, subtraction images that yielded negative average flow values across the gray matter were thrown out and not included in subsequent analysis.

Gray and white matter masks were formed from segmenting the T1 structural scan using FSL's FAST software (Zhang et al., 2001). The gray and white matter masks were then transformed into the subject's pTILT space using a registration between the control image in pTILT and the MPRAGE from FSL's FLIRT (Jenkinson & Smith, 2001). Because the parietal region covered by the pTILT slices had the greatest relationship to age and cardiorespiratory fitness (CRF) in chapter 2, we utilized the same region to look at the time course of the breath holding reactivity here.

The Harvard-Oxford cortical and subcortical structural atlas provided by FSL was used to isolate activity in the parietal cortex. A linear registration between the subject's MPRAGE space was used to bring the parietal region back into the invidual subject's MPRAGE space and then on to pTILT space. The parietal region was determined by combining five parietal areas in the atlas including the postcentral gyrus, superior parietal lobule, supramarginal gyrus, anterior division, posterior division, and the angular gyrus. CBF was averaged in the gray matter from this region.

CBF quantification

Baseline perfusion images in mL/100 g/min units were calculated according to recent modelling quantification guidelines (Alsop et al., 2014):

$$CBF = \frac{\Delta M}{M_0} \cdot \frac{6000 \cdot \lambda \cdot e^{\frac{\omega}{T_{1,blood}}}}{T_{1,blood} \cdot (1 - e^{-\frac{\tau}{T_{1,blood}}})}$$

where ΔM is the estimated coefficient of the tag-control difference (i.e., the perfusion-weighted control-tag image); M_0 is the estimated coefficient of the static tissue signal (i.e. the control image); λ is the blood-brain partition coefficient (0.9; Herscovitch & Raichle, 1985); ω is the post-labeling delay (0.5 seconds); $T_{1,blood}$ is the longitudinal relaxation rate of blood (1650 ms at 3T; Lu, Clingman, Golay & van Zijl, 2004); τ is the label duration (3 seconds).

Breath-holding task

The optical data were obtained during a breath-holding task concurrently with magnetic resonance imaging reported separately. The advantage of the breath-holding task is that no additional apparatus, such as a capnograph or breathing mask, need to be used, and no medical doctor is necessary to oversee the experiment. However, BH itself is very dependent on how well participants follow instructions and so there may be error and extra noise introduced. In order to help alleviate this risk, we instructed subjects how to perform the BH ahead of time and allowed for an opportunity to practice. Participants underwent a total of six end-expiratory BH trials in two blocks of three sequentially, with a brief pause between each block. All stimuli were presented using E-Prime 2.0 (Schneider, Eschman, & Zuccolotto, 2002) on a white screen with black letters. Participants were asked to breathe normally for twenty-nine seconds, followed by 2 seconds of a get ready prompt, 2 seconds instructing them to breathe out, 2 seconds of breathing in followed by a final instruction (4 seconds duration) to breathe out and hold their breath for 14 seconds. We define the beginning of the breath-holding period as the onset of the final instruction. During the period of breath-holding, a concentric circle presented at fixation shrinks in six gradations to provide visual feedback on how much longer the participants needed to hold their breath.

NIRS Acquisition

Concentrations of HbO_2 and Hb were measured with a near-infrared spectroscopy device (OxiplexTS; ISS Inc., Champaign, IL) capable of deriving absolute concentrations of HbO_2 and HHb with at least two wavelengths of light and at least two source-detector distances. 4 emitting source probes containing two fibers each (one emitting 690nm light and another emitting 830nm light) and a single detector of the NIRS equipment was placed on the right forehead of the participants. The source-detector distances were set at 22.5mm, 27mm, 33.5mm and 38mm, sufficiently far apart to allow chromophore detection on the surface of the cerebral cortex. The forehead was chosen because of the frontal lobe's importance in cognitive function lost with aging and because of the absence of hairs, which absorb light. Light was modulated at a radio frequency of 110MHz and acquired continuously at a sampling rate of 50Hz. The OxiplexTS device calculates the scattering and absorption coefficients and then determines the concentrations of HbO_2 and HHb in the probed area. During the procedure, we monitored the participant's compliance with BH procedure by observing the movements of the abdomen with a respiratory strain gauge.

