EXERCISE-INDUCED CEREBROVASULAR RESPONSIVENESS AND BRAIN AGING MARKERS

BY

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Abstract

At the interface of heart function and brain function is cerebrovascular function. The hundreds of miles of cerebrovasculature within our compact skulls exists to assure delivery of vital life substrates to brain tissue. A healthy cerebrovasculature is important to aging, as evidenced in age-related afflictions, including stroke and dementias. Cerebrovascular function is of tremendous interest to numerous fields, including, but not limited to, gerontology, physical therapy, rehabilitation medicine, psychology, neurology, and neurosurgery. Although we have made substantial advancements in understanding the aging brain, there are unanswered questions regarding cerebrovascular function which could ultimately impact our understanding of brain aging.

This body of work addresses gaps regarding the role of cerebrovascular function in the aging brain, and it has potential clinical implications regarding cerebrovascular dysfunction in stroke and dementias. The aim of this work was to elucidate the links between resting and exercise-induced cerebrovascular function and three markers of brain aging: executive function, brain structural integrity, and β-amyloid, a cellular hallmark of Alzheimer's disease pathology. Our analyses of exercise-induced cerebrovascular functions revealed links between cerebrovascular responsiveness and these brain aging markers. Hence, the findings are a prelude to further investigation into cerebrovascular responsiveness and its links to brain aging.

Long-term development of markers of cerebrovascular responsiveness may provide researchers and clinicians with surrogate markers of brain pathological risk, i.e., a non-invasive marker of stroke or dementia risk. This could then serve as the basis for implementing brain-sparing interventions, particularly exercise interventions in high-risk populations. Therefore, this

work lays the foundation for assessing whether longitudinal exercise-induced cerebrovascular functions could serve as potential indicators of brain aging.

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"For the love of science!" – R.E.A.C.H. Laboratory motto

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Chapter 1

Cerebrovascular Aging:

Relationships with Cognition and Brain Structural Integrity

1.1. Overview

Cerebrovascular function is an important component for maintaining healthy brain aging. The cerebrovasculature's primary function is to meet the disproportionately high metabolic demands of brain tissue through the flow-pressure relationship. This balance between blood pressure and blood flow allows for an adequate and steady supply of blood to neurons and glial cells (1). While prior work has focused primarily on neuronal networks that may promote healthy brain aging, relatively few studies have concentrated on cerebrovascular mechanisms that might underlie improved cognitive function (2). Further, our knowledge of how to preserve or enhance cerebrovascular function longitudinally across the aging spectrum is lacking. Modifiable cardiovascular risk factors such as maintaining normal blood pressure (3), avoiding diabetes (4) and being physical active (5, 6), may have significant roles to maximize cerebrovascular function. Cerebrovascular function measures are used as surrogate markers for brain health (2, 7). Cerebrovascular reserve capacity (CVRC), of the middle cerebral artery (MCA) has been used as a marker of cerebrovascular function. Cerebrovascular reserve capacity has been calculated as the change in mean MCA velocity (MCAv mean, in $cm*sec^{-1}$) from a resting condition in response to some stimulus, such as carbon dioxide or exercise (2, 8-11). A second measure of cerebrovascular function is the cerebrovascular conductance index (CVCI), which describes the relationship of MCAv to mean arterial pressure (12), thus capturing the flow-pressure relationship in response to physiological stress (13-15). To measure the MCAv mean, Transcranial Doppler (TCD) ultrasound is used as a non-invasive alternative to scanner-based neuroimaging. The main goal of this project is to better understand whether MCAv mean, CVCI, and CVRC is related to cognition and brain structural integrity, specifically white matter lesions (WML) burden, in people who were considered to be cognitively normal. Our work lays

the foundation for using MCAv mean, CVCI, and CVRC as measures of cerebrovascular health and as potential biomarkers of brain aging.

1.2. Brain Blood Flow and the Aging Brain

There is strong evidence suggesting cerebrovascular health is an essential component of brain aging (7, 16-20). The brain's cerebrovasculature is a vast network of blood vessels, with an estimated 400 miles of capillaries, designed to maintain brain hemodynamics, or brain blood flow (1, 21, 22). The cerebrovasculature burdens the responsibility of continual transportation of oxygen and energy, from aerobic metabolism, to brain tissue. This is because the brain demands a tremendous portion of the body's energy, with an estimated 20% of cardiac output dedicated to brain function, yet sufficient brain energy storage is lacking (21). Without this continued supply of vital materials, brain function ceases within seconds, and the brain is at risk of cell death within minutes (23). Specifically, the normal hemodynamic rate is between 50-55 ml/100g/min of brain tissue, and at rates between 25-30 ml/100g/min altered consciousness and electroencephalogram brain wave abnormalities occur. When the hemodynamic rate drops below 20 ml/100g/min, neurons convert to anaerobic metabolism, which leads to increases in lactate and H^+ concentrations. At rates between 10-12 ml/100g/min, neurotransmission ceases, Na^+/K^+ pumps stop, and cytotoxic neuronal edema begins. Glutamate and Ca^+-dependent neuronal death begin once rates reach 6-10 ml/100g/min (24).

One of the age-associated risks is decreased brain hemodynamics, seen in both pathological and nonpathological aging. A review of the literature shows decreased hemodynamics as an important factor in a multitude of brain diseases including Parkinson's disease, Huntington's, dementia, and stroke (25-28). The average yearly global and regional

brain hemodynamic decreases vary depending on study. In healthy adults, one study established an average regional decrease of approximately 0.5% a year. This was seen in a study of 34 healthy volunteers, ages 22-82, who underwent positron emission tomography (PET) (29) . However, more recent studies suggest that the decrease is between 0.78% and 1.2% per year (30, 31). This is in stark contrast to the estimated 6.2%, 8.1%, and 8.6% yearly decreases in computer tomography (CT)-measured global brain blood flow, parietal cortex regional brain blood flow, and frontal cortex regional brain blood flow , respectively, seen in older adult with cognitive decline and dementia (32). One possible reason for the discrepancies may be due to differing methodology, as each study used a different imaging modality (PET vs. CT vs. magnetic resonance imaging (MRI) vs. magnetic resonance angiography). A second possible reason may be due to non-standardized quantification of blood flow, as some studies use ml/100g/min as the flow unit while other studies use mL/min. Regardless of the imaging tool or unit of measure used, the decades of evidence suggests that brain hemodynamics declines across the lifespan.

1.3. Cerebrovascular Regulation

Cerebrovascular regulation is an intricate process that is largely affected by the partial pressure of arterial carbon dioxide (P_aCO_2), mean arterial pressure (MAP), neurovascular coupling, and autonomic nervous system functioning (33). Therefore, this process is integrative, balancing neural, cardiovascular, and pulmonary functions. Regional brain blood flow regulation is associated with local neural metabolic demand. Hence, hemodynamics and neurovascular coupling have reciprocating effects on each other. Neurovascular coupling describes the functional relationship between neurons, glia, cerebrovasculature (particularly the pial vessels), and local metabolic demand. For instance, the response to hypoxia relies on excitation of the

astrocytes, which stimulate the production of vasodilators in order to affect increased hemodynamics (34).

The cerebrovasculature is particularly sensitive to changes in carbon dioxide. For instance, there is up to a 6% increase in hemodynamics BBF velocity for every 1 mg Hg increase *above* eupneic P_aCO_2 and up to a 3% decrease in hemodynamics for every 1 mm Hg decrease *below* eupneic P_aCO_2 (33). Hemodynamics increases with increasing levels of hypoxia, but the levels of P_aCO_2 also controls the cerebrovasculature's response to hypoxia, such that hypercapnia increases hemodynamics, but hypocapnia decreases hemodynamics (35). Although the cerebral arteries are sensitive to changes in blood gas concentrations and perfusion pressure, the pial arterioles are responsible for modulating cerebrovascular resistance, and therefore, regional hemodynamics. For instance, in hypocapnia, the smooth muscles contract, leading to vessel constriction and increased cerebrovascular resistance, which ultimately leads to a decrease in hemodynamics. However, hypercapnia results in relaxation of the smooth muscles and pial artery dilatation (up to 40%) to effect a net increase in hemodynamics (36).

The relationship between MAP and hemodynamics (see section *1.5.2. Flow-pressure Relationships and the Vasomotor Response*) has provided the basis for studying cerebrovascular regulation, the homeostatic process in which hemodynamics remain stable over a wide range of arterial pressures. Lassen's classic review paper and autoregulatory graph (**Figure 1A,** adapted from Willie's review paper (33)) plotting brain blood flow vs blood pressure (plotted using data from seven studies) shows that hemodynamics rises as arterial pressures rises, but a plateau is reached and maintained at pressures between 60 mm Hg and 150 mm Hg. "Lassen's curve" has become a cornerstone concept to those studying in the fields of anesthesia, neurosonology, and neurosurgery (37-39). However, separate work by Tan and Willie and colleagues have

challenged this convention by suggesting the stable autoregulatory plateau is much thinner (**Figure 1B**), and therefore smaller than Lassen's hypothetical 90 mm Hg gap (33, 40). Willie points out that this may be due to different methodologies interacting with established physiology, mainly the baroreflex. Specifically, the baroreflex limits the range of blood pressures which can be studied (41), which then constrains experimental manipulation of hemodynamics via use of vasoactive drugs, gases (see next section), postural changes, or exercise. Hence, the cerebrovascular response is not fully understood, but there are current efforts such as this work and others (see Appendix) (42), which strive to add to our understanding of the dynamic response to a stimulus such as exercise.

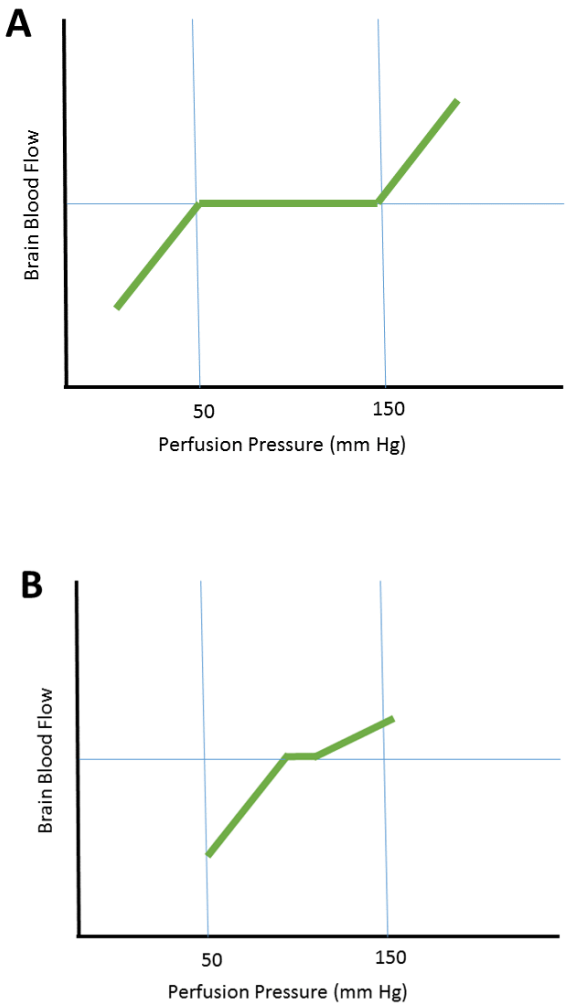


Figure 1. The Relationships between Perfusion Pressure and Hemodynamics. Classic and contemporary autoregulatory models adapted from Willie's review article, page 487, figure 3 (33). **A)** Lassen's classic autoregulatory curve, showing brain blood flow remains stable over a wide range of pressures, and a plateau is reached and maintained between 50-60 mm Hg and 150 mm Hg. **B**) Recent work challenges the classic autoregulatory curve by suggesting the stable autoregulatory plateau is a much smaller range (33, 40). Recreated with permission. © 2014 The Authors. The Journal of Physiology © 2014 The Physiological Society.

1.4. Neuroimaging markers of cerebrovascular aging

1.4.1. White matter lesion burden

White matter lesions (WML) are a phenomenon associated with aging (43). These areas of infarct-like lesions appear as hyperintensities on T2-weighted FLAIR neuroimages, and the

volume of WMLs increase as individuals surpass 60 years of age. One report suggests that over 90% of adults over age 65 have WMLs (44). Clinically, WMLs are associated with Alzheimer's disease (AD), dementia, impaired motor function and reduced cognitive capacity (44, 45). Impaired cerebrovascular infrastructure is implicated in WMLs, as histopathology studies provide evidence of brain blood barrier leakage associated with WMLs in AD and cognitively normal aging (46, 47). White matter lesions are associated with an increased risk for cerebrovascular disease, stroke, and dementia (48). Furthermore, because WMLs are associated with classic cardiovascular risk factors, such as hypertension and elevated plasma cholesterol (49), WML burden is used to identify cerebrovascular pathology in the elderly (50).

There is a body of work suggesting that WMLs are important in discerning pathological brain aging. For instance, having moderate to severe WML burden may be sufficient to characterize an individual as having vascular cognitive impairment (51). This is substantiated by reports of individuals with moderate to severe WML burden being at high risk for developing more lesions and cognitive decline (51, 52). One study suggests WMLs can differentiate between mild cognitive impairment (MCI) subtypes. A report on the Washington Heights Inwood Columbia Aging Program (WHICAP) published in 2009, assessed the relationship between WML and MCI subtypes in 679 adults over the age of 65 (53). The 97 amnestic MCI and 74 non-amnestic MCI adults were compared to 508 cognitively normal older adults on appearance of infarcts and WML volume, as a percentage of total intracranial volume. A T-test on the log-transformed WML values revealed that the amnestic MCI group had a significantly higher total WML intracranial percentage than the normal cognition group. Second, regression analysis revealed that the amnestic MCI group had higher odds of having WMLs (OR = 1.9, 1.1-3.4 95% CI) compared to the normal cognition group. Third, in regards to cognitive performance,

regression analysis showed WMLs to be significantly and negatively related to language ($r = -0.16$, $p < 0.05$), and a negative trend emerged between WMLs and memory ($r = -0.10$, $p = 0.07$). Fourth, adults with non-amnestic MCI were almost twice as likely to have infarcts as adults with amnestic MCI (53). Adults with MCI are considered to have significant impairment that is less than the impairment that is associated with Alzheimer's. Therefore, this data suggests that adults who have preclinical AD have more WMLs than adults who do not have preclinical AD. Furthermore, adults whose main cognitive impairment was amnesia were about twice more likely to have WMLs than adults without impairment and 20% were more likely to have WMLs than adults categorized as non-amnestic. Finally, the data suggests that differentiation between amnestic and non-amnestic MCI can be determined by the infarct or WML appearance. Specifically, infarcts appear to be the distinguishing neuroimaging factor in labeling an individual as having non-amnestic MCI, while WMLs are more likely to identify adults with amnestic MCI. However, this conclusion should be viewed with caution as infarcts and WMLs are not easily differentiated when viewing MRI scans. A separate study looked to parse out WMLs and infarcts, but the data suggests difficulty in separating the two. Authors reported that a third of adults who had the most WMLs also had infarcts, suggesting there was significant overlap in WMLs and infarcts (54). Therefore, WMLs may not be the differentiating factor between amnestic subtypes, which is what the authors of the WHICAP study suggest. Nonetheless, the WHICAP study showed that WMLs can be a distinguishing marker between normal and pathological aging.

Although the WHICAP study suggests using WML burden as a marker for dementia progression, there are important criticisms against using WML burden as a specific marker for cerebrovascular function. The criticisms date back to over 30 years ago to the first study to

establish the link between WMLs and normal aging (43, 55). The first part of the study looked at 240 patients over a 6 month period and looked at the severity of WMLs. These patients spanned a wide age of characteristics, including age (range was 5 to 82 years; median age = 46 years) and clinical presentation, as only patients with head trauma, craniotomy, and demyelinating disease were excluded. The WMLs were graded in increasing severity from 0-3. The percentage of patients with WMLs increased with age, with 51% of patients 41-60 years and 92% of patients over 65 having at least one WML. As age increased over 40 years, WML frequency and severity increased. Interestingly, WML frequency was the same in the 14 elderly patients with dementia and 68 patients without dementia. Multiple relationships between WMLs and vascular health were discovered. In patients over 50 years, a history of brain ischemia was related to increases in WML incidence, specifically an increase in multiple, bilateral confluent WMLs. Independent of age, hypertension was related to increased frequency of WMLs, which were mostly multiple, non-confluent lesions. Age was related to WML incidence and severity in the 33 elderly patients without a prior history of cerebrovascular disease, hypertensions diabetes, coronary artery disease, or dementia. However, when breaking down the elderly cohort into three groups (51-60 years, 61-70 years, and older than 70 years), there were no significant relationships with WML frequency and severity. Regression analysis revealed that age, history of brain ischemia, and hypertension were significant predictors of WML presence and severity. The second part of this study looked at seven post-mortem and one pre-mortem brain in patients who were asymptomatic for neurological disease (55). Subcortical lesions were mostly concentrated around the ventricles, and ventricular WMLs were the most prevalent hyperintensities, showing up in 6 of the 8 brains. The lesions were associated with a variety of histological pathology features. The "mild" pathology included enlarged spaces surrounding the perivascular tissues and ectasia

(distention of the vascular tubular structures of the cerebral arteries and veins); "severe" pathology included myelin degradation and glial death. However, the histopathology and MRI data were not related. The authors concluded that the MRI data could not differentiate between mild, vascular histopathology and severe, non-vascular histopathology (43).