Hemodynamic Response Quantification

The hemodynamic response from the OxiplexTS output was corrected for motion artifacts using a procedure developed by Chiarelli, Maclin, Fabiani & Gratton (2015). Then the data is down-sampled to one data point per second. We then separate the data into five epochs (excluding the 6^{th} breath hold due to noise at the end of the procedure), and average the epochs together. Finally, we baseline correct the time course by averaging the 10s preceding BH and setting this average to zero. The concentrations are therefore expressed in the change in µM/L from baseline. The figures are presented as 9 point moving averages to provide clearer representations of the overall response without high-frequency noise.

Data Analysis

We sought to determine whether there were differences in the timing of the hemodynamic response to BH by both age and arterial compliance. Our compliance measure was derived for each subject by utilizing the shape of the pulse waveform in the optical signal measured separately across the head, described fully in Fabiani et al. (2014). The measurement is based on the average shape of the heart beat when measured with near-infrared light. Since haemoglobin in blood absorbs light at this wavelength, the systolic phase of the heart beat significantly reduces the amount of light that reaches the detector. Healthier, compliant arteries expand during the systole and rebound during the diastole creating a greater area underneath the line between a systolic peak and the subsequent diastolic peak. After normalizing this area by the amplitude of the of the pulse, this technique can provide a non-invasive measurement of arterial compliance across the cortex.

To test if there were reactivity differences based on age and arterial compliance, we sought to compare the timing differences of the reactivity to BH after performing median splits on the sample by both age and again by compliance using both the concurrently recorded ASL data and NIRS data. In order to determine whether there was a timing difference between the two groups for both age and elasticity, we used the "finddelay" function in the MATLAB and Signal Processing Toolbox (The MathWorks, Inc. Natick, Massachusetts, United States). This function determines the normalized cross-correlation between each pair of signals at all possible lags and then returns the lag for which the cross-correlation is largest. If there is no significant correlation between the two signals at any lag, then the default delay is 0.

Results

Breath holding task

Although there was a numerical increase in the average CBF values during the breath holding period compared to the rest period, the difference was not significant, $t(46) = -1.35$, $p = .18$. However, the greatest increase in CBF actually occurred after the end of the breath holding period. The difference between this Rec1 period and rest was significant, $t(46) = -4.13$, $p < .001$).

In the optical data, the change in HbO_2 during the last ten seconds of the breath holding task was significantly different from 0, $t(46) = 4.0$, $p < .001$). Figure 5.2 shows the average CBF for the four periods and the average HbO_2 waveform for all of the subjects.

Age-related lag of the hemodynamic response to breath holding

Figure 5.3 shows the average time course of the hemodynamic response separated by age-groups determined by median split. The younger age group ($M_{age} = 62.41$, $SD = 4.01$) had a hemodynamic response that was 3 seconds ahead of the older age group ($M_{age} = 76.73$, $SD = 4.38$) in the NIRS data. The same analysis yielded no timing difference in the ASL data.

Compliance-related lag of the hemodynamic response to breath holding

Figure 5.4 shows the average time course of the hemodynamic response separated by level of compliance determined by median split. The more compliant group had a hemodynamic response that was 1 second ahead of the less compliant group. The same analysis again yielded no timing difference in the ASL data. Notably, the compliance measures were negatively correlated with age in this sample, $r(44) = -.32$, $p = .03$.

Discussion

The primary result of this study is that the added temporal resolution from diffusive optical imaging measures can add potentially important timing information about the hemodynamic response to a compensatory challenge. Indeed, we observed that in the NIRS data, there was a 3 second difference in the average response between the younger and older halves of the participants and a 1 second difference between the more compliant and stiffer halves. These timing differences were not seen in the ASL data,

but that would be expected since each image represents a full 9 seconds. These results highlight the usefulness of utilizing multiple methodologies to investigate cerebrovascular health and dynamics.

We observed an overall response to BH, which included a peak and plateau in the HbO_2 response to BH, but an even larger response to the recovery period immediately following BH. This may be due to oxygen flooding the already vasodilated vessels, since BH includes a period of hypoxia along with the hypercapnia. The observed timing differences in the HbO_2 signal may reflect the ability of the vasculature to react quickly and appropriately to changes in oxygenation.

Many factors influence the neurovascular response. A multitude of vasoactive factors from both neurons and glia, and also distal physiological factors, such as blood pH and sympathetic tone act in concert on endothelial cells, pericytes, and smooth muscle to control CBF (Girouard & Iadecola, 2006; Fierstra et al., 2013; Laan et al., 2013). In the previous chapter, we observed a relationship between arterial compliance and vascular reactivity, so we were particularly interested in factors influencing the neurovascular response that could also relate to arterial compliance. Neurovascular reactivity and arterial compliance may be related indirectly. For instance, it is known that aging is related to both a decrease in arterial compliance (Jani & Rajkumar, 2006), and with endothelial dysfunction, which may have the effect of reducing signaling factors that are necessary for appropriate vasodilation (Herrera et al., 2010)

It is also possible that some connecting factor is influencing both compliance and neurovascular reactivity. For example, chronic hypertension both alters the structure of stiffness of cerebral arteries and also impairs cerebrovascular autoregulation and neurovascular coupling (Girouard & Iadecola, 2006).

Finally, there may be direct mechanical reasons for a delayed functional hyperemia. Blood flow through the arteries is kept steady and consistent through the compliance of the arterial walls. The arterial walls absorb some of the increase in pressure by expanding. Then during diastole, the walls contract, which continues to propel blood forward through the arterial system. The effect of this is that the stiffer the arteries are, the more that blood flow will depend on the systolic phase of the heartbeat instead of being spread more equally across all of the phases of the cardiac cycle. Therefore, functional hyperemia may also be more dependent on the systolic phase of the cardiac cycle. Systole is shorter than diastole,

composing about 1/3 of the time of the cardiac cycle compared to the 2/3 of the diastole. Therefore, as compliance decreases, on average, it would take a little bit longer for blood flow to increase, since the increase would become more phase dependent. The phase of the cardiac cycle would matter much less for healthy compliant arteries.

One problematic aspect of this analysis is that the effect of age may be masking the effects of specific factors that contribute to the overall hemodynamic response. This interpretation implies that other factors are contributing more to the age-related lag that we observed than arterial compliance. Our results seem to suggest that at least for the timing differences in the hemodynamic response, the myriad physiological changes that accompany aging matter more than compliance alone.

In conclusion, we demonstrated the possibility and potential value of concurrently acquiring NIRS data to assess hemodynamic information with ASL. Utilizing this multi-modal approach may be important to gaining further insights into impact of cerebrovascular health on cognition. Future work should explore utilizing this approach during a cognitive task, particularly to determine whether the amplitude of reactivity, assessed by ASL, provides different predictive value than timing differences in reactivity, assessed by optical imaging.

Table

Table 5.1. Demographic Information

% Female	51.06
Mean age (SD) (years)	69.42 (8.34)
Mean education (SD) (years)	16.73 (2.96)