This work was very important to our understanding of neuropathology and aging, but there are some important limitations. First, although there was no difference between WML frequencies in older adults with dementia compared to those without, it is important to note that this cohort included adults over 50 years of age. Hence they compared WML frequency in adults with dementia to adults without, even though more than half of these nondemented adults were below the age of 60. Dementia prevalence increases with age, and dementia prevalence for adults under age 65 is approximately 0.5%-1% (56). Therefore the analysis may have erroneously included adults who were not at high enough age risk for developing dementia. Furthermore, grade 1 and grade 3 lesions appear to increase with age, and grade 2 lesions seem to decrease with age. Thus, the inclusion of the 51-60 age group may have confounded the analysis and prevented any significant relationships from being discovered. Therefore, the authors' conclusion of WMLs not differing between demented and nondemented aging must be accepted with caution. Second, although WMLs were related to cerebrovascular disease, in scans of patients over 40, WMLs were also related to patients with headaches and brain tumors. This suggests that different, unrelated clinical complaints are associated with WMLs, which further suggests that different pathologies can manifest as WMLs. This idea was corroborated by their second part of their study because their data suggests vascular and non-vascular pathology were both related to WMLs, and neuroimaging could not differentiate between the mild lesions versus the severe lesions. Hence, using WMLs are likely one of several measures to index cerebrovascular

function. Therefore, testing other measures such as MCAv mean, CVCI, and CVRC may prove to be valuable cerebrovascular function measures since: (1) the WML hyperintense signal reflects both vascular (mild, perivascular-related changes) and non-vascular (myelin degradation and gliosis) brain tissue changes, and (2) the WML hyperintense signal relates to non-cerebrovascular conditions such as brain tumors and headaches.

1.4.2. Neuroimaging Assessment of Brain Blood Flow

Scanner-based functional neuroimaging with and without gas administration has been an important tool for investigating brain hemodynamics across aging. Hemodynamics can be measured via various imaging techniques, including single photon emission computed tomography (SPECT), PET, and arterial-spin labeling (ASL) MRI. Briefly, SPECT and PET have been used to study brain hemodynamics since the 1980s (57). In the mid-to- late 20th century, gas administration, such as O_2-inhalation, in conjunction with emerging imaging modalities allowed investigators to study brain hemodynamics in humans. This eventually led to the discovery of age-related hemodynamic decreases (58, 59).

There are limitations associated with using neuroimaging and gas administration for hemodynamic assessment. There are two major limitations associated with PET: participants are exposed to radiation, and PET has a temporal resolution on the scale of tens of seconds (60). A limitation of fMRI is that it captures an indirect measure of brain blood flow. Second, the administration of the fMRI exam involves subjecting a participant to a loud and often closed-in space. Finally, poor temporal resolution is a major disadvantage associated with fMRI (61). The limitations of gas administration include participant hesitation to inhale radioactive gas and/or

delivery of the radioisotope via puncturing of neck tissue. Although these methods are widely used and acceptable, there are alternative noninvasive, measures with good temporal resolution that is used for studying brain blood flow: transcranial Doppler ultrasound (62-64). From this point forward, our discussion of cerebrovascular function will focus on transcranial Doppler ultrasound outcomes, particularly MCAv-centered measures.

1.5. Middle Cerebral Artery Velocity and the Cerebrovascular Conductance Index

1.5.1. Transcranial Doppler Ultrasound

Transcranial Doppler (TCD) ultrasound is used to determine brain blood flow *velocity* in the vertebral arteries comprising the Circle of Willis. The artery most studied is the middle cerebral artery (MCA), which can be insonated at depths around 50 mm (64, 65). In research studies, the MCA is the standard vessel of measurement during a TCD exam (2, 12, 66), and it is important because it perfuses 60-70% of the cerebral lateral surface (67). The upper MCA branches perfuse the frontal lobe, and the lower branches perfuse the parietal and temporal lobes. These branches penetrate into the brain, forming the lateral striate and lenticulostriate branches, which perfuse the internal capsule, global pallidus, basal ganglia, and putamen (67-69). The primary outcome of the research TCD exam is the MCAv (64).

The MCA velocity (MCAv)-centered outcomes obtained from TCD are considered valid surrogate measures of brain blood flow (2, 7). Multiple studies suggest the diameter of large cerebral vessels remain constant during changes in blood pressure and end-tidal CO_2 ($ETCO_2$) (62, 70-72). Additionally, there is evidence from MRI/PET/^{133}Xe -TCD comparisons that changes in MCAv correlate to changes in global hemodynamics (72-76). For instance, a ^{133}Xe

study by Bishop showed a significant, moderate relationship, r = 0.424, between MCAv peak and global hemodynamics. Exposure to CO_2 has also been used to show changes in MCAv peak and MCAv mean in multiple reports (77-81). For example, Piepgras and colleagues showed that acetazolamide injection increased the absolute MCAv by approximately 25 cm/s (percent change of 39%) over baseline MCAv (80). This blood flow velocity change attributed to vasoactive stimuli is the cerebrovascular reserve capacity (CVRC) (80, 81).

1.5.2. Flow-pressure Relationships and the Vasomotor Response

Changes in hemodynamics require a coordinated vasomotor response from both the large extracranial arteries, specifically the vertebral and internal carotid arteries, and the pial vessels (1). Two, reciprocal terms have been used to quantify the vasomotor response: conductance describes the relationship of flow over pressure, and has been used interchangeably with its reciprocal, resistance (14). However, these terms should not be used liberally and interchangeably without regard to knowing how vasoactive substances, activation of the baroreflex, or stimulating the sympathetic nervous system affect the flow-pressure relationship, as using one term over the other can lead to erroneous conclusions (14). Furthermore, it has been suggested that conductance is the correct measure to use when there is a need to describe the flow-pressure relationship in regards to changes in vascular tone, changes in perfusion pressure gradient, or changes in mean arterial pressure as a result of the baroreflex or exercise-induced vasoconstriction (13-15).

In terms of cerebrovascular flow-pressure relationships, Claassen makes a sound case for the use of the cerebrovascular conductance index (CVCI) vs the cerebrovascular resistance index

(12). Particularly, he argues using resistance, instead of conductance, would lead to difficulties in data interpretation, especially when considering numbers resulting from increased hemodynamics. For example, vasodilation during low resistance and high brain blood flow would reflect a large increase in CVCI and a small decrease in the cerebrovascular resistance index, so an analysis of this vasomotor response relying on the small decrease in cerebrovascular resistance would be incorrectly interpreted as a small increase in vasodilation. Thus, Claassen argues the use of the CVCI as a marker of the cerebrovascular response to stress because vasomotor testing increases hemodynamics (12), and it may be a useful marker of cerebrovascular aging because older adults have a 30% lower CVCI value compared to younger adults (10).

1.5.3. The Cerebrovascular Response to Physiological Stress

The cerebrovascular response to physiological stress varies by age and is not linear. MCAv reactivity and CVCI reactivity to hypercapnic gas showed 35% lower MCAv and 53% lower CVCI in older adults compared to young adults (10). A recent study looked at healthy young adults and their MCAv and CVCI response to 3% and 6% CO_2 hypercapnic gas challenge. In response to 3% CO_2, there was an absolute percent increase of 10% in MCAv and a 17% absolute percent change decrease in CVCI. In response to 6% CO_2 challenge, there was an absolute percent change increase of 52% and an absolute percent decrease of 25% CVCI compared. Hence the MCAv and CVCI response to CO_2 increase is not linear, although MAP increases to CO_2 increases are linear (11).

1.5.4. The Cerebrovascular Response to Exercise

Challenge to the cerebrovasculature, such as breath holding, can produce a hemodynamic response that is different across the spectrum of brain aging (82). Aerobic exercise provides an alternative stimulus to breath-holding and hypercapnic gas, because exercise provides a challenge to cerebrovascular function with changes in carbon dioxide output and blood pressure (83, 84). One of the advantages of acquiring data with TCD ultrasound versus a MRI imaging is that participants can engage in physical activity real-time during the assessment. Hence, there is a growing body of work looking at exercise-induced changes in hemodynamics measures. The work of Ainslie and colleagues showed that MCAv mean is maintained during hypoxic (80% arterial oxygen saturation) rest and during hypoxic cycling exercise compared to normoxic rest and exercise (66). Further work from this group showed age differences in MCAv mean changes during exercise. In a comparison between 14 older adults and 21 young adults, young adults had almost a 100% greater MCAv mean increase when going from rest to 50% of peak oxygen uptake (VO_{2peak}) (28% younger adults vs 15% older adults) (85). This has been corroborated by other work which has shown that exercise-induced MCAv mean remains lower in older adults compared to younger adults across submaximal and maximal exercise (8, 86, 87).

Our understanding of exercise-induced CVCI is not as well understood. In one study comparing MCAv-centered outcomes between older and younger adults during maximal and submaximal exercise (8), the CVCI had different trajectories between the two groups. In young adults, the CVCI increased at submaximal levels over rest, then went below resting levels at maximal intensity. In older adults, the CVCI did not change significantly from resting levels at submaximal level, but, similar to the younger adults, the CVCI fell below resting level at maximal intensity. Further, the CVCI was significantly lower in all adults at rest and during both

exercise intensities. In contrast, a previous study showed no differences in CVCI at rest and low intensity exercise between age groups, but older adults had lower CVCI at moderate intensity (86). One possible explanation between the two studies may be the age of the older adults. Closer inspection of the TCD indices between the two studies shows a resting MCAv mean percent difference of 48% and a resting CVCI percent difference of 56% values between the two older adult groups. Although there were only 19 older adults in the two studies, such stark differences suggests that in less than 10 years of aging, cerebrovascular function can decline by about 50%.

1.6. Cerebrovascular Function and Cognition

1.6.1. MCAv and Cognitive Domains

The MCAv has been used to study to the link between cerebrovascular function and cognition, particularly in tasks involving planning, language, and visuospatial abilities in healthy individuals (88-91). Strong associations between the MCAv mean and cognition has also been reported across a variety of neurological conditions, such as chronic traumatic brain injury (TBI), stroke, epilepsy, and dementia (92-95). In a study of boxers versus controls, boxers had lower cognitive performance, lower MCAv mean, and diminished CVRC, suggesting that MCAv-centered outcomes may be sensitive enough to capture the cerebrovascular deficits associated with chronic TBI (92). The MCAv mean has been used to assess and predict language lateralization in epilepsy and recovering stroke patients. In the former case, MCAv mean was assessed in people with epilepsy undergoing a word test (96); in the latter, MCAv mean in the hemisphere ipsilesional to the stroke lesion was a predictor of recovery from aphasia(94). In the Rotterdam Study, cognitively normal participants had greater MCAv mean, which was related to less cognitive decline and larger hippocampal volumes than adults with dementia (93).

1.6.2. MCAv and Executive Function

Executive function (EF) is an umbrella term which encompasses various cognitive skills, such as task switching, planning, inhibition, working memory, sustained attention, and selective attention (97). EF deficits can show up early in AD (98), and there is evidence suggesting EF deficits precede memory deficits prior to the diagnosis of AD (preclinical phase) (99, 100). The EF deficits can manifest to impact daily functioning, as a meta-analysis of executive function and AD suggested that the moderate relationship between EF and activities of daily living (ADLs) explains why AD patients with executive dysfunction have trouble with day-to-day living (101). However, lesions in the frontal cortex, particularly in the frontal-striatal tracts underlying EF, develop in conjunction with atrophy of medial temporal areas, which underlie memory (102). Hence, capturing the early decline of executive function is important in identifying the time window of burgeoning memory impairment in AD development.

The interplay between MCAv and EF is not completely understood. There is some research which supports the role of decreased MCAv in the development of executive dysfunction. For instance, in Bailey and colleagues' work comparing active professional boxers to controls, boxers showed impaired cerebrovascular function, in particular lower MCAv reactivity to hypercapnic gas. Moreover, boxers had marked EF performance deficits across three tests. Specifically, the boxers had a 36% slower response time in Stroop performance, a 38% slower completion time in the Trail Making Test A (TMTA), and a 43% slower completion time in the Trail Making Test B (TMTB) (92). However, in Lucas' study, the authors reported that MCAv was significantly inversely related to Stroop performance ($r = -0.69$, $p < 0.001$), suggesting that EF performance, particularly an increase in attentional interference, coincides

with increased brain blood flow (103). *Therefore, two contradictory interpretations can be made: The Bailey interpretation is the greater the MCAv, the better the cognitive outcome, but the Lucas interpretation is the lower the MCAv, the better the EF performance.* Thus, there is a need to better characterize the relationship between cerebrovascular function, particularly MCAv, and EF.

In chapters 2 and 4, we use different iterations of the widely-used TMT to examine the relationships between MCAv-centered indices and EF in nondemented aging (chapter 2) and preclinical AD (chapter 4). The TMTA and TMTB are two iterations of a timed test. The TMTA requires takers to draw lines between consecutively numbered circles. The TMTB requires takers to draw lines between consecutively numbered and lettered circles. Hence, the TMTA tests number recognition, visual scanning, processing speed, and number sequencing. The TMTB, in addition, tests set shifting and cognitive flexibility in handling multiple stimuli simultaneously. The raw TMTA and TMTB scores can be manipulated to derive other scores. One iteration is the delta TMT (TMTD), the time difference between TMTA and TMTB. The TMTD assesses set shifting ability, which decreases as a function of age (104). The TMTD score may provide a more accurate EF score (105) by accounting for the performance differences due to arm speed, simple sequencing, visual scanning, and processing speed (104). Another derived iteration score is the TMT ratio score (TMTR). The TMTR is calculated by dividing the raw TMTB score over the raw TMTA score. This score reflects the cost of set shifting. Specifically, an increase in TMTR is associated with increases in response time to switching between tasks, due to the inability to suppress attention from a previously engaged task (106). Higher TMT z-scores and lower TMTD and TMTR scores are indicative of better EF performance, but there is no consensus as to which TMT iteration provides the best EF measure.

1.6.3. White Matter Lesions and Brain Blood Flow

Tzourio and colleagues reported on the relationships between brain blood flow velocity outcomes and grades of WMLs (107). The Epidemiology of Vascular Aging study was a population-based longitudinal study aimed at studying cognitive decline and vascular aging. Researchers recruited 1,389 adults ages 59-71 years and followed over a period of four years (108). Of these participants, 628 participants over the age of 63 had their resting MCAv mean recorded at rest and underwent an MRI scan in a 1.0 T scanner. They reported that higher quartiles of WMLs had lower MCAv mean (107), thereby suggesting that diminished MCAv is a risk factor for WMLs. Having captured bilateral MCA signals in a large number of older adults is a strength of this study. However, the investigators were unable to obtain bilateral MCA signals in 206 participants. The age and WMLs differences between these participants and the 628 participants should be noted. *Specifically the participants with missing bilateral MCA signals were significantly older and significantly had more severe WMLs. They did not report how many of the participants had useable MCA signal from just one side. Therefore, they missed an opportunity to establish the relationships between unilateral MCAv and WMLs.* A second limitation was that the MCA signal was obtained at supine, which produces a less reliable measurement compared to a seated position (77).

Tzourio's work is contradicted by recent work from Shim and colleagues, which suggested no relationship between TCD measures and WMLs (82). In this 2015 study, 67 AD patients, 75 MCI patients, and 52 controls were recruited to assess how hemodynamics related to cognition. Measurements included the MMSE and CDR; WMLs, graded on severity; and TCD-obtained MCAv mean and breath-holding-induced CVRC. Initially, there were significant

differences between the groups in TCD measures, such that the AD group had lower MCAv mean and lower CVRC than both controls and MCI. However, after adjusting for age, education, and WML severity, there were differences between the groups in MCAv mean. After the adjustment, the AD group had significantly reduced CVRC compared to controls and MCI. Regression analysis showed that WMLs were not significant indicators of MCAv mean or CVRC, contradicting the findings of Tzourio and colleagues. Thus, the relationships between WMLs and MCAv-centered indices are not fully understood.

In chapter 3, we present our work regarding the relationships between cerebrovascular function, WML burden, and cognition. Although WML burden is used as a marker for cerebrovascular aging, not all WMLs are caused by vascular dysfunction. We set out to show that MCAv-centered outcomes and WML burden were related, and then followed up with an analysis to determine whether MCAv-centered outcomes or WML burden better predicted global cognition. Hence, the work presented in chapter 3 provides needed insight as to whether an alternative marker to WML burden can better characterize the relationship between cerebrovascular function and cognition.

1.7. Applications and Significance

This work has important implications to brain aging, and we aimed to expand upon the relationships cerebrovascular function, as measured by MCAv, CVCI, and CVRC, has with cognitive function, brain structural integrity, and preclinical AD neuropathology. Compared to the existing literature focused on neuronal mechanisms and health, less is known regarding exercise, cerebrovascular function, and cognition, specifically executive function, and how they relate to each other in nondemented aging. In chapters 2 and 4, we aimed to untangle these

relationships by studying MCAv mean responses to moderate intensity exercise. The work in chapters 2 and 4 characterizes the relationship between TCD outcomes and EF because EF deficits may precede memory deficits during pathological brain aging (99, 100). We looked at the relationships between exercise-induced cerebrovascular function indices (MCAv, CVCI and CVRC) with cognitive scores, providing us the opportunity to assess whether MCAv, CVCI, and CVRC measures could be used as markers for cognitive aging in nondemented aging (chapter 2) and preclinical AD (chapter 4). In chapter 3, we aimed to characterize the relationship between MCAv mean, CVCI, and CVRC with white matter lesion (WML) burden, a cerebrovascular biomarker which does completely correspond to cerebrovascular function, but has been nonetheless used to characterized cerebrovascular dysfunction across the spectrum of aging. In chapter 4, we discuss new findings pertaining to the role cerebrovascular function has in preclinical AD, which is defined as individuals having elevated levels of the protein, β-amyloid. Hence the work in chapter 4 sheds light on how amyloid load relates to MCAv mean, CVCI, and CVRC. In total, the work presented here lays the foundation to support using a physical challenge such as exercise may have indices as biomarkers across the spectrum of nondemented aging, which could have impact on how we understand stroke, TBI, and dementias.