Figures

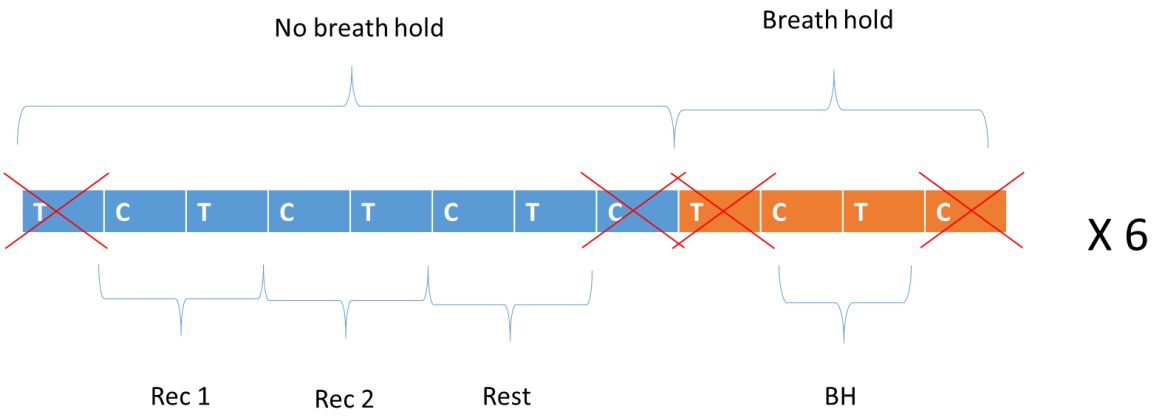
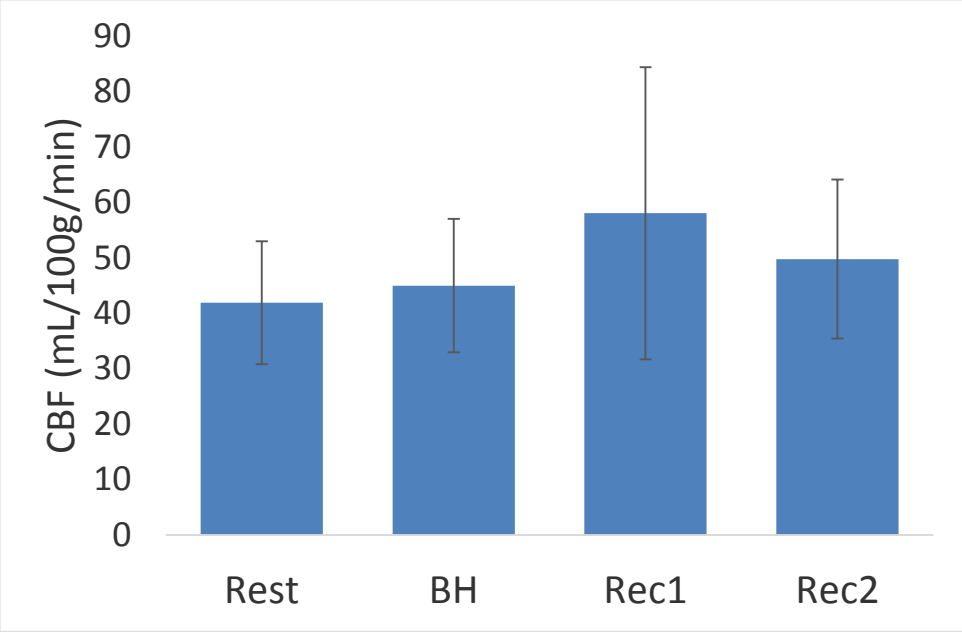


Figure 5.1. Schematic of the tag and control images (T and C) acquired during the breath holding task. Because of motion accompanying transition periods into and out of the breath holding, tag-control image pairs during the transition periods were discarded. This left 4 difference images for each of the 6 breath holding epochs, including one image during the breath hold, and 3 images of no breath hold. Those images were labeled recovery 1 (Rec1), recovery 2 (Rec 2), and rest.



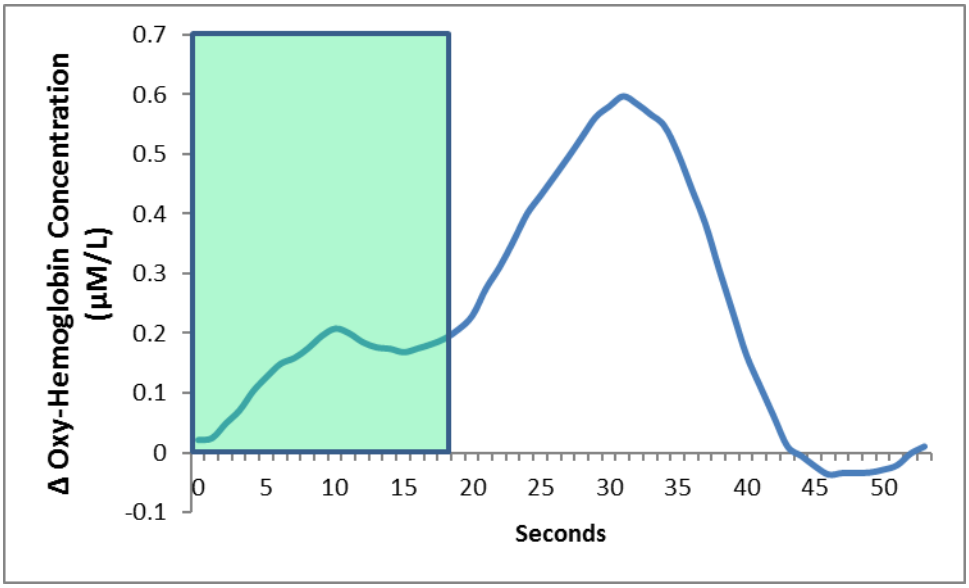


Figure 5.2. Top panel: shows the average gray matter CBF across all subjects throughout the breath holding epoch. CBF rises slightly, but non-significantly during the breath holding period, continues to rise for the first recovery image (Rec1) and begins to fall by the second recovery image (Rec 2). Bottom panel: shows the average time course of the