1.8. Aims

In summary, the literature is lacking important information regarding how to preserve cerebrovascular function throughout the course of aging. Preserving and enhancing brain blood flow may be the key. Yet, before therapeutics can be developed, the field must first develop biomarkers sensitive to changes in cerebrovascular function and cognition. In regards to combatting Alzheimer's, as the evidence against the Amyloid Cascade Hypothesis accumulates,

it is imperative we develop a biomarker that is not totally dependent on amyloid load, yet robust enough to distinguish between nondemented older adults with/without elevated levels of amyloid. The main goal of this project is to better understand whether measures of cerebrovascular function indices at rest and during exercise are related to cognition and brain structural integrity (WML burden) during cognitively normal aging. To accomplish this task we had 3 aims via which we sought to answer our primary hypotheses **(H)**.

Aim 1: To determine whether cerebrovascular health is related to cognition in cognitively normal older adults.

We will study n = 37 older adults without cognitive impairment and who have completed cognitive testing on the Uniform Data Set (UDS). We will study their cerebrovascular function using TCD and beat-to-beat blood pressure. We will obtain two measures of cerebrovascular health, mean middle cerebral artery velocity (MCAv mean) and mean cerebrovascular conductance index (CVCI), which describes the MCAv mean in relation to mean arterial pressure (MAP) (12). We first hypothesized that resting MCAv mean and CVCI would be positively correlated to an age, sex and education adjusted TMTB score (TMTB-Z), our measure of EF **(H1)**. We will calculate percent change in MCAv mean (i.e., the CVRC) and CVCI between rest and moderate intensity exercise for analysis of our secondary hypotheses. We hypothesize that exercise-induced CVRC and CVCI percent change would have positive relationships with TMTB-Z **(H2)** and negative relationships with the secondary EF measures, delta TMT score (TMTD) and TMT ratio score (TMTR) **(H3)**.

Aim 2: To determine whether white matter lesion burden is related to the cerebrovascular function in cognitively normal older adults.

Although WML burden is used as a marker for cerebrovascular aging, not all WMLs are caused by vascular dysfunction. First, we set out to show that resting and exercise-induced cerebrovascular function and WML burden were related because inducing hemodynamic changes may elucidate the relationship between perfusion and WMLs (82). Second, we will conduct a secondary analysis to determine whether cerebrovascular function or WML burden better predicted global cognition (Mini-Mental State Exam z-scores). Finally, we set to determine if resting and the exercise-induced cerebrovascular function can predict WML burden and determine if exercise-induced cerebrovascular functions are better predictors of global cognition than WML burden. Using the same cohort in Aim 1, we hypothesize that WML burden will be negatively related to our resting and exercise-induced measures of cerebrovascular function, MCAv mean, CVRC, and CVCI **(H4)**. Further, we expect both resting and exercise-induced cerebrovascular function measures to predict WML burden **(H5)**. Finally, we expect exercise-induced cerebrovascular function to be better predictors of MMSE z-scores than WML burden **(H6)**.

Aim 3: To determine whether the cerebrovascular function is altered by the presence of β-amyloid in cognitively normal older adults.

For this aim, we will enroll 34 cognitively normal older adults who have undergone Amyvid PET imaging and have been characterized as having elevated (β+, n = 17) or non-elevated (β+-, n = 17) cerebral β-amyloid levels. We will match these adults by age ($\pm$ 3 years),

gender, and cardiac risk disease classification (moderate or high risk). Participants will have cerebrovascular TCD outcomes recorded at rest and during moderate intensity exercise. We will determine if CVCI is altered in older adults with elevated levels of cerebral β-amyloid compared to older adults with non-elevated levels of cerebral β-amyloid. We hypothesize that β+ older adults will show lower MCAv mean and lower CVCI compared to β- older adults **(H7)**. Second, we expected the β+ group to have lower global cognition scores and executive function **(H8)**. Finally, we expected exercise-induced changes in CVRC and CVCI would positively relate to better scores of EF, specifically with higher TMTB z-scores (TMTB-Z), lower TMT delta scores (TMTD), and lower TMT ratio scores (TMTR) **(H9)**. As we progressed through the project for this aim, we decided to examine a larger group (vs matched pairs) and examine CVRC and global amyloid burden.

Chapter 2

The Partial Relationships Between Cerebrovascular Function At Rest and During Exercise In Nondemented Aging

2.1. Abstract

The aging brain is accompanied by decreases in cerebrovascular function and executive function (EF). We lack a thorough understanding between cerebrovascular function and executive function during aging. Using beat-to-beat transcranial Doppler ultrasound, we set out to elucidate the relationships between resting and exercise-induced cerebrovascular function and EF in a cohort of 45 nondemented older adults over 65. We expected our resting and exercise-induced cerebrovascular measures would positively correlate with better EF performance. The cerebrovascular measures were the MCAv mean, cerebrovascular conductance index (CVCI), and cerebrovascular reserve capacity (CVRC). We used the Trail Making Test A and B (TMTA, TMTB) to obtain three EF scores, the TMTB z-score (TMTBZ), the delta score between TMTB and TMTA (TMTD), and the TMTB:TMTA ratio score (TMTR). There were no relationships between the TMTBZ and resting and exercise-induced cerebrovascular function. Spearman correlations showed a direct relationship between resting MCAv mean and TMTR ($r = 0.314$, $p = 0.018$) and an inverse relationship between CVRC and TMTD (spearman $r = -0.293$, $p = 0.025$) but no other cerebrovascular function measures were significantly related to EF. These two findings suggest resting MCAv mean and a greater cerebrovascular reserve correlates to better EF using the TMTR and TMTD, respectively. However, because no relationships were found between resting and exercise-induced cerebrovascular function and the TMTBZ score, we cannot generalize these results as applicable to other nondemented, elderly populations. Further characterization of the relationships between exercise-induced cerebrovascular function and executive function may lead to a cerebrovascular marker sensitive to executive function decline during aging.

2.2. Introduction

One of the age-associated risks with profound implications in both healthy and pathological brain aging is decreased cerebral perfusion, or brain blood flow hemodynamics. The average yearly brain blood flow decrease during normal aging is initially estimated to be 0.05%. More recent studies suggest that the decrease is between 0.78% and 1.2% per year (30, 31). In contrast, there is an estimated 6.2% yearly decrease in global brain blood flow in older adults with cognitive decline and dementia (32). Further, decreased hemodynamics is as an important factor in a multitude of brain diseases such as Parkinson's disease, Huntington's disease, and stroke (25-28). Through multiple neuroimaging modalities, we have gained important insights into how cerebrovascular function is impacted across the continuum of aging. For instance, the use of arterial spin labeling imaging has shown that impairments in regional brain blood flow can be a distinguishing factor between nondemented older adults, those with mild cognitive impairments, and those with Alzheimer's disease (109-111). This discrepancy in brain hemodynamics has given us key insight into the potential contributions of the vasculature to normal and pathological aging (16, 112-115).

Transcranial Doppler (TCD) ultrasound is used to assess brain blood flow velocity at rest, during hypercapnic conditions and during exercise. In a comparison between older adults (n = 14) and young adults (n = 21), young adults had almost a 100% greater MCAv mean increase over older adults when going from rest to 50% of peak oxygen uptake (VO_{2peak}) (85). This has been corroborated by other work which has shown that exercise-induced MCAv mean remains lower in older adults compared to younger adults across submaximal and maximal exercise (8, 86, 87). Hence, this suggests that the cerebral vascular reserve capacity (CVRC), or ability to increase blood flow in response to a stimulus such as exercise (80), declines with aging. Another

MCAv-centered outcome is the cerebrovascular conductance index (CVCI), which describes the brain's flow-pressure relationship and has been used to quantify the cerebrovascular vasomotor response to stress, such as exercise (8, 12, 62, 86). Similar to the MCAv, the resting, hypercapnic-induced, and exercise-induced CVCI is significantly lower in older adults compared to younger adults (10, 86) largely due to the increased in mean arterial blood pressure.

Cerebrovascular function, in general, may underlie executive dysfunction (EF) development during aging (116). The relationship between cerebrovascular function using MCAv through TCD ultrasound and EF decline is not clear. For instance, in Bailey and colleagues' work comparing active professional boxers to active athletes not engaging in sparring activities, boxers showed impaired cerebrovascular function in response to a hypercapnic condition (given a higher CO_2 gas mixture). In addition to impaired cerebrovascular function, those categorized as boxers demonstrated worse performance on EF tests with a 36% slower response time in Stroop performance, a 38% slower completion time in the Trail Making Test A (TMTA), and a 43% slower completion time in the Trail Making Test B (TMTB) (92).

Studies measures such as MCAv and the TMT have provided insight into EF and brain hemodynamics in stroke and hypertension, suggesting the roles of brain injury and blood pressure in the development of executive dysfunction (117, 118). However, to date, we do not have a clear understanding of how hemodynamic characteristics influence EF during normal aging. Elucidating how both resting and exercise-induced MCAv-centered measures relate to EF may provide additional insight regarding cerebrovascular function in individuals at risk for pathological brain aging. The purpose of this study was to investigate the relationship between resting and exercise-induced MCAv-centered outcome measures and EF in healthy, non-demented older adults. The MCAv-centered outcome measures were MCAv mean,

cerebrovascular conductance index (CVCI; MCAv mean/MAP), and cerebrovascular reserve capacity (CVRC) which was the percent change in in MCAv mean from rest to exercise. We collected TMTA and TMTB scores, and calculated multiple TMT score iterations to get indices of EF performance. Our primary EF measure was an age, sex, and education adjusted TMTB z-score (TMTBZ) (119). Secondary EF measures were the delta TMT score (TMTD, calculated as TMTB-TMTA) (105) and TMT ratio score (TMTR, calculated as TMTB/TMTA) (106). We hypothesized that resting MCAv mean and CVCI would positively correlate to TMTBZ. We calculated percent change in MCAv mean (the CVRC) and CVCI between rest and moderate intensity exercise. We hypothesized that greater exercise-induced CVRC and CVCI percent change would relate to better performance on the EF scores, specifically higher TMTBZ scores and lower TMTD and TMTR scores.

2.3. Methods

2.3.1. Participants

Older adults were recruited from the KU Alzheimer's Disease Center's Alzheimer's Prevention Program from August 2014 to September 2016. Inclusion criteria were age over 65 years, classification as cognitively normal/nondemented with a Clinical Rating Score = 0, sedentary or underactive lifestyle, and completion of Amyvid PET and MRI scans. Exclusion criteria were DSM-IV defined drug or alcohol abuse within the prior 2 years, clinically significant depression or anxiety, insulin-dependent diabetes, myocardial infarction or symptoms of coronary artery disease within the prior two years, acute decompensated congestive heart failure or class IV heart failure, major orthopedic disability, inability to exercise, inability to travel to the KUMC Research in Exercise and Cardiovascular Health (REACH) laboratory, and

inability for researchers to obtain the MCA signal from the transcranial window. Individuals who met the inclusion criteria provided institutionally approved written informed consent.

2.3.2. Cerebrovascular Function Assessment

We used an experimental protocol similar to previously published work (86). This protocol captures MCAv mean and mean arterial pressure (MAP) for every cardiac cycle over an eight-minute rest period and during eight minutes of steady state moderate intensity exercise. A ROBOTC2MD robotic TCD headset (Multigon, White Plains, NY) was placed on the head and the ultrasound probes (2Hz) were used to capture the MCA signal; a Finometer Pro finger plethysmography system (Finapres Medical Systems, Amsterdam, Netherlands) was placed on the left brachial arm and left middle finger, to record beat-to-beat MAP; a Capnocheck 9400 capnograph (Smiths Medical, St. Paul, MN) and cannula was used to measure breath-by-breath end-tidal CO_2 ($ETCO_2$); and a 5-lead electrocardiogram (EKG) (CardioCard, Nasiff Associates, Central Square, NY) was placed according to appropriate anatomical marks to collect heart rate.

2.3.2.1 Set Up. **Figure 1** shows the experimental set up. Once EKG leads were placed, participants were instructed to sit upright on the NuStep T5XR recumbent stepper (NuStep, Inc., Ann Arbor, MI), and extend out both their arms to the side onto tables. The TCD headset, Finapres left arm and left finger cuff, capnograph nasal cannula, and Tango M2 right arm brachial cuff (SunTech Medical, Inc., Morissville, NC) were placed on the participant. Before onset of recording, the Finapres values were compared to the Tango automated blood pressure cuff to ensure accurate values with long-term recording (120). If the difference between Finapres

and Tango values were greater than 15 mm Hg, the Finapres finger cuff was removed then repositioned, until the difference was below the recommended 15 mm Hg (121).

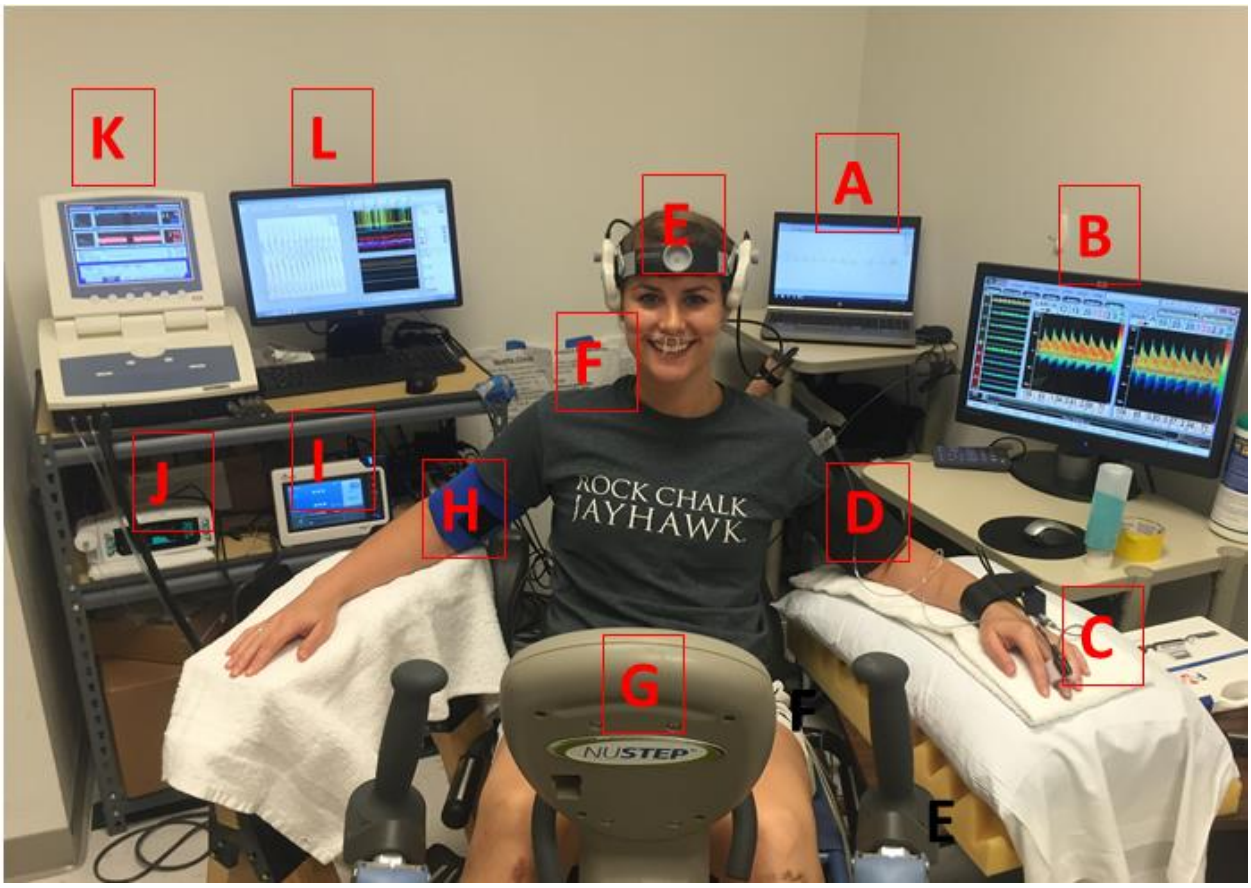


Figure 1. Experimental Set Up. Beat-to-beat assesment depends on the synchronization of mutliple pieces of equipment. A= Cardiocard EKG software; B = TCD output; C = Finapres finger cuff; D = Finapres brachial cuff; E = Multigon TCD headset; F = nasal cannula; G = NuStep T5XR recumbent stepper; H = Tango M2 brachial cuff; I = Tango M2 module; J = Capnograph; K = Finapres module; L = Matlab collection program.

2.3.2.2. Resting and Exercise Recordings. After completion of set up, participants were instructed to remain seated, breathe through the nose, fix their gaze forward, and refrain from talking and movement. Then eight minutes of data were recorded. After rest, participants completed one bout of moderate intensity exercise. Moderate intensity exercise was defined as

an exercise target zone between 40%-60% heart rate (HR) reserve (122). Age predicted HR maximum (220-age) was used in the calculation.

THR = [(age-predicted maximum HR− resting HR) × % Exercise Intensity)] + resting HR

Participants were instructed to use only their legs during the exercise session. As participants pedaled at a cadence of 90-100 steps per minute, the resistance was increased until the THR was reached and maintained for one minute then recording commenced. Beat-to-beat values were recorded for eight minutes during exercise. Upon completion of eight minutes of exercise, participants cooled down for one minute at 15 watts.

2.3.2.3 Data Collection and Computations. Analog signals were fed through an analog-to-digital signal converter (NI USB-6212, National Instruments Corporation, Austin TX), collected and displayed in real time with a custom MATLAB program (The MathWorks, Inc., Natick, MA). We collected an R-wave trigger signal, MCAv and MAP at 500 Hz **(Figure 2).** Beat-to-beat Finapres and TCD values were calculated via integration of each cardiac cycle and then dividing the integral by length of the cardiac cycle. A custom MATLAB post-processing program computed our primary cerebrovascular outcome variables. Percent changes in the primary cerebrovascular measures were calculated. Percent change was calculated using the formula:

Percent Change X = (|(X at moderate intensity exercise – X at rest)/X at rest| x 100)

where X denotes a primary cerebrovascular measure. Percent change was calculated for $ETCO_2$, MAP, MCAv mean (i.e., CVRC), and CVCI.