change in HbO_2 over the breath holding epoch averaged over all subjects. The green box represents the time that the participant is holding their breath. There is an initial rise in HbO_2 during the breath hold, and then a steep rise immediately after the breath holding period, followed by a fall back to baseline.

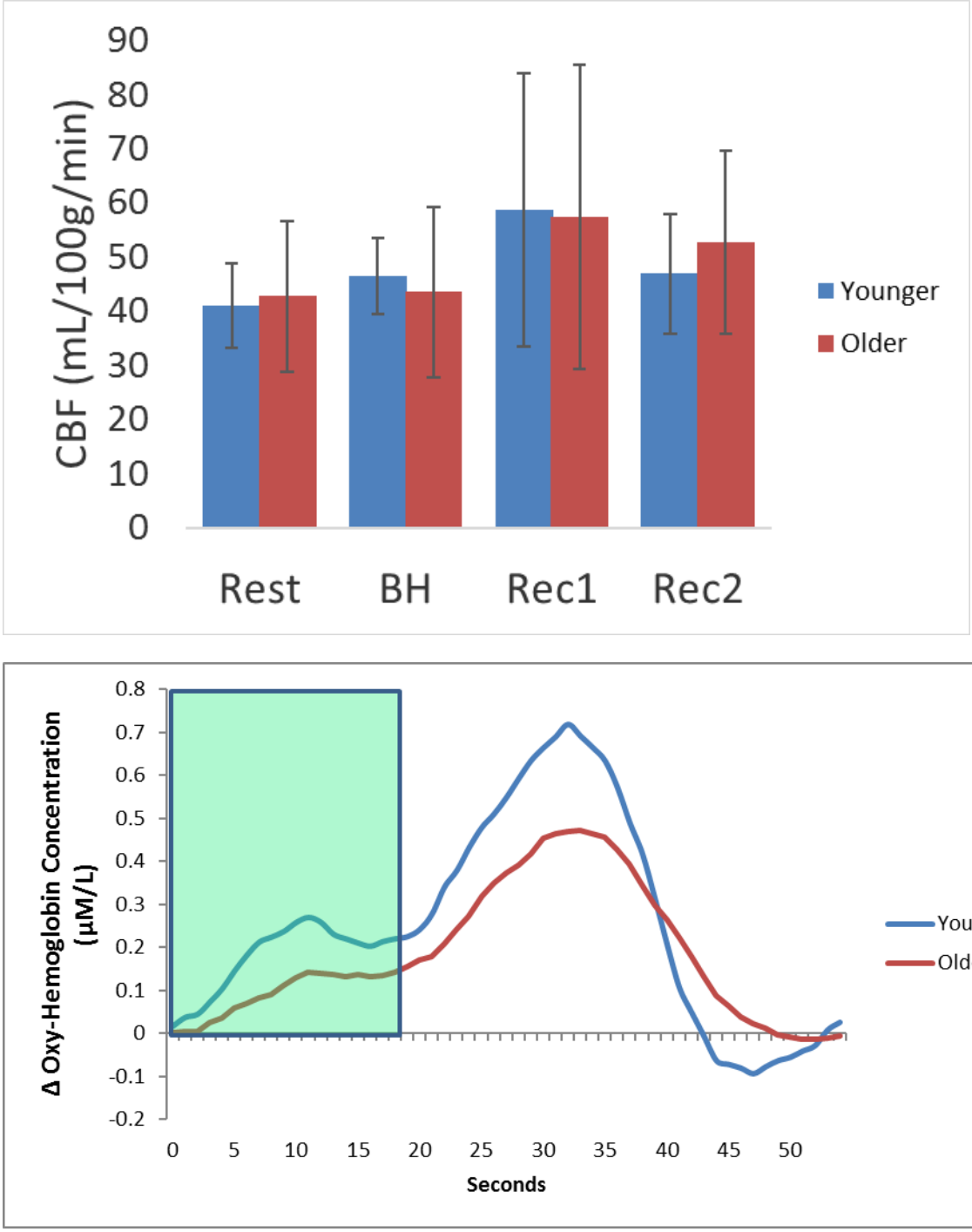
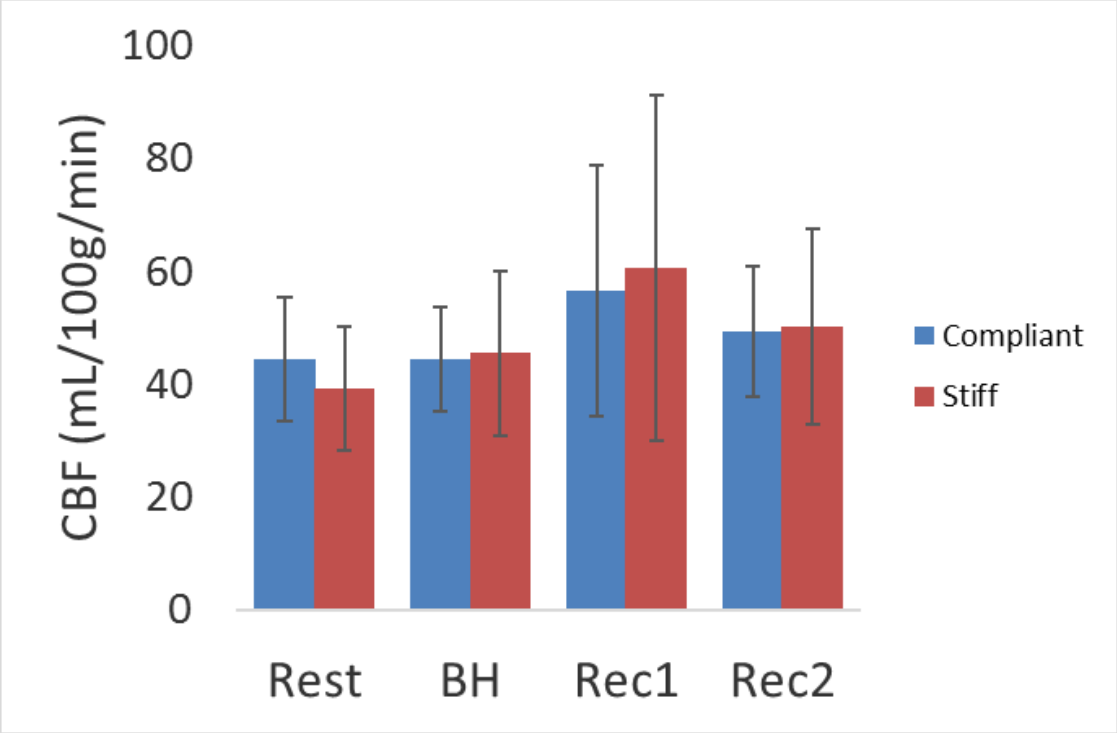