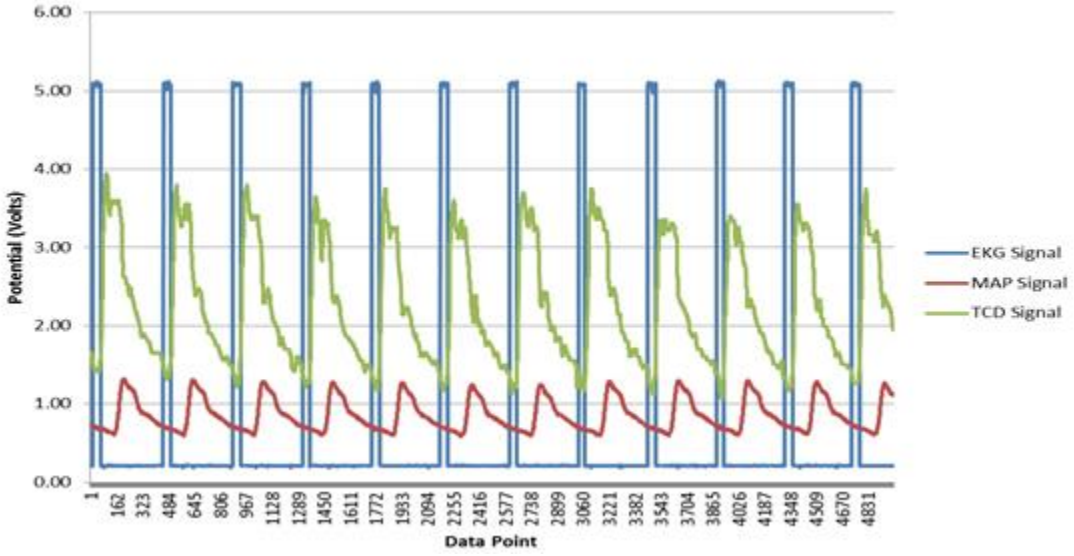


Figure 2. Example of Data Acquisition Using Custom MATLAB Data Collection Program. Finapres (red line) and TCD (green line) signals were aligned to onset of a trigger signal based on detected R-waves (blue line) for each beat over eight minutes of recording. A processing script converted the analog voltage (Y-axis) into appropriate physiological units for mean arterial pressure and mean MCA velocity.

2.3.3 Executive Function

As part of the Alzheimer's Prevention Program, our study participants underwent cognitive testing by a trained psychometrician and completed the Uniform Data Set (UDS) version 2.0 (123). The standard battery consists of multiple cognitive tests, including Mini-Mental State Exam, Immediate and Delayed Logical Memory, Digit Span Forward/Backward, Animal and Vegetables Category Fluency, WAIS- Digit Symbol, Boston Naming, and the Trail Making Test A/B (TMTA, TMTB).

2.3.3.1 Trail Making Tests. The TMTA and TMTB are timed tests and assess different aspects of executive function. The TMTA requires takers to draw lines between consecutively numbered circles; in the TMTB, takers draw lines between consecutively numbered and lettered circles. Hence, the TMTA tests number recognition, visual scanning, psychomotor speed, and number sequencing. The TMTB, in addition, tests set shifting and cognitive flexibility in handling multiple stimuli simultaneously. Delta TMT (TMTD) is the time difference between TMTA and TMTB, and it reflects set-shifting ability, which decreases as a function of age (104). Raw TMTB test scores for each participant were normalized into our primary cognitive measure, TMTB Z-scores (TMTBZ), adjusted for age, sex, and education according to previously published methods (119).

In addition to the TMTBZ, we calculated secondary TMT measures using both the TMTA and TMTB raw scores. We calculated the TMT delta score (TMTD) by subtracting the raw TMTA score from the raw TMTB score. The TMTD score may provide a more accurate measure of executive function (105) by accounting for the performance differences due to arm speed, simple sequencing, visual scanning, and psychomotor speed (104). Finally, the TMT ratio score (TMTR), was calculated by dividing the raw TMTB score over the raw TMTA score. This score reflects the cost of set-shifting. Specifically, an increase in TMTR is associated with increases in response time to switching between tasks, due to the inability to suppress attention from a previously engaged task (106). For both TMTD and TMTR, lower scores signal better EF performance.

2.3.4 Data Analysis

Analyses were completed using SPSS version 23 (SPSS, version 23, IBM Corporation, Armonk, NY). For our first step in testing our hypotheses, we assessed homogeneity of variance and normality using Levene's test for equality of variance and the Shapiro-Wilk test, respectively. One-tailed Pearson correlation was used to assess the relationship between two normally distributed variables. One-tailed, non-parametric Spearman correlation (124) was used for data that violated normality or homogeneity of variance. We conducted paired samples T-tests to assess differences between rest and moderate intensity exercise. When assumptions of normality and homogeneity variance were violated, we used non-parametric related samples Wilcoxon signed rank tests. For all analyses, $\alpha < 0.05$ was used as the criterion for statistical significance.

2.4. Results

We recruited 64 older adults from the Alzheimer's Prevention Program into our study. We successfully insonated the MCA and obtained the MCAv signal in 51 (79.7%) participants. We were unable to use data from an additional 6 participants due to unattainable Finpares and capnograph signal. Specifically, one participant did not have a useable Finapres signal both at rest and during exercise; one participant did not have a useable capnograph signal at rest and during exercise; two participants did not have a useable capnograph signal during exercise; and two participants only completed rest Twenty-one participants were on blood pressure medications, and fourteen participants had resting blood pressures above the normotensive range (140/90 mm Hg) (125). **Table 1** depicts the descriptive statistics pertaining to participant characteristics, cerebrovascular function, and executive function scores. The mean raw TMTA

and TMTB scores used for calculation of our EF scores were, respectively, 29.1 ± 10.7 s and 73.8 ± 31.5 s.

Measure	Mean ± SD	Range [minimum, maximum]
Age, years	71.3 ± 5.1	21 [65,86]
BMI, kg/m^2	26.5 ± 4.5	22.9 [18.6, 42.7]
Education, years	16.9 ± 2.8	13 [12,25]
TMTBZ	0.081 ± 0.77	4.3 [-2.64, 1.61]
TMTD, s	44.8 ± 27.0	135 [13, 148]
TMTR	2.63 ± 0.95	5.32 [1.48, 6.80]
Resting MCAv mean, $cm*s^{-1}$	48.4 ± 9.2	39.7 [25.7, 65.4]
Resting CVCI, $cm*s^{-1}$ *mm Hg^{-1}	0.668 ± 0.18	0.76 [0.365, 1.13]

Table 1. Descriptive Statistics of Participant Characteristics, Cerebrovascular Function, and Executive Function Scores. BMI= body mass index; TMTBZ = Trail Making Test B z-score; TMTD = Trail Making Test Delta score; and TMTR = Trail Making Test Ratio score; MCAv = middle cerebral artery velocity; CVCI = cerebrovascular conductance index.

Shapiro-Wilk tests were used to determine normality. Resting $EtCO_2$ ($p = 0.801$), resting MCAv mean ($p = 0.544$), and resting CVCI ($p = 0.179$) were normally distributed, but not resting MAP ($p = 0.004$). With exercise, $ETCO_2$ ($p = 0.014$) and MAP ($p < 0.001$) were not normally distributed but MCAv mean ($p = 0.632$) and CVCI ($p = 0.184$) were normally distributed. Percent change values for MAP ($p = 0.390$) were normally distributed, but not the percent change values for $ETCO_2$ ($p < 0.001$) and CVCI ($p = 0.039$). The CVRC was not normally distributed ($p = 0.001$). The primary EF measure, TMTBZ, was not normally distributed ($p = 0.003$). Neither of the secondary EF measures, TMTD and TMTR, were normally distributed (both p's < 0.05).

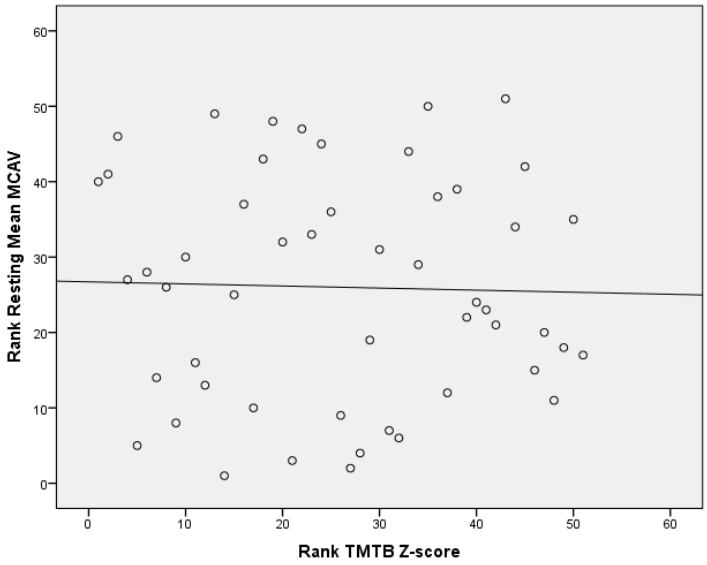
2.4.1. Resting Cerebrovascular Function & Executive Function

We report no relationships between the TMTBZ and resting cerebrovascular function, but we found one relationship between resting cerebrovascular function and a secondary EF measure, the TMTR. **Table 2** shows the relationships between resting and exercise-induced cerebrovascular function and executive function scores. The relationships between resting cerebrovascular function and our primary EF scores is shown in **Figure 3**. **Figure 3A** shows the rank MCAv mean and rank TMTBZ. Spearman correlation analysis did not reveal a significant positive correlation between MCAv mean and TMTBZ (spearman r = -0.070, p = 0.325). **Figure 3B** shows the relationship between rank CVCI and rank TMTBZ. Spearman analysis revealed a small positive, but non-significant relationship between CVCI and TMTBZ (spearman r = 0.043, p = 0.389). No significant relationships were found between cerebrovascular function and secondary EF measures, except for TMTR. **Figure 4** shows the significant and positive relationship between rank MCAv mean and rank TMTR (spearman r = 0.314, p = 0.018).

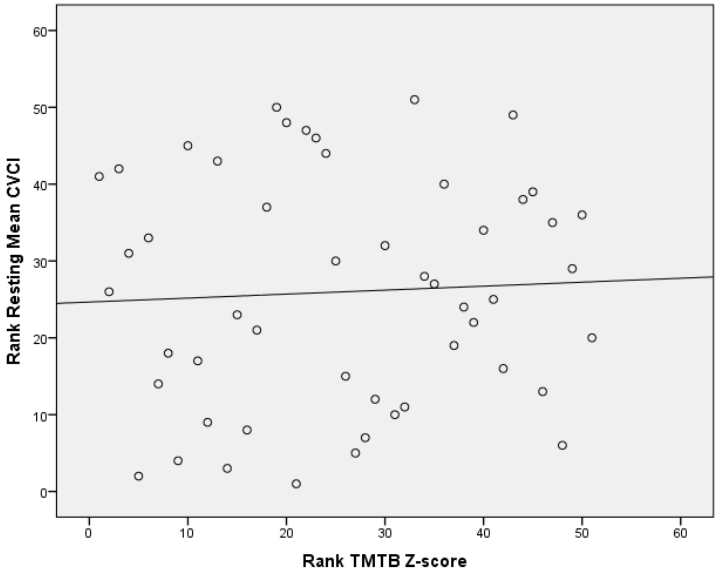
		Executive Function Measure	
Cerebrovascular Function Outcome	**TMTBZ**	**TMTD**	**TMTR**
Resting MCAv mean	r = -0.070, p = 0.325	r = 0.147, p = 0.167	**r = 0.314, p = 0.018**
Resting CVCI	r = 0.043, p = 0.389	r = -0.008, p = 0.479	r = r = 0.182. p = 0.116
CVRC	r = 0.162, p = 0.144	**r = -0.293, p = 0.025**	r = -0.177, p = 0.122
Percent Change CVCI	r = -0.120, p = 0.216	r = 0.163, p = 0.142	r = 0.073, p = 0.317

Table 2. Relationships between Resting and Exercise-induced Cerebrovascular Function and Executive Function Scores. Spearman correlations between cerebrovascular function and executive function. MCAv = middle cerebral artery velocity; CVCI = cerebrovascular conductance index; CVRC = cerebrovascular conductance index; TMTBZ = Trail Making Test B z-score; TMTD = Trail Making Test Delta score; and TMTR = Trail Making Test Ratio score.

A)



B)



<u>Figure 3. Relationship between Resting Cerebrovascular Function and Trail Making Test B Z-scores.</u> A) This plot depicts the relationship between TMTBZ ranks against the resting MCAv mean ranks (spearman r = -0.070, p = 0.325). B) This plot depicts the relationship between the TMTBZ ranks against the resting CVCI ranks (spearman r = 0.043, p = 0.389).

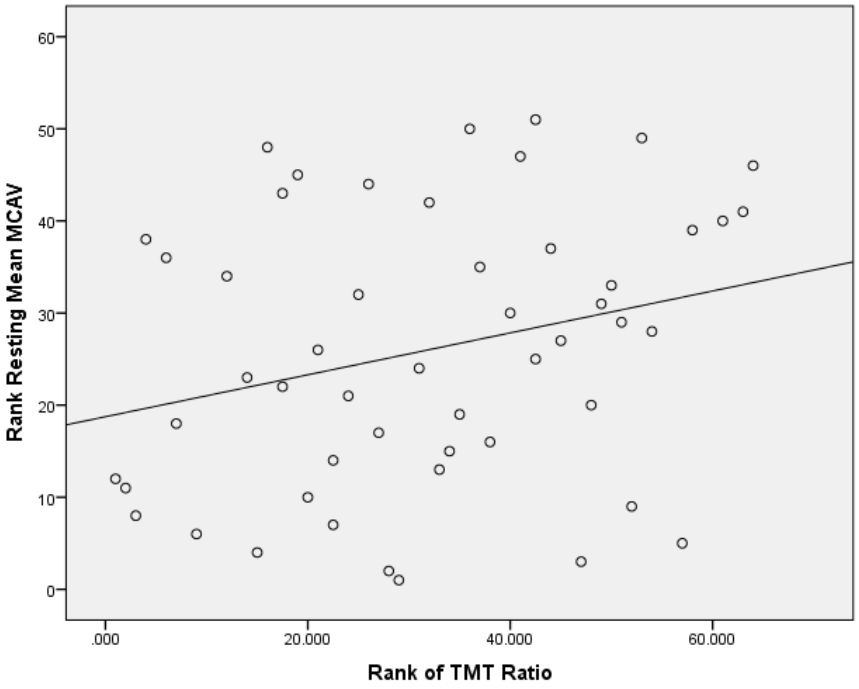


Figure 4. Relationship between Resting Cerebrovascular Function and Trail Making Test Ratio Scores. This scatterplot shows the significant and positive relationship between resting rank MCAv mean and rank TMTR (spearman r = 0.314, p = 0.018).

2.4.2. Exercise-induced Cerebrovascular Function and Executive Function

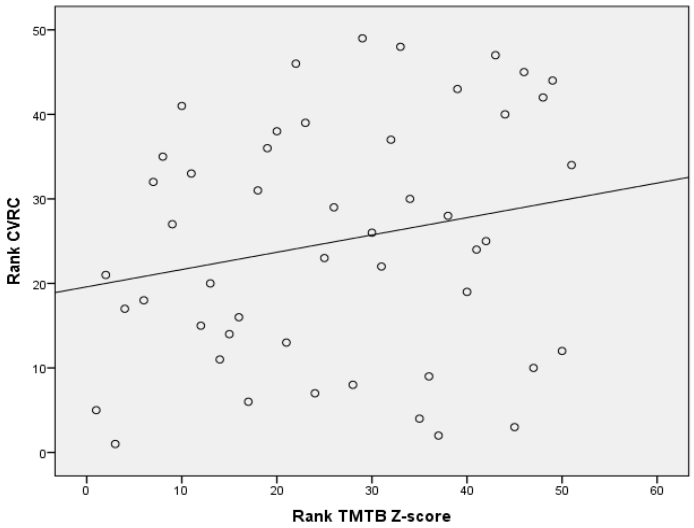
We report no relationships between the TMTBZ and exercise-induced cerebrovascular function, but we found one relationship between exercise-induced cerebrovascular function and a secondary EF measure, the TMTD. **Table 3** compares the cerebrovascular function measures obtained at rest and during moderate intensity exercise.

Measure	Resting (± SD)	Exercise (± SD)	Percent Change (± SD)
$ETCO_2$, mm Hg	33.3 ± 5.4	37.4 ± 5.3	16.9 ± 12.3
MAP, mm Hg	74.2 ± 12.3	104.7 ± 22.6	41.8 ± 22.6
MCAv mean, $cm*s^{-1}$	48.4 ± 9.2	54.7 ± 13.2	17.2 ± 10.6
CVCI, $cm*s^{-1}$ *mm Hg^{-1}	0.668 ± 0.18	0.527 ± 0.15	-21.4 ± 14.8

Table 3. Primary Measures at Rest and during Moderate Intensity Exercise. $ETCO_2$ = End-tidal CO_2; MAP = mean arterial pressure; MCAv = middle cerebral artery; CVCI = cerebrovascular conductance index.

Figure 5 shows the relationships between the exercise-induced cerebrovascular outcomes and the primary EF score. **Figure 5A** shows the relationship between the rank CVRC (percent change in MCAv mean) and rank TMTBZ, which was positive, but non-significant (spearman r = 0.162, p = 0.144). **Figure 5B** shows the relationship between rank percent change CVCI and rank TMTBZ (spearman r = -0.120, p = 0.216).