Figure 5.3. Top panel: shows the average gray matter CBF throughout the breath holding epoch split by the younger and older half of the sample. The older group seems to have a slower response, but it is not extreme enough to come out in the cross-correlation analysis. Bottom panel: shows the average time course of the change in HbO_2 throughout the breath holding task and subsequent rest. The green box represents the time that the participant is holding their breath. The older group has a 3 second lag compared to the younger group.



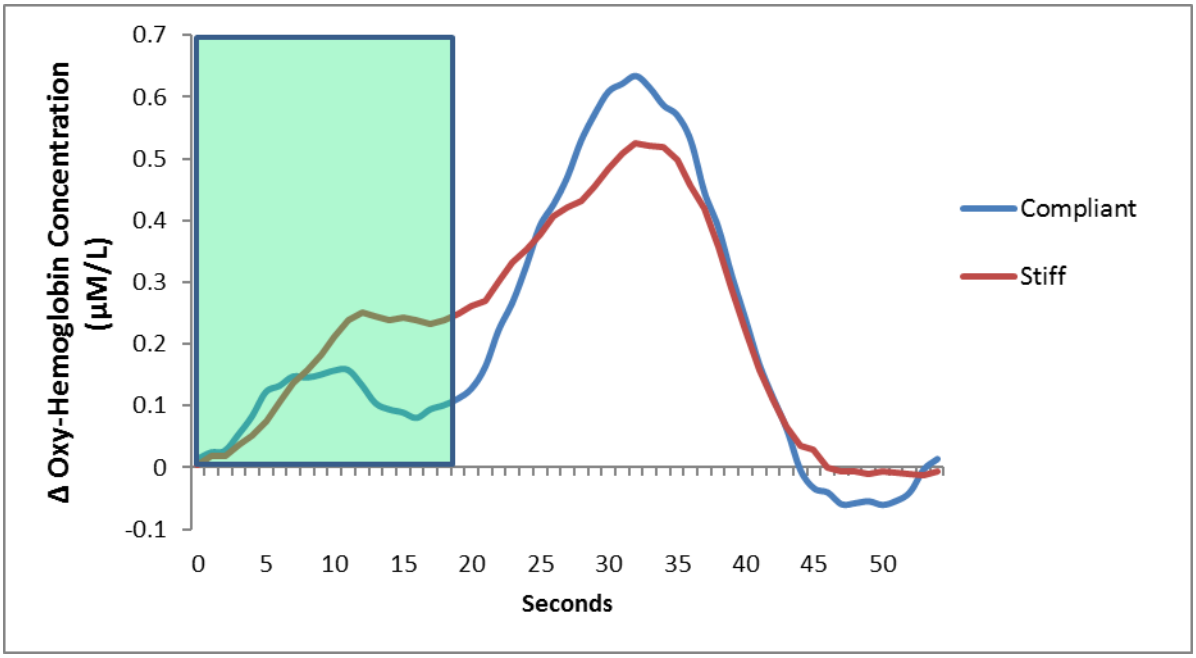


Figure 5.4. Top panel: shows the average gray matter CBF throughout the breath holding epoch split by compliance. There is no clear difference in the time course between the more compliant and stiffer participants. Bottom panel: shows the average time course of the change in HbO_2 throughout the breath holding task and subsequent rest. The green box represents the time that the participant is holding their breath. The stiffer group has a 1 second lag compared to the more compliant group.

CHAPTER 6

CONCLUSIONS

Overall, this set of experiments highlights the complexity of measures of cerebrovascular health both across the lifespan and in their relationships to cognition. These complexities should be carefully considered in both the design and interpretation of studies that seek to intervene on cerebrovascular health and to more deeply understand the role of cerebrovascular health on cognitive aging. In this research program, a number of unexpected results came out of perhaps a too simplistic view of the measurements of cerebrovascular function.

In the first experiment, our results conformed to our expectations. We demonstrated that in older adults, resting CBF was related to CRF, and CRF mediated age-related declines in CBF. This result highlighted the importance of fitness in a measure of cerebrovascular functioning. In addition, the observation of regional correlations between gray matter volume and blood flow made us optimistic about the usefulness of resting blood flow as a regionally specific tool to investigate cognition.

However, the relationship between CBF and CRF reversed in a sample of younger adults. Although this effect was very small, the theoretical importance of this significant result was that higher CBF may not always be a positive result, and at least that the relationship between CBF and CRF is not as simple as we believed. More research should be done to better understand how CRF modulates CBF, and if that changes across the lifespan.

Another experiment revealed that resting CBF did not predict cognitive decline in a sample of older adults. In contrast, the level of task-related change in CBF did positively relate to executive functioning. This result pointed to a greater role in reactivity, which was related to pulse pressure, than resting blood flow in impacting normal age-related cognitive decline. We hypothesized that the role of resting CBF would become more important after some lower-bound for acceptable perfusion was reached, which would likely be accompanied by disease.

Pursuing measures of reactivity further, we found that adding NIRS measures provided temporal resolution that allowed us to see differences in the timing of cerebrovascular reactivity across age and arterial compliance in the brain. These timing differences may be complementary to the amplitude differences found through ASL, and future research will continue to resolve these separable components of cerebrovascular health and their relationship to cognition.

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