A)



B)

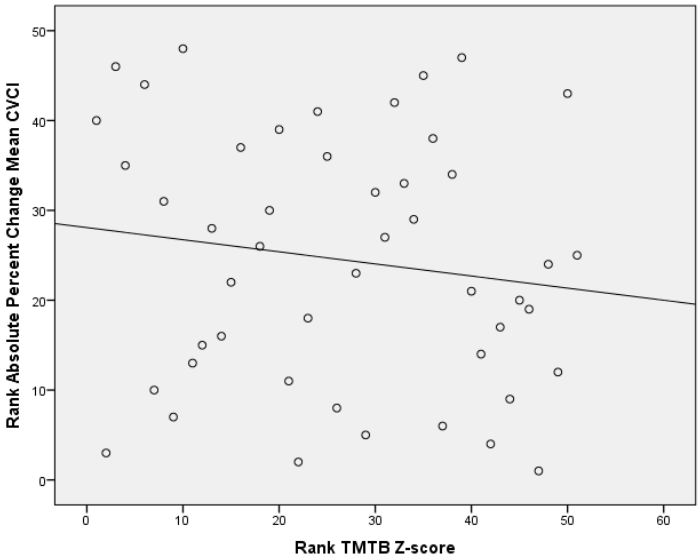


Figure 5. Relationship between Cerebrovascular Function during Exercise and Trail Making Test B Z-scores. A) Scatterplot of the TMTBZ ranks against the CVRC ranks (spearman r = 0.043, p =0.389). B) Scatterplot showing the relationship between TMTBZ ranks and percent change CVCI ranks (spearman r = -0.120, p = 0.216).

Spearman analysis of secondary EF scores did not show any significant relationships with exercise-induced cerebrovascular measures, except between CVRC and TMTD. **Figure 6** shows a significant and negative relationship between rank CVRC and rank TMTD (spearman r = -0.293, p =0.025).

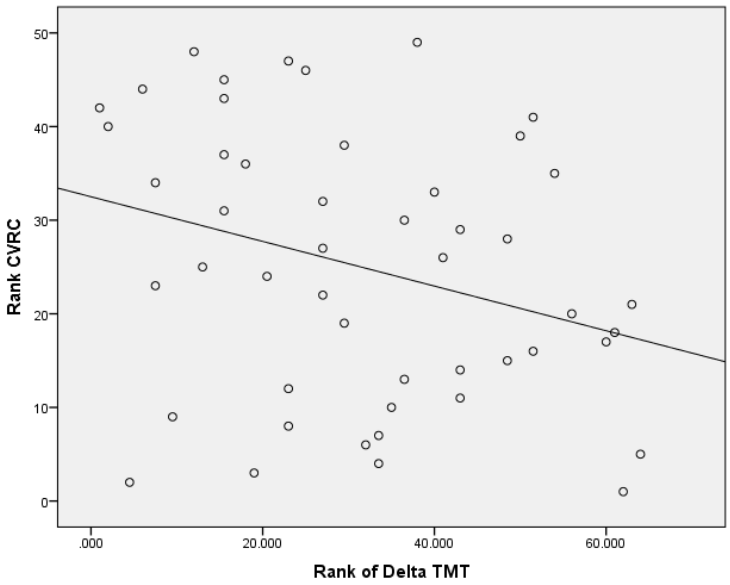


Figure 6. Relationship between Cerebrovascular Reserve Capacity and Trail Making Test Delta Scores. Scatterplot showing the relationship between TMTD ranks and CVRC ranks (spearman r = -0.293, p =0.025).

2.5. Discussion

Decreases in brain blood flow and cognitive performance are features of the aging brain. Executive dysfunction may be prevented by addressing vascular risk factors (126), so it is plausible that identifying a marker at the interface of vascular function and brain function may prove useful in combatting age-related executive function (EF) decline. Since little is known concerning the relationships between cerebrovascular function and EF, we conducted an investigation to elucidate how cerebrovascular function relates to EF in nondemented older adults. Our overall aim was to determine if measures of resting cerebrovascular health and measures of exercise-induced cerebrovascular reserve could be used as markers of EF during nondemented aging. By studying MCAV-centered outcomes, we specifically tested whether resting cerebrovascular function and exercise-induced cerebrovascular function linked to EF

performance using three iterations of the widely-used Trail Making Test (part B z-score, TMTBZ; delta score, TMTD, and TMTA:TMTB ratio score, TMTR).

Our data provides partial support for our hypotheses. Particularly, we have partial evidence that the Trail Making Test is related to resting and exercise-induced MCAv function, but is not related to resting or exercise-induced cerebrovascular conductance (CVCI). *Further, our results suggest that MCAv has different relationships with EF performance depending whether the cerebrovasculature is at rest or is being challenged with exercise: higher resting MCAv relates to poorer EF performance, but higher exercise-induced CVRC relates to better EF performance.* Higher MCAv mean is seen in hypertension (127), and approximately half of our cohort was on blood pressure medications, and approximately 1/3 exhibited resting blood pressures above the normotensive range (125). Our results support one study which originally showed a relationship between hypertension and poor EF and also showed a link between greater cerebrovascular reserve and better TMT performance (128). Hence, our results may reflect the relationship between EF and hypertension's mechanical effects on arterial function and/or endothleial function (114, 128). MCAv-centered measures are widely studied and have been used to assess EF performance, and we are the first to report a significant relationship between higher exercise-induced CVRC and better EF performance in a cohort of nondemented older adults using iterations of the TMT. However, the absence of significant relationships between all TMT iteration scores and resting MCAv and exercise-induced CVRC precludes the assertion of definitive, generalizable relationships between resting cerebrovascular function and exercised-induced cerebrovascular reserve with EF.

2.5.1. Resting Cerebrovascular Function & Executive Function

Contrary to our hypotheses, we report non-significant correlations between resting MCAv mean and resting CVCI with our primary EF measure, TMTBZ. Further analyses looking at resting cerebrovascular function and secondary EF measures showed one significant relationship, a positive correlation between resting MCAv and TMTR. This relationship was not expected and does not support our hypothesis. This finding suggests higher resting brain blood flow velocity relates to poorer EF performance. Therefore, our results contradict three studies which link diminished cerebrovascular function to poorer EF performance, particularly on the TMTB (116, 129, 130). The first study looked at the relationships between arterial spin labeling perfusion imaging and cognition in 52 nondemented older adults. The TMT scores were adjusted for age and sex, and linear regression showed that global hypoperfusion and frontal cortex hypoperfusion related to poorer TMTB performance (116). The second study assessed perfusion via single photon emission computed tomography (SPECT) in older adults with mild cognitive impairment and adults with Alzheimer's disease (130). These older adults were divided into a "poor" or "good" TMTB group based on raw TMTB scores. The poor TMTB group had hypoperfusion in the anterior cingulate cortex, caudate nucleus, caudate, and putamen, suggesting that regional cerebrovascular function underlies set-shifting performance deficits (130). The third study comes from recent work from the MOBILIZE Boston Study, which compared older adults with cognitive impairment to older adults without cognitive impairment (129). Older adults with cognitive impairment had significantly worse performance on the TMTB coupled with significantly lower MCAv mean and higher cerebrovascular resistance index (the mathematical reciprocal of the CVCI). Although the authors did not report TMTR or TMTD, calculation of both shows the cognitively impaired group had a TMTR of 2.35 and TMTD of 91.7s, compared to the cognitively normal group's TMTR of 2.04 and TMTD of 61.8

s. Hence, in MOBILIZE Boston, the group with the lower brain blood flow velocity and lower cerebrovascular conductance had higher TMTR and lower TMTD scores, signaling worse EF performance and diminished cerebrovascular function. It is plausible that we did not see relationships between resting cerebrovascular function and all TMT iterations because lower MCAv mean is linked to higher white matter lesion burden (107), yet, our cohort had lower than average white matter lesion burden (data presented in Chapter 3) (131, 132). *Thus, our cohort may not have been as impacted by the effects of age-related vascular risk factors, such as white matter lesions, which are known to deleteriously impact cerebrovascular function during aging (113)*.

There are differences in TMT performance and resting cerebrovascular function between the cognitively healthy older adults in the MOBILIZE Boston Study and this current study. Specifically, we report a MCAv mean of 48 cm/s, whereas the MCAv mean reported in MOBILIZE Boston was 41 cm/s. This 7 cm/s difference between the two studies is almost double the MCAv mean difference between MOBILIZE Boston's cognitively normal and cognitively impaired groups (difference = 3.6 cm/s). Therefore, our cohort of nondemented older adults may have better cerebrovascular function compared to the MOBILIZE Boston Study cognitively normal cohort. Pertaining to the EF performances, we report that the nondemented older adults had a TMTR of 2.63 and a TMTD of 44.8 s, juxtaposed to MOBILIZE Boston's TMTR of 2.04 and TMTD of 61.8 s. Thus, at first glance, these numbers lead to a *paradoxical conclusion*: our cohort had better EF performance than the MOBILIZE Boston cognitively normal group if considering only the TMTD scores, but worse EF performance if considering only the TMTR scores. Resolution of this discrepancy relies on considering the raw TMTA and TMTB scores from both studies. We report a mean raw TMTA of 29.1 $\pm$ 10.7 s and a raw TMTB

of 73.8 ± 31.5 s, juxtaposed to MOBILIZE Boston's raw TMTA score of 59.3 ± 32.2 s and raw TMTB score of 121.1 ± 79 s. Hence, our nondemented cohort has more than a 100% performance TMTA increase and almost a 40% TMTB performance increase over the MOBILIZE cognitively normal cohort. On average, our cohort's age was seven years younger than the MOBILIZE Boston Study cohort, so to account for age, as well as gender and education, a crude calculation of the MOBILIZE Boston data yields a TMTBZ of -0.0567, which is lower than our reported TMTBZ of 0.081. Hence, via both closer inspection of the raw TMT scores and the use of a normative calculator, our nondemented older cohort had faster (better) performance on the TMT. Compared to normative TMT performance in the 70-74 age range (133), our TMTD score is roughly equivalent, and our TMTR score is slightly lower (better performance). However, our resting MCAv mean, 48.4 cm/s, is higher compared to previously reported resting MCAv means in nondemented aging, which has been reported in the mid-30s to mid-40s cm/s (134, 135). *Thus, it is plausible that we did not find statistically significant relationships between resting cerebrovascular functions and all TMT scores due to greater brain blood flow velocity and better executive function observed in our cohort compared to previous reports.* Conversely, one methodological difference may explain the difference between our resting MCAv mean average and previously reported MCAv mean averages. Our protocol captures beat-to-beat MCAv mean over an eight minute period, whereas previous work reported beat-to-beat MCAv mean averages in the captured data over one to two minutes (85, 136). Hence, we recorded resting cerebrovascular function over a longer duration than previous work, therefore, we are confident our protocol adequately captures the resting cerebrovascular state.

2.5.2. Exercise-induced Cerebrovascular Function & Executive Function

The current data corroborates previous work in older adults in which moderate intensity exercise elicited changes in cerebrovascular function, particularly an increase in MCAv (84, 85) and a decrease in CVCI, compared to rest (84, 86). Previous work showed exercised-induced CVRC of 16% -21% in older adults (84, 85). A study by Fisher and colleagues showed an absolute CVCI percent decrease of 16.4% in older adults completing moderate exercise on a cycle ergometer accompanied by a 1 cm/s MCAv mean increase over rest (86). This drive in CVCI percent change was due to the 24% percent increase in MAP over rest, suggesting that the body maintains brain blood flow velocity in response to physiological stress by increasing arterial pressure (86). Therefore, our data suggests that the 42% increase in MAP is in response to the 17% increase in MCAv mean, resulting in a 21% exercise-induced decrease in CVCI. An exercise-induced decrease in CVCI indicates the possible vasoconstriction of cerebral vessels, a normal autoregulatory response needed to counteract steep exercise-induced increases in MAP (86). Previous exercise-induced increases in MCAv mean have been reported, and these increases range from 1.5% -20.5%, with accompanying MAP increases between 3%-24% over rest in nondemented older adults (8, 85, 86). There are important methodological differences between this study and previous studies. First, the present study has 3 to 4.5 times the number of older adults than the previous studies (8, 85, 86). Second, compared to the older adults in one study (86), our older adults were older by an average of 14 years. Therefore, our work focuses on the exercise-induced cerebrovascular response in sexa-, septa-, and octogenarians, while the previous work assessed adults in late midlife to early retirement age. Finally, one study only assessed older males (85), therefore, limiting the generalizability of the cerebrovascular changes

observed in that cohort. Nevertheless, these studies provide a valuable basis on which one can design investigations into exercise-induced cerebrovascular functions across the aging spectrum.

Contrary to our hypotheses, we did not find significant and positive relationships between CVRC and TMTBZ or between CVCI percent change and TMTBZ. However, our secondary hypothesis found a significant, negative relationship between the CVRC and TMTD, corroborating previous work showing cerebrovascular capacity inversely correlates to TMTD performance (128). It has been postulated the TMTD may be a better indicator of EF than the raw TMTB score because it subtracts the psychomotor and visual component of the TMTA score from the TMTB score (137). However, the TMTD is based off the raw TMTA and TMTB scores. Therefore, the TMTD score does not take age, sex, or education into consideration, which are important demographic factors which influence cognitive reserve and preservation of cognitive faculties during aging (138-140). Hence, our findings suggest greater percent changes in perfusion velocity are related to better raw EF scores, but these scores did not account for the influence of important cognitive reserve variables. Accordingly, we cannot state a generalization that cerebrovascular reserve is related to better EF performance during nondemented aging.

2.5.3. Methodological Considerations of Assessing Executive Function

There is an inherent challenge to studying EF due to its component cognitive facets, such as working memory, attention, and set-shifting: these components have different operational definitions which reflect many task-based tests (141). Unsurprisingly, there is no definitive EF test. *We chose the TMT as our EF measure because it is widely used, easily administered, and, in a comparison of EF tests administered during a functional TCD examination, it elicited the*

greatest bilateral MCA response (142, 143). However, there is no consensus on which version of the TMT is best used for EF assessment, and previous studies have reported using multiple iterations of the TMT (143). We chose the TMTBZ as our primary EF measure because it will be the primary EF measure used by the National Alzheimer's Coordinating Center (NACC), which coordinates research projects among the nation's 29 NIH-designated Alzheimer's Disease Centers. We recruited all of our older adults from one of these select national centers. The normative calculator which we used to obtain the age, sex, and education-adjusted TMTBZ was created to establish reliable cognitive normative data sensitive to demographic characteristics among the Alzheimer's Disease Centers' research participants. Hence, unlike the raw TMT scores and the iterations derived from the raw TMT scores, the *strength of TMTBZ is that it provides a generalizable measure of EF (119).* Further, the normative calculator is designed for the Uniform Data Set (UDS), the cognitive battery used by the NACC. There are tests within the UDS which probe other aspects of EF, aside from set-shifting, and future investigations could capitalize on these tests to further our understanding of EF. For instance calculating z-scores for Digit Forward, Digit Forward Length, Digit Backward, and Digit Backward Length tests would provide the factor score for attention (119).

2.6. Conclusion

We investigated a well characterized cohort of nondemented older adults to determine the relationships between cerebrovascular function and executive function. Our results suggest that MCA velocity has different relationships with executive function performance depending whether the cerebrovasculature is at rest or is being challenged. First, higher resting MCAv mean relates to poorer executive function performance, but closer inspection of the resting

cerebrovascular function and raw executive function scores suggest greater brain blood flow velocity and better executive function in our cohort compared to previously studied nondemented cohorts. Second, higher exercise-induced CVRC relates to better executive function performance, suggesting that greater cerebrovascular reserve correlates to better executive function. However, these relationships between MCAv-centered measures and executive function were found using raw Trail Making Test scores, but no relationships were found between resting and exercise-induced cerebrovascular function and the Trail Making Test score normalized for age, sex, and education level. This prevents generalizing the results to other nondemented, elderly populations. Further characterization of the relationships between exercise-induced cerebrovascular function and executive function may lead to a vascular marker sensitive to age-related executive decline.

Chapter 3

Assessing White Matter Lesion Burden Relationships With Cerebrovascular Function In Nondemented Aging

3.1. Abstract

White matter lesion (WML) burden is a marker of brain health and cognitive aging. Diminished cerebrovascular function has been implicated in the development of WML burden, but the evidence relating resting and dynamic cerebrovascular function to WML burden is inconclusive. Hemodynamic measures of cerebrovascular function, specifically middle cerebral artery velocity (MCAv) - centered measures, may be useful in elucidating the relationships cerebrovascular function and WML burden. Therefore, we set out to elucidate the relationships between resting and exercise-induced cerebrovascular function and WML burden in 43 nondemented adults over age 65 using beat-to-beat transcranial Doppler (TCD) ultrasound at rest and during moderate intensity exercise on a recumbent stepper. We obtained the resting and exercised-induced cerebrovascular functions, specifically resting MCAv mean, resting cerebrovascular conductance index (CVCI), the exercise-induced cerebrovascular reserve capacity (CVRC), and the exercise-induced CVCI percent change. We hypothesized that WML burden, quantified as lesion volume and lesion count, would negatively relate to resting and exercise-induced cerebrovascular functions. Cross-sectional analysis showed a) no significant relationships between WML burden and resting cerebrovascular function, b) an inverse relationship between CVRC and WML count ($r = -0.260$, $p = 0.046$), and c) a direct relationship between the CVCI percent change decrease and WML count ($r = 0.348$, $p = 0.011$). These results suggest that increases in WML quantity decreases with increases in MCAv change, and WML quantity increases as the CVCI percent decrease diminishes. These results partially support our hypothesis that cerebrovascular responsiveness to exercise relates to WML burden, because no relationships were found between WML volume and exercise-induced cerebrovascular function. Hence, we cannot definitively assert that cerebrovascular responsiveness is related to WML

burden. Nonetheless, this is the first report to show exercise-induced cerebrovascular responsiveness relates to a measure of WML burden.

3.2. Background

As the brain ages, it is more likely to have damage to its structural integrity. Aging associated damage includes white matter lesions (WML) which appear as hyperintensities in MRI scans (43). Up to 90% of adults over age 65 have WMLs (44). Clinically, WMLs are associated with cerebrovascular disease, Alzheimer's disease (AD), dementia, impaired motor function and reduced cognitive capacity (44, 45, 48). Impaired cerebrovascular infrastructure is implicated in WMLs, as histopathology studies provide evidence of brain blood barrier leakage associated with WML burden in AD and cognitively normal aging (46, 47). WML burden is associated with classic cardiovascular risk factors, such as hypertension and elevated plasma cholesterol (49). Consequently, WML burden is often used to identify cerebrovascular pathology in the elderly (50).

3.2.1. White Matter Lesion Burden and Brain Hemodynamics

There has been speculation that elevated cerebrovascular resistance, or diminished cerebrovascular conductance, in AD-related regions may interact with WML burden to affect brain aging (144). However, a recent meta-analysis suggests that the diminished hemodynamics is a consequence, not the cause, of WMLs (145). Thus, the relationship between WML and hemodynamics is not fully understood. We propose assessing hemodynamics during an exercise challenge as a way to add valuable knowledge regarding WML burden and cerebrovascular

health. A major advantage for using TCD lies in its *high temporal resolution* (142). This affords the opportunity to study dynamic cerebrovascular responses to physiological, vasoactive stressors, such as gas inhalation and exercise (8, 73, 86, 146, 147).

The standard TCD assessment is an examination of the middle cerebral artery velocity (MCAv) (72, 73, 148). The MCAv can be incorporated with mean arterial blood pressure (MAP) into a ratio termed the cerebrovascular conductance index (CVCI, mathematically MCAv mean/MAP), which describes the flow-pressure relationship during a vasomotor response (12). Claassen posited the CVCI as the hemodynamic measure which best describes the vasomotor response (12). The MCAv mean change reflects the cerebrovasculature's ability to increase blood flow in response to vasoactive stimuli, termed the cerebrovascular reserve capacity (CVRC)(80). Diminished CVRC has been evidenced in stroke, diabetes, and coronary artery disease (149).

There is lack of understanding how cerebrovascular function and WML burden interact with or cause one another (107, 145). Although lower resting MCAv mean has been linked to more WML burden (107) , there is lack of information regarding the relationships between resting CVCI and WML burden. Further, injected acetazolamide and gas-induced CVRC has been linked to WML burden (150, 151) , but there is no report on the relationships between WML burden and exercise-induced cerebrovascular functions such as CVRC or CVCI. Hence we seek to address these knowledge gaps in an effort to increase to elucidate the relationships between resting and exercise-induced cerebrovascular function and WML burden.

3.2.2. Hypotheses

It is plausible that inducing MCAv-centered changes may elucidate the relationships between brain hemodynamics and WML burden (82). This would add valuable information regarding the development of a cerebrovascular marker which describes both brain hemodynamic function and white matter lesion impact. The main goal of this study was to assess the relationship between brain structural integrity and cerebrovascular function by assessing WML burden and MCAv-centered outcomes at rest and during exercise. We hypothesized that WML burden would inversely relate to our resting and exercise-induced cerebrovascular functions, MCAv mean, CVCI, and CVRC.

3.3. Methods

3.3.1. Participants

We used a cross-sectional study design to assess the relationships between brain structural integrity and cerebrovascular function. Participants were recruited into the Trial for Assessing Cerebrovascular Regulation and Vascular Risk (TrACR) from the University of Kansas Alzheimer's Disease Center (KU ADC) Alzheimer's Prevention Program (APP) from August 2014 to September 2016. Inclusion criteria were age over 65 years, classification as cognitively normal/nondemented with a Clinical Dementia Rating of 0, sedentary or underactive lifestyle. Exclusion criteria were DSM-IV defined drug or alcohol abuse within the prior 2 years, clinically significant depression or anxiety, insulin-dependent diabetes, myocardial infarction or symptoms of coronary artery disease within the prior two years, acute decompensated congestive heart failure or class IV heart failure, inability to travel to the KUMC Research in Exercise and Cardiovascular Health (REACH) laboratory, and inability for researchers to obtain the MCA signal from the transcranial window. Individuals who met the inclusion criteria provided written

informed consent. KU ADC recruitment personnel approached those individuals who met the TrACR inclusion/exclusion criteria for interest in the study. If the participant was interested, then the KU ADC provided a list of potential TrACR participants to the study coordinator for the REACH lab member. This study coordinator then contacted potential participants. If a potential participant decided to participate in TrACR, a morning appointment was scheduled. Prior to participation in study procedures, all participants provided written informed consent. All study procedures were approved by the University of Kansas Medical Center Human Subjects Committee.

Participants made one visit to the REACH laboratory, which lasted for approximately 4 hours (earliest possible appointment start time was 7:00 am; latest, 9:00 am). Participants were asked to refrain from caffeinated beverages the night before and morning of their study visit and refrain from strenuous physical activity 24 hours before study visit.

3.3.2. WML Burden Collection and Analysis

White matter lesion burden is currently being collected as part of the KU ADC's APP. Neuroimaging was done under the direction of the KU ADC's neuroimaging core at the KUMC Hoglund Brain Imaging Center. Fluid-attenuated inversion recovery (FLAIR) and anatomical (MPRAGE) sequences were acquired.

We used SPM8 (Welcomme Department of Imaging Neuroscience, London, UK) and the VBM8 toolbox (Structural Brain Mapping Group, University of Jena, Jena, Germany) for anatomical image pre-processing including segmentation of the image into tissue classes, including gray matter, white matter, or cerebrospinal fluid using the 'new segment' procedure.

We then used the Lesion Segment Tool, Version 2.0.13 (152), to automatically calculate white matter lesion segmentation and generate total lesion volume maps. Briefly, the LST algorithm coregistered FLAIR images to the native space anatomical image. FLAIR intensity distribution was calculated for each tissue class, and these distributions were summed to create belief maps. Based on pilot work and visual inspection we selected a k threshold = 0.125 as the cutoff point on the gray matter belief map. This threshold was independently corroborated in a cohort with multiple sclerosis (data not shown). The LST algorithm generates the white matter lesion segments and total lesion volume (mL) and count based on the selected k threshold (153). The WML volumes (mL) and counts (quantity) were the primary WML burden measures used for correlational and regression analysis.

3.3.3. Cerebrovascular Function Assessment and Analysis

Set up. Participants were fitted at rest. We used transcranial Doppler (TCD) ultrasound and beat-to-beat-finger plethysmography (154) to get measures of cerebrovascular health. Using beat-to-beat measures allowed us to capture changes in mean arterial pressure (MAP) and brain blood flow velocity with high temporal resolution (66, 86, 155). We used a protocol similar to previously published work (86). This protocol captures mean MCAv and MAP for every cardiac cycle over an eight-minute rest period and during eight minutes of steady state moderate intensity exercise. A ROBOTC2MD robotic TCD headset (Multigon, White Plains, NY) was placed on the head to capture the left MCA signal. For those participants whose left MCA signal was unattainable, we measured right MCAv mean. A Finometer Pro finger plethysmography system (Finapres Medical Systems, Amsterdam, Netherlands) brachial cuff was placed on the left brachial arm, and the finger cuff was placed on the left middle finger at heart level, to record

beat-to-beat MAP. A Tango M2 brachial cuff (SunTech Medical, Inc., Morrisville, NC) was placed on the right arm. Finapres values were compared to the Tango automated blood pressure cuff before the onset of testing (120). If the difference between Finapres and Tango values were greater than 15 mm Hg, the Finapres finger cuff was removed then repositioned, until the difference was below 15 mm Hg (121). A Capnocheck 9400 capnograph (Smiths Medical, St. Paul, MN) and cannula was used to measure breath-by-breath end-tidal CO_2 ($ETCO_2$); and a 5-lead electrocardiogram (EKG) (CardioCard, Nasiff Associates, Central Square, NY) was used to collect heart rate.

3.3.4. Resting and Exercise Recordings. Resting and exercise cerebrovascular measures were collected while participants remained in a seated position. After completing set up, the participants were instructed to remain seated for up to fifteen minutes, breathe through the nose, fix their gaze forward, and refrain from talking and movement. We then began to record resting cerebrovascular measures for 8 minutes. After completing the rest recording, participants completed one bout of moderate intensity exercise. Moderate intensity exercise was between 40%-60% of an individual's heart rate (HR) reserve (122), and exercise target heart rate (THR) range was calculated using the Karvonen equation (156):

THR = ((age-predicted maximum HR− resting HR) × % Exercise Intensity) + resting HR

To get participants to reach the THR, we used an exercise protocol that has been adapted for use on the NuStep (86). Participants were instructed to use only their legs. As participants pedaled at a cadence of 90-100 steps per minute, the resistance was increased until the THR was reached and maintained for one minute. Beat-to-beat values were recorded for eight minutes

during exercise. Upon completion of eight minutes of exercise, participants cooled down for one minute against the lowest resistance (15 watts).

3.3.5. Data Collection and Computations

Analog signals were delivered through an analog to digital signal converter (NI USB-6212, National Instruments Corporation, Austin TX), collected and displayed in real time with a custom MATLAB program (The MathWorks, Inc., Natick, MA). We collected an R-wave trigger signal, MCAV and MAP at 500 Hz. The Finapress and TCD beat-to-beat averages were aligned with the R-wave signal. We used a custom MATLAB program (R. Maletsky, Lawrence, KS) to generate data values. Beat-to-beat Finapres and TCD values were calculated via integration of each cardiac cycle and then dividing the integral by length of the cardiac cycle. A custom MATLAB post-processing program computed our primary outcome variables, ($ETCO_2$, MAP, MCAv mean and CVCI (mathematically, MCAv mean/MAP).Percent changes in the primary cerebrovascular measures were calculated to provide exercise-induced measures. Percent change was calculated using the formula:

Percent Change X = (|(X at moderate intensity exercise – X at rest)|/X at rest) X 100

where **X** denotes a primary cerebrovascular measure. Percent change was calculated for $ETCO_2$, MAP, MCAv mean (CVRC), and CVCI.

3.3.7. Statistical Analyses

For our first step in testing our hypotheses, we assessed homogeneity of variance and normality using Levene's test for equality of variance and the Shapiro-Wilk test, respectively. Non-parametric Spearman correlation was used for data that violated normality or homogeneity of variance. One-tailed Pearson correlation was used to assess the relationship between two normally distributed variables. One-tailed, non-parametric Spearman correlation (124) was used for data that violated normality or homogeneity of variance. For cerebrovascular measures, we conducted paired sampled T-tests to assess differences between rest and moderate intensity exercise. When assumptions of normality and homogeneity were not met, we used log-transformation. For all analyses, $\alpha < 0.05$ was used as the criterion for statistical significance. All analyses were completed with Statistical Package for the Social Sciences software (SPSS, version 23, IBM Corporation, Armonk, NY).

3.4. Results

We recruited 64 older adults from the Alzheimer's Prevention Program into TrACR. We successfully insonated the MCA and obtained the MCAv signal in 51 participants (79.7%). We were unable to use data from an additional 8 participants due to unattainable Finpares and capnograph signal. Specifically, one participant did not have a useable Finapres signal both at rest and during exercise; one participant did not have a useable capnograph signal at both rest and during exercise; two participants did not have a useable capnograph signal during exercise; two participants only completed resting recordings; and two participants had completed cerebrovascular measures but did not complete MRI. Of the 43 analyzed participants, 28 were female. These participants were 71.2 $\pm$ 5.1 years old, had a body mass index of 26.5 $\pm$ 4.6 kg/m^2, and had 17.0 $\pm$ 2.7 years of education.

Shapiro-Wilk tests were used to determine normality. Resting MCAv mean (p = 0.216), and resting CVCI (p = 0.135) were normally distributed. The exercise-induced CVCI percent change (p = 0.055) was normally distributed, but the exercise-induced CVRC was not (p = 0.001). Neither WML burden measure was normally distributed: WML volume (p < 0.001), WML count (p = 0.009) Log-transformation of WML burden yielded normal distributions for WML volume (logWML volume, p = 0.194, **Figure 1**) and WML count (logWML count, p = 0.230, **Figure 2**). Log transformation of CVRC yielded a normal distribution (logCVRC, p = 0.031). Hence, the log-transformations allowed us to conduct sets of Pearson correlations.

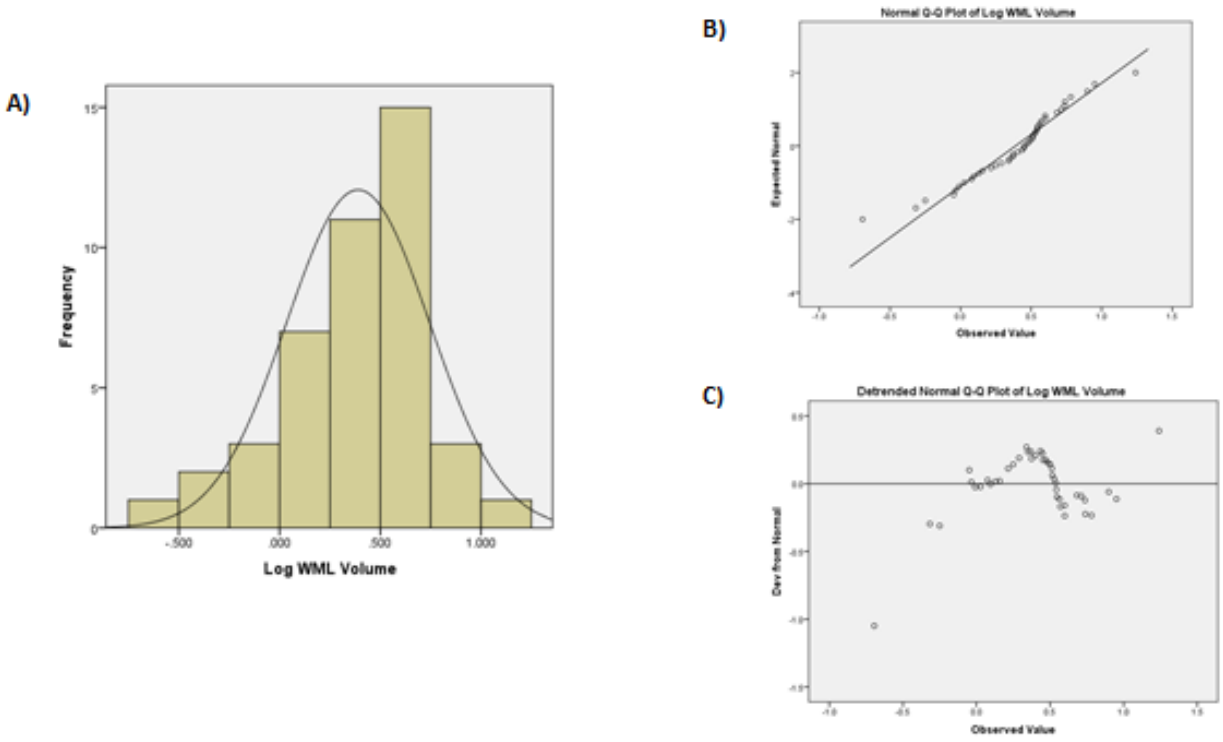


Figure 1: Normal Distribution of Log-transformed WML Volume (n = 43). Visual depiction of non-normal distribution of logWML volume after removal of 1 outlier: A) Right-skewed histogram of lowWML volume frequencies; B) Normal Q-Q plot of observed logWML volume vs expected normal value; C) and Detrended Normal Q-Q plot of observed logWML volume value vs deviation from normal.

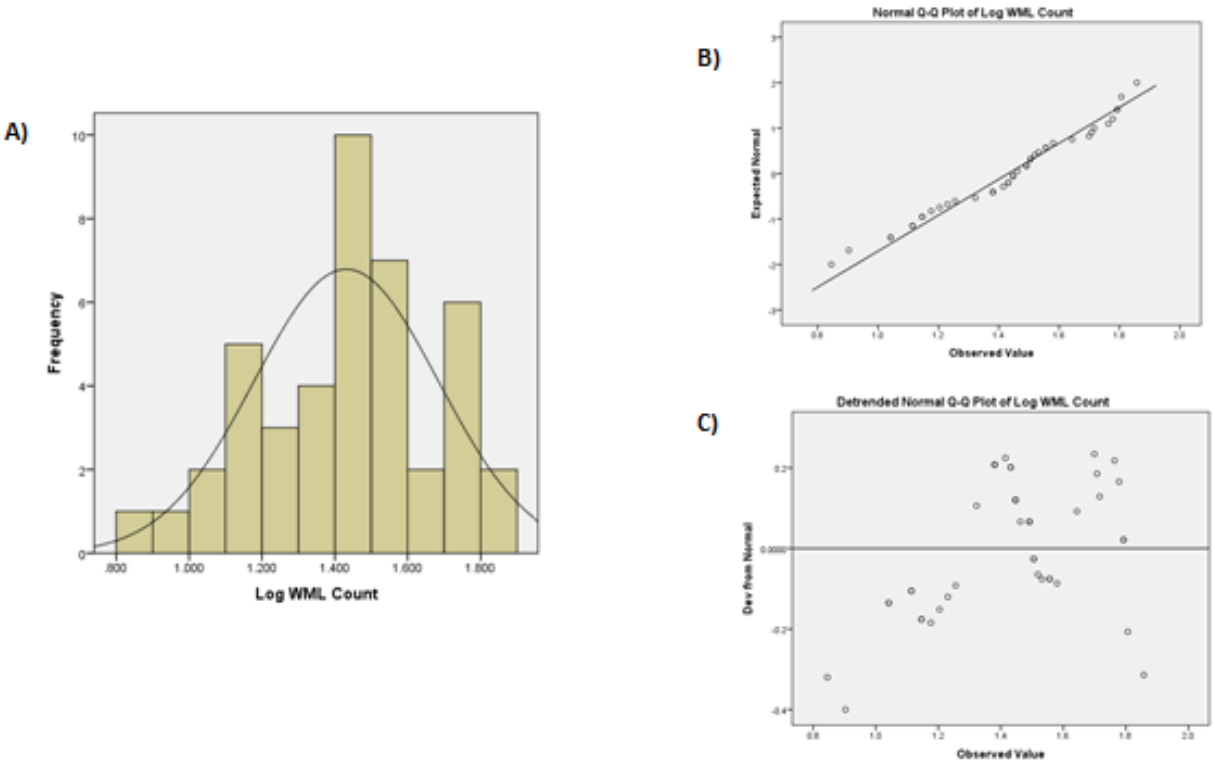


Figure 2: Normal Distribution of Log-transformed WML Count (n = 43). Visual depiction of normal distribution of logWML count. A) Right-skewed histogram of lowWML count frequencies; B) Normal Q-Q plot of observed logWML count vs expected normal value; C) and Detrended Normal Q-Q plot of observed logWML count value vs deviation from normal.

3.4.1. WML Burden Relationships with Resting and Exercise-induced Cerebrovascular Function

All participants had WMLs. Mean WML volume was 3.3 ± 2.8 mL (range 0.20-17.4 mL) and the mean WML count was 31.4 ± 16.9 lesions (range 7-72 lesions). **Table 1** shows significant differences between the cerebrovascular measures at rest versus during moderate intensity exercise. There was a significant exercise-induced MCAv increase, or CVRC ($p < 0.001$), and a significant exercise-induced decrease in CVCI ($p < 0.001$).

Measure	Resting (± SD)	Exercise (± SD)	Percent Difference (± SD)
$ETCO_2$, mm Hg	33.3 ± 5.4	37.4 ± 5.3	16.5 ± 16.0
MAP, mm Hg	74.4 ± 12.5	104.8 ± 23.1	41.5 ± 23.0
MCAv mean, $cm*s^{-1}$	48.1 ± 9.0	54.0 ± 12.3	16.8 ± 10.6
CVCI, $cm*s^{-1}$ *mm Hg^{-1}	0.664 ± 0.17	0.521 ± 0.15	21.6 ± 16.6

Table 1. Resting and Moderate Intensity Exercise Cerebrovascular Measures (n = 43). Exercise-induced differences over rest in capnography, MAP, and measures of cerebrovascular function. The values presented are absolute percent changes. $ETCO_2$ = end-tidal carbon dioxide; MAP = mean arterial pressure; MCAv = middle cerebral artery velocity; CVCI = cerebrovascular conductance index.

Table 2 shows the relationships between cerebrovascular function and WML burden. No significant relationships were found between WML burden and resting cerebrovascular measures.

Cerebrovascular Function	logWML Volume	logWML Count
Resting MCAv	r = 0.058, p = 0.355	r = 0.023, p = 0.442
Resting CVCI	r = 0.185, p = 0.118	r = 0.078, p = 0.309
log CVRC	r= 0.081, p = 0.303	**r = -0.260, p = 0.046***
CVCI Percent Change	r = 0.073, p = 0.322	**r = 0.348, p = 0.011***

Table 2. Relationships between White Matter Lesion Burden and Resting and Exercise-induced Cerebrovascular Function (n = 43). logWML = log white matter lesion; MCAv = middle cerebral artery velocity; logCVRC = log cerebrovascular reserve capacity; CVCI = cerebrovascular conductance index.

Two significant relationships were found between WML burden and exercise-induced cerebrovascular function. First. There was a significant, inverse relationship between CVRC and WML count (r = -0.260, p = 0.046, **Figure 3**). Second, there was a significant, direct relationship between logWML count and negative CVCI percent change (Pearson r = 0.348, p = 0.011, **Figure 4**). Hence, we found an inverse relationship between WML count and the CVRC, suggesting that increases in WML quantity decreases with increases in MCAv change. Further, we found a direct relationship between WML count and the CVCI percent change. The CVCI

decreased from rest to exercise; therefore, this finding suggests WML quantity increases as the CVCI percent decrease diminishes in value.

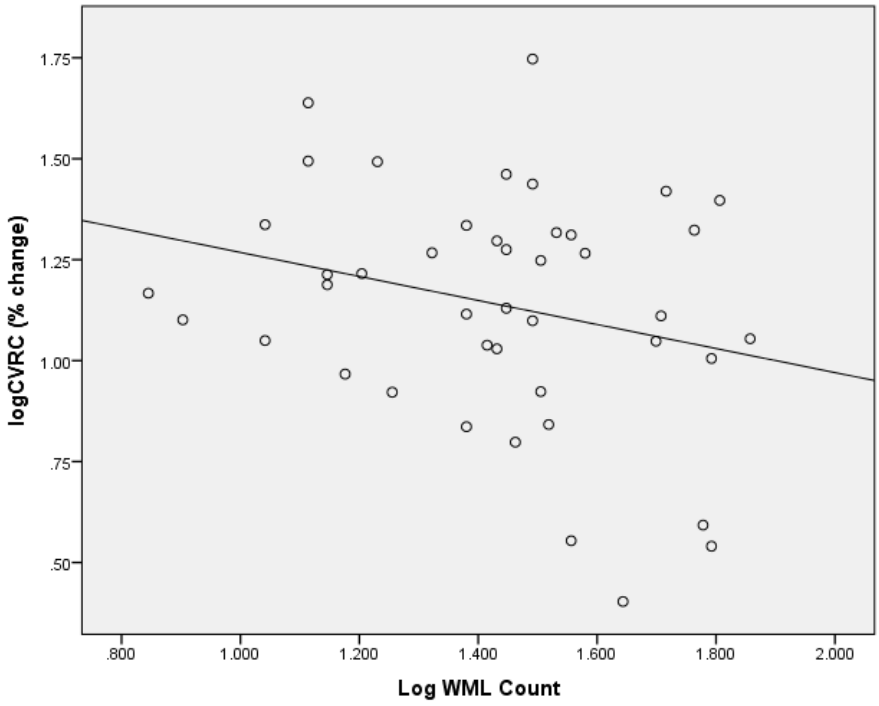


Figure 3: Relationship between WML Count and CVRC (n = 43). A significant, inverse relationship was found between logWML count and logCVRC ($r = -0.260$, $p = 0.046$).

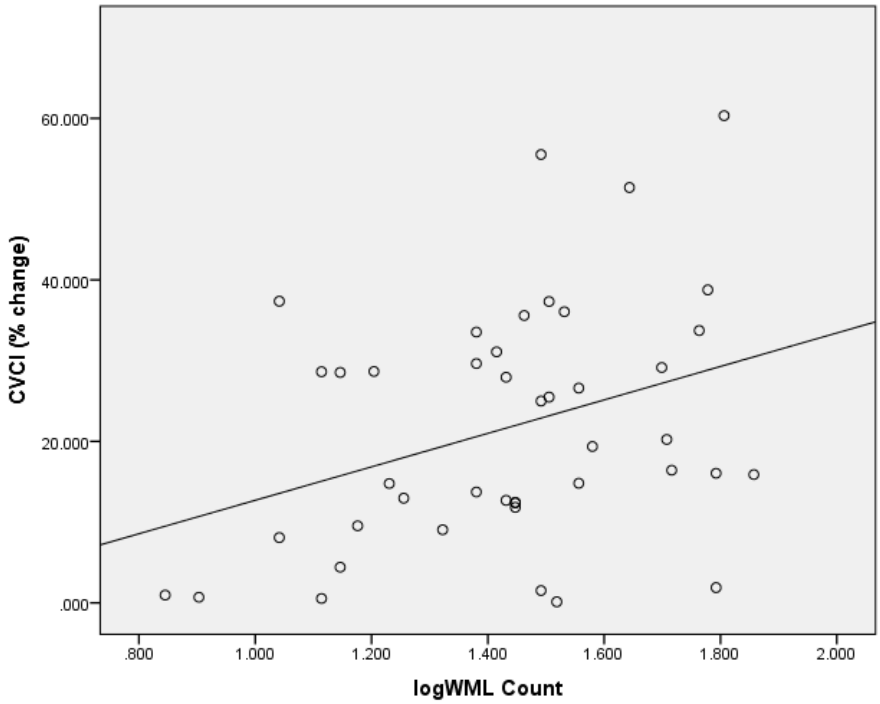


Figure 4: Relationship between WML Count and CVCI Percent Change (n = 43). A significant positive relationship between logWML count and CVCI percent change ($r = 0.348$, $p = 0.011$).

3.5. Discussion

We investigated relationships between measures of WML burden and resting and exercise-induced cerebrovascular function in an attempt to further our understanding of the brain's hemodynamic relationship to brain aging. Although there were no significant relationships between WML burden and resting cerebrovascular function, we report two main findings between WML count and exercise-induced cerebrovascular function, which suggests greater WML count relates to lower cerebrovascular responsiveness. Hence, these results partially support our hypothesis that cerebrovascular responsiveness to exercise relates to WML burden. We are the first to report relationships between WML burden, specifically the quantity of WMLs, and exercise-induced cerebrovascular function in a cohort of nondemented, older adults.

However, because there were no significant relationships between WML volume and exercise-induced cerebrovascular function, we cannot make a strong assertion that cerebrovascular responsiveness is related to WML burden.

3.5.1. WML Burden and Resting Cerebrovascular Function

A recent review and meta-analysis by Shi and colleagues suggests lower brain blood flow is related to more WML burden across various clinical and non-clinical populations using various techniques, including TCD (145). For instance, a positron emission tomography study showed older adults with asymptomatic WMLs had reduced perfusion in white matter and the basal ganglia compared to control subjects, suggesting a link between hypoperfusion and brain structural damage in a nonclinical population (157). Tzourio and colleagues assessed bilateral MCAv mean in 628 elderly subjects to show a link between resting cerebrovascular function and WML severity. Further, a regression analyses done by Tzourio and colleagues showed subjects with the lowest MCAv mean were more than 4 times more likely to develop severe WML burden and more than twice as likely to develop moderate WML burden compared to subjects with the highest MCAv mean (107).

Contrary to our hypotheses, there were positive, non-significant relationships between our resting measures of cerebrovascular function and measures of WML burden. Hence, we did not reproduce prior work which detected inverse relationships between WML burden and resting brain blood flow using neuroimaing or between WML and brain blood flow velocity using TCD (107, 150, 157). Prior studies investigating the link cerebrovascular function and WML burden did not quantify WML burden by volume or quantity; instead the WML burden was classified by

severity using graded scales. Further, in a majority of these studies, clinical populations were studied, and the symptomatic manifestations of WMLs were linked to the clinical conditions (158-161). In contrast, we studied a non-clinical, relatively healthy cohort of nondemented older adults. Hence, the lack of a relationship between resting cerebrovascular measures and WML burden may be a result of studying the cerebrovasculature of a healthy cohort without comparing this cohort to a clinical group. This idea is further supported by the WML volume of our cohort. Specifically, our cohort had lower than average WML volume compared to other studies using the Lesion Segmentation Toolbox software (131, 132). For instance, in one study of nondemented older adults over 55 years, the average WML volume among the four subject groups ranged from 13.6 mL to 25.8 mL (131). In another study of adults aged 32-85 and free of neurological disease, the average WML volume was 37.9 mL, and the range was between 3 to 99 mL. *Thus, it is plausible that our cohort of older adults may have been, on average, spared by the age-related accumulation of WML volume.* One possible explanation may be the incidence of hypertension and use of blood pressure medications in our cohort. Hypertension is known to be a factor in the link between hypoperfusion and WML burden (161). Forty-three percent (19/43) of these older adults were on blood pressure medications, which could have possibly tempered the development of WML volume in this cohort. A second explanation may be education. In one study, there was relationship between more severe WMLs and poorer cognitive performance in older adults with lower levels of education (11 years and under), yet no relationships were seen between WML and cognition in adults with higher levels of education (162). Thus, higher education levels may protect against cognitive decline and brain damage. Our cohort had, on average 17 years of education and were cognitively normal, so it is possible these older adults' higher education levels factored into sparing cognitive performance and brain structure

3.5.2. WML Burden and Exercise-induced Cerebrovascular Function

To our knowledge, this is the first report showing relationships between WML burden and exercise-induced cerebrovascular function. However, because these relationships were formed between exercise-induced cerebrovascular function and one of two WML burden measures, our data partially supports our hypotheses. Nonetheless, we recapitulated the relationship between cerebrovascular reserve and brain structural integrity (150, 163). Cerebrovascular reserve reflects the cerebral arterioles compensatory ability to affect perfusion changes in response to a physiological stressor, such as exercise or carbon dioxide exposure (80, 149). Prior studies have shown an inverse link between WML burden and CVRC (150, 151), but no study has reported on possible links between exercise-induced cerebrovascular function and WML burden. Neuroimaging studies posit the possibility that perfusion is compromised within the WMLs. For instance, one study of 21 nondemented 85-year olds reported a 47% CVRC within the grey matter, a 52% CVRC within normal-appearing white matter, and 30% CVRC within WMLs (150). Hence, perfusion was compromised within the WMLs compared to normal-appearing white matter and grey matter. Further, a recent MRI study showed that the normal white matter which eventually become WMLs have prolonged vascular response times and lower CVRC compared to normal white matter which did not become WMLs (151). Thus, it is likely that areas with WMLs have cerebrovasculature that has been subjugated to damage associated with a combination of ischemia, atherosclerosis, hypertension, or hypoperfusion (150, 151, 164).

We demonstrate that the CVCI percent change can be a cerebrovascular reserve marker. The CVCI describes the flow-pressure relationship within the cerebrovasculature (103), and may best capture the vasomotor response to physiological stress (12). The CVCI decreased between

rest and exercise; therefore, the 22% change between rest and exercise in CVCI reflects the magnitude of this decrease, and this decrease was directly related to WML count. Previous research has shown exercise-induced CVCI changes coinciding with CVRC values between 1.5% - 21% and MAP percent changes between 3%-24% in older adults (84-86). Fisher and colleagues showed an absolute CVCI percent decrease of 16.4% in older adults completing moderate exercise on a cycle ergometer (86). This drive in CVCI percent change was due to the 24% increase in MAP over rest, suggesting that the body maintains brain blood flow velocity in response to physiological stress by increasing arterial pressure (86). However, their cohort only had a 1 cm/s MCAv mean increase over rest, which was a 1.6% CVRC. In juxtaposition, our cohort had a 17% CVRC, a 21% CVCI decrease, and a 42% MAP increase from rest. Hence, compared to Fisher et al, our cohort had a much larger CVRC and lower CVCI percent change driven by a larger MAP change. This could be explained by the age discrepancy between the two studies, as our cohort was on average 14 years older than Fisher et al older adult cohort. Regardless, in both elderly cohorts, the exercise-induced CVRC is more conservative than the MAP change. This mathematically explains why the CVCI decreased during exercise, suggesting that vasoconstriction occurs to counteract exercise-induced increases in MAP.

Comprehensive understanding of exercise-induced cerebrovascular markers requires further characterization of the MCAv increases and the flow-pressure relationship across the spectrum of aging. Accomplishing this could provide further knowledge of which exercise workloads produce comparable cerebrovascular effects across different age groups. For instance, Ogoh and colleagues conducted a cycling study that did report CVCI numbers (146). They compared the effect of cycling intensity on MCAv-centered measures in a sample of seven young adults. Participants cycled at mild (target heart rate of 90 beats per minute), moderate (120

beats per minute), and heavy (150 beats per minute) workloads for eight minutes each. Compared to rest, the CVRC at mild, moderate, and heavy intensities were 5%, 11.4%, and 16.4%, respectively. The respective MAP changes were 1.1%, 8.0%, and 20.4%. The respective CVCI percent changes were 5.8%, 4.3%, and -2.8% (146). Hence, only heavy exercise induces a negative CVCI percent change in young adults. However, this change was not as large as the CVCI decrease we report in older adults. We report a 22% decrease in CVCI as result of exercise, and this decrease is accompanied by a 42% increase in MAP. In Ogoh and colleagues' work, the exercise-induced 3% decrease in CVCI is accompanied by a 20% increase in MAP. Therefore, *when older adults face vascular stress, there is a more drastic drive to increase MAP in order to maintain the cerebral flow-pressure relationship (85, 86).* This MAP increase may partially explain why WML burden increases with age. Elevated blood pressure is linked to WML in normal older adults (164). The bioavailability of nitric oxide declines with age and sedentary behavior, and this compromises endothelial function during aging, ultimately contributing to vascular stiffening and hypertension (165, 166). The older adults in our cohort were sedentary, and 14 of the 43 (32%) of these adults had a pre-exercise blood pressure above normotensive. Hence, we cannot remove the possibility of increased vascular stiffening and/or altered endothelial function in a substantial proportion of our cohort. Therefore, our reported relationships between exercise-induced cerebrovascular functions and WML count may be more indicative of vascular aging mechanisms than brain structural atrophy.

Our data shows a link between exercise-induced cerebrovascular function and WML burden quantity in a nondemented elderly cohort. Most research has focused on cerebrovascular function at rest, during gas inhalation/rebreathing, or during sit-to-stand (12, 92, 167), but there is a lack of information regarding exercise-induced cerebrovascular functions. In this small

sample, we present data suggesting that exercise-induced CVRC and CVCI percent changer are cerebrovascular functions linking cerebrovascular responsiveness and brain structural integrity. Hence, future work should determine if exercise-induced cerebrovascular functions can be used as long-term markers of the vasomotor-brain structural integrity relationship during normal aging. For example, recapitulating this relationship throughout the course of an exercise intervention would add further evidence of exercise's capacity to preserve cerebrovascular function (168), with preservation of brain structural integrity being linked to cerebrovascular preservation.

3.5.3. Methodological Considerations

The cross-sectional design limits our interpretation and generalizability of our results. We aimed to study nondemented older adults, but our use of MCAv-centered outcomes as biomarkers only provides a snapshot of the relationships between brain hemodynamics and brain structural integrity. Further, participants only completed one exercise session, so we cannot draw conclusions as to what effect repeated exercise sessions would have on the relationship between brain hemodynamics and WML burden. Therefore, the use of MCAv-centered outcomes as long-term markers of these relationships needs to be established in larger studies across multiple time points in a variety of clinical conditions including, but not limited to mild cognitive impairment, Alzheimer's disease, vascular dementia, and stroke. Additionally, future work is needed to determine if an exercise-intervention affects these relationships in larger samples across the spectrum of healthy and pathological brain aging. This could set the foundation for using exercise-induced cerebrovascular markers as indicators of cerebrovascular and structural decline, preservation, or improvement. Recent work by Spencer showed that MCAv-centered responses

to a hypercapnic challenge remain stable over 6 months in adults over age 55, and their pending work will assess the effects of a six month exercise intervention on MCAv-centered outcomes (42). Therefore, future work could potentially supplement Spencer's work and could help determine whether or not long-term exercise-induced cerebrovascular percent changes predict WML burden.

3.6. Conclusion

A cross-section of nondemented older adults completed one exercise session so that we could determine the relationships between cerebrovascular function and WML burden. Through assessment of middle cerebral artery velocity-centered measures at rest and during exercise, we showed that exercise-induced percent changes in cerebrovascular function, particularly the cerebrovascular conductance index and cerebrovascular reserve capacity, relate to WML burden count, but not WML volume. Thus, we only have partial support of our hypotheses, mitigating the potential use of exercise-induced cerebrovascular measures as markers of brain structural integrity. Future work is needed to determine if these relationships can be reproduced along the spectrum of brain aging, including adults under 65 years, across multiple time points, and in a larger cohort, which would determine the utility of using these cerebrovascular measures as long-term indicators of brain structural integrity and global health.

Chapter 4

Cerebrovascular Reserve Capacity Is Blunted During Exercise in People with Elevated Beta-Amyloid

Sisante J-F, Vidoni E, Kwapizewski S, Maletsky R, Burns J, Billinger S. *Cerebrovascular Reserve Capacity Is Blunted During Exercise in People with Elevated Beta-Amyloid.* To be Submitted, *Stroke* February, 2017.

4.1. Abstract

It has become increasingly clear that cerebrovascular dysfunction is an important driver of cognitive decline. Mounting evidence suggest that accumulation of Alzheimer's disease pathology, beta-amyloid, interferes with and may be worsened by cerebrovascular dysfunction. However, little is known about how beta-amyloid accumulation directly impacts cerebrovascular regulation when homeostasis of the system is challenged. We report on the cerebrovascular regulation of older individuals with normal cognition. We characterized beta-amyloid with florbetapir PET scans. Brain blood flow velocity of each participant was measured at rest and during moderate intensity exercise, allowing us to calculate a reserve capacity. Cognitive testing was also performed at a separate visit. We found that greater beta-amyloid load was associated with worse cerebrovascular reserve capacity (CVRC; b=-9.3, p=0.03). Beta-amyloid loads but not CVRC were related to aspects of cognition. These the results extend the growing literate in humans that cerebrovascular dysregulation and beta-amyloid accumulation are closely linked. CVRC may be an important early marker of AD pathology.

4.2. Introduction

Continuous regulation of brain blood flow is essential at rest, during daily activity and physical demand (exercise) for optimal brain health (83, 169-176). A healthy cerebrovascular system maintains constant global brain blood flow across multiple conditions despite changes in metabolic demands, carbon dioxide production and perfusion pressure changes (177). Age-related decline in cerebrovascular regulation may be associated with decreased oxygen delivery and suboptimal removal of metabolic by-products that lead to white matter hyperintensities and eventually impaired cognitive function (16, 178).

There is evidence in animal models that the presence of beta-amyloid, a hallmark pathology of Alzheimer's disease (179), may interfere with endothelial-dependent response of the cerebral arteries and impair cerebrovascular autoregulation (180, 181). Specifically, one study reported transgenic mice that expressed higher levels of beta-amyloid showed the greatest disruption in cerebrovascular autoregulation across various ranges of blood pressure (180). If impaired cerebrovascular function is one mechanism by which beta-amyloid accumulation causes neurodegeneration (115), replication in humans would have profound implications regarding brain health especially during daily activities and exercise.

Exercise challenges the body to respond to an increase in metabolic demand. Therefore, blood flow and oxygen delivery must match the brain's metabolic needs despite changes in blood pressure and carbon dioxide (CO_2) levels (83). In this study, we present a novel experimental protocol for assessing cerebrovascular regulation in cognitively normal individuals with and without elevated beta-amyloid. We employed a moderate intensity exercise protocol to assess cerebrovascular reserve capacity (CVRC) in the middle cerebral artery. We hypothesized that greater amyloid loads would be associated with lower CVRC. Using established criteria for amyloid load (SUVR), we then divided the participants into 2 groups (elevated, non-elevated) to examine whether group differences were present for CVRC. We also sought to explore the relationship of CVRC, amyloid burden and cognition, hypothesizing that CVRC would mediate any relationship between amyloid burden and cognition, especially executive function, in our participants.

4.3. Methods

Individuals were recruited from the University of Kansas Alzheimer's Disease Center's (KU ADC) Alzheimer's Prevention Program. To be included in the study, individuals were: 1) 65-90 years of age, 2) classified as cognitively normal/nondemented with a Clinical Dementia Rating = 0, 3) sedentary or underactive lifestyle, and 4) completion of Amyvid PET scan within 6 months of our experimental procedures. Exclusion criteria were: 1) DSM-IV defined drug or alcohol abuse within the prior 2 years, 2) clinically significant depression or anxiety, 3) insulin-dependent diabetes, 4) myocardial infarction or symptoms of coronary artery disease within the prior two years, 5) acute decompensated congestive heart failure or class IV heart failure, 6) major orthopedic disability, 7) inability to exercise due to pain or restrictions from physician. All individuals provided written informed consent. The KU institutional review board provided approval for all study procedures.

Before coming to our laboratory at KU Medical Center, participants refrained from caffeinated beverages for 12 hours, physical activity for 24 hours and no large meal within 2 hours of the procedures (2). Procedures began in the morning for all participants. After consent, each individual was classified as having low, moderate or high cardiovascular risk according the American College of Sports Medicine (182). The laboratory room for the experimental session was dimly lit, quiet and temperature maintained between 22-24 degrees Celsius (183, 184). External stimuli were kept to a minimum during the testing session.

The left middle cerebral artery was used for the TCD ultrasound with a 2-MHz probe (RobotoC2MD, Multigon) placed over the temporal window and fixed in place using a robotic TCD headpiece. If the left MCA was not obtainable, then the right side was used. Once the optimal signal was identified, we began the imaging process for mean MCA velocity

($MCAV_{mean}$). Individuals performing the TCD data collection were blinded to the beta-amyloid status (elevated, non-elevated).

A finger plethysomograph (Finometer Pro) was placed on the middle finger of the left hand supported at the level of the heart on an adjustable padded bedside table. The finger plethysomograph collected continuous measures for mean arterial pressure (MAP). It has been reported that MAP values measured at the finger are typically lower than intrabrachial pressure at rest (185) and during exercise (186). End-tidal CO_2 ($ETCO_2$) was assessed using a nasal cannula and capnograph (BCI Capnocheck 9004, Smiths Medical St. Paul, MN). We used a 5-lead electrocardiogram (ECG) for heart rate (HR). The 8-minute average MAP, $ETCO_2$ and $MCAV_{mean}$ for each condition (resting and moderate intensity) were used.

4.3.1. Resting Protocol. Once signal acquisition occurred and was stable for the ECG, Finometer Pro, TCD and $ETCO_2$, participants sat quietly on the recumbent stepper (NuStep, T5XR) to rest for 15 minutes. During the last 8 minutes of rest, baseline data for all variables was recorded.

4.3.2. Exercise Protocol. After the 8-minute resting data collection, the participant performed a single bout of exercise at moderate intensity using the recumbent stepper (187). Moderate intensity exercise was defined as 40% - 60% of age-predicted heart rate (HR) reserve (187). Participants maintained a step rate of 90 steps per minute (188). All participants began at 40 watts while the resistance was increased until the targeted HR range was reached. Data collection commenced when a steady state HR was maintained for one minute. The participant exercised at moderate intensity for 8 minutes. Data were sampled at 500 Hz.

4.3.3. Vascular Measures. $MCAv_{mean}$, MAP and $ETCO_2$ were resampled to 10Hz. We calculated cerebrovascular reserve capacity (CVRC) as the change in $MCAv_{mean}$ (cm/s) from rest to moderate intensity exercise. We reported mean $MCAv_{mean}$, MAP and $ETCO_2$ at rest and baseline.

4.3.4. Dementia and Cognition. All participants were well-characterized as having normal function and cognition during a clinical consensus conference based on a Clinical Dementia Rating (189) and neuropsychological test battery. The neuropsychological test battery is common to all National Institute on Aging-designated Alzheimer's Disease Centers, the Uniform Data Set (119). We created cognitive composite scores of these domains by averaging unadjusted standardized scores in each domain: executive function, attention, language, and processing speed (119). Memory tests in the Uniform Data Set (UDS) were changed during our study by the National Alzheimer's Coordinating Center. Consequently, we did not have consistent UDS memory tests for all participants. Therefore, we used the Free and Cued Selective Reminding Test as a measure of memory (190). We standardized the total number of items recalled without cue to a similar, previously characterized sample (191).

4.3.5. Brain Imaging. To assess beta-amyloid, participants were administered intravenous florbetapir F-18 (370 MBq). Florbetapir PET images were obtained on a GE Discovery ST-16 PET/CT scanner. Two five-minute duration PET brain frames were acquired continuously, approximately 50 minutes after florbetapir administration. Frames were then summed and attenuation corrected.

Three trained raters (192) considered both visual and quantitative information on each scan. Quantitative analysis was performed using MIMneuro software (MIM Software Inc, Cleveland, OH), normalizing the PET image to a template and standardizing florbetapir uptake to the whole cerebellum, creating a standardized uptake value ratio (SUVR). Final determination of scan status, elevated vs. non-elevated, was the majority determination of the 3 raters. We calculated the mean of the anterior cingulate, posterior cingulate, precuneus, inferior medial frontal, lateral temporal, and superior parietal cortex as the global beta-amyloid burden.

4.3.6. Analyses. All statistical analyses were performed using R (version 3.2.4 (193)). We performed linear regression to explore relationships between our vascular, cognitive and brain amyloid measures. Between group differences (amyloid-elevated, non-elevated) were assessed using Welch's two-sample tests (due to different sample sizes and variance characteristics) or chi-square tests as appropriate. We set α=0.05 to protect against Type I error and did not correct for multiple comparisons due to the exploratory nature of this study.

4.4. Results

We consented 73 individuals into our experimental protocol. Of those consented, we were unable to acquire a quality signal at baseline and during moderate intensity exercise on 17 individuals. These individuals were not different in age, sex or cardiovascular risk factor classification from those individuals we were able to acquire a signal on. Signal failure was due to: 1) unable to insonate the MCA (n = 16), 2) significant artifact during exercise (n =1).

Of the remaining 56 individuals (see **Table 1**; 14 elevated, 42 non-elevated), the groups were similar in age, sex and cardiovascular risk ($p>0.1$). Individuals with elevated amyloid performed notably worse in the memory ($F=8.99$, $p=0.006$) and processing speed ($F=10.2$, $p=0.003$) cognitive domains, with a trend for better scores in the language domain ($F=3.4$, $p=0.08$).

	Non-elevated (n=42)	Elevated (n=14)	All Participants (n=56)
Age, years	70.0 [4.7]	72.9 [5.6]	70.7 [5.0]
Female	28 [66.7%]	9 [64.3%]	41 [66.1%]
Cardiovascular Risk, n	18 Moderate 24 High	3 Moderate 11 High	21 Moderate 35 High
Attention z-score	-0.14 [0.81]	-0.34 [0.64]	-0.19 [0.77]
Memory z-score	0.78 [1.01]	-0.11 [0.93]	0.56 [1.06]*
Language z-score	-0.37 [0.73]	0.07 [0.78]	-0.26 [0.78]
Executive Function z-score	0.29 [0.63]	0.22 [0.46]	0.27 [0.58]
Processing Speed z-score	0.41 [0.61]	-0.11 [0.49]	0.28 [0.62]*

Table 1. Baseline demographics and cognitive performance scores. Values are mean [standard deviation] unless otherwise noted. *$p<0.01$

Because blood flow is sensitive to CO_2 ($ETCO_2$) and blood pressure (MAP) we first checked for group differences in these measures (**Table 2**). Neither baseline $ETCO_2$ (F=0.003, p=0.95) nor moderate intensity exercise $ETCO_2$ (F=1.08, p=0.44) were different between groups. Baseline MAP was different at rest (F=4.5, p=0.04) with the non-elevated individuals having a higher mean MAP. But the groups were not at moderate intensity exercise MAP (F=2.65, p=0.11).

	Non-elevated (n=41)	Elevated (n=14)	All Participants (n=55)
Cerebrovascular Reserve Capacity (cm/s)	6.9 [5.5]	4.4 [3.7]	6.2 [5.2]†
Mean Middle Cerebral Artery Velocity at Rest (cm/s)	48.5 [8.8]	45.6 [10.5]	47.8 [9.3]
Mean Middle Cerebral Artery Velocity during Mod. Exercise (cm/s)	55.4 [11.1]	50.0 [11.1]	54.1 [11.2]
Mean Arterial Pressure at Rest (mmHg)	74.8 [13.4]	67.7 [9.9]	73.1 [12.9]*
Mean Arterial Pressure during Mod. Exercise (mmHg)	105.0 [24.0]	95.3 [17.3]	102.6 [22.7]
Mean End-tidal CO_2 at Rest (mmHg)	33.1 [5.9]	33.1 [4.4]	33.1 [5.6]
Mean End-tidal CO_2 during Mod. Exercise (mmHg)	37.5 [4.9]	38.4 [3.2]	37.5 [4.9]

Table 2. Rest and Exercise Measures for CVRC, MAP and $ETCO_2$. All measures mean [standard deviation]. *p=0.04; †p=0.06

4.4.1. Vascular Measures. Our primary measure of cerebrovascular health was cerebrovascular reserve capacity (CVRC), the change in $MCAv_{mean}$ (80) between resting and moderate intensity exercise. We assessed the direct relationship of CVRC and amyloid burden. Lower CVRC was significantly associated with greater global amyloid burden (b=--9.3, p=0.03; **Figure 1**). That is, each increase in SUVR of 0.1 units was associated with a 0.9 cm/s decline in CVRC ([95% confidence interval [-17.6 -0.89]). This relationship held even when controlling for age and blood pressure (b=-10.4, p=0.02). We did find that CVRC was 36% lower for individuals with elevated amyloid compared to those who were non-elevated but was not significant (F=3.69, p=0.06).

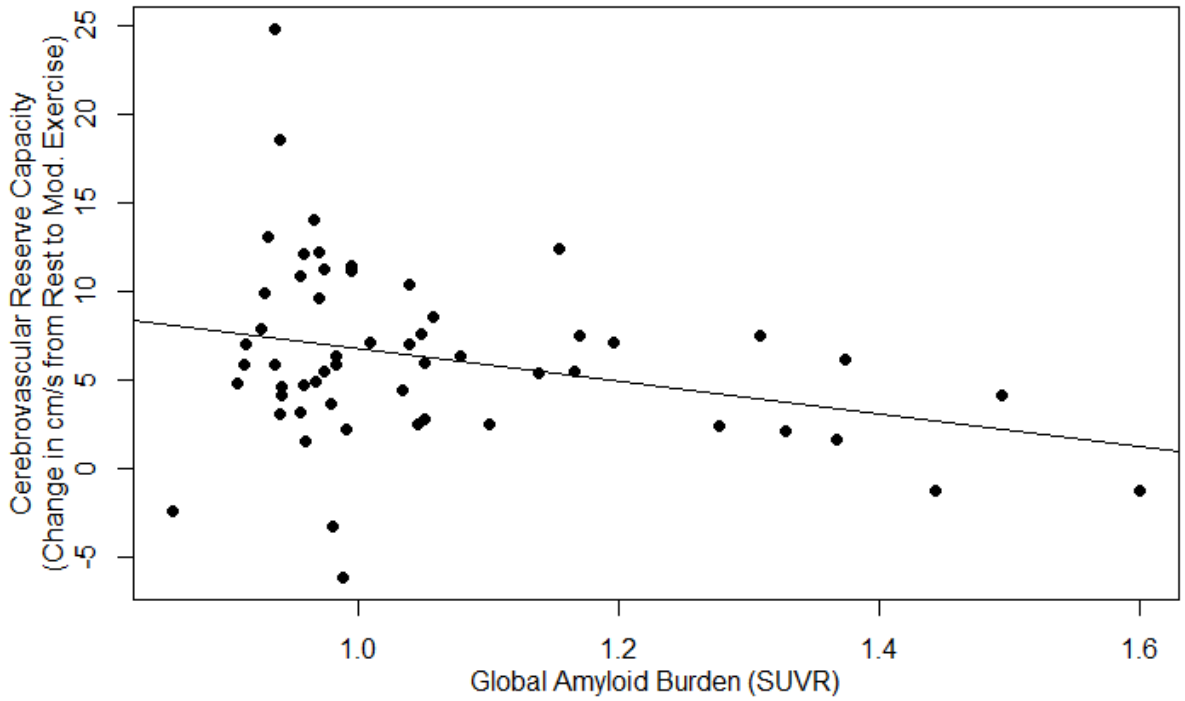


Figure 1. Cerebrovascular reserve capacity was associated with global amyloid burden.

4.4.2 Cognitive Measures. We then assessed the relationship of CVRC to our cognitive measures with linear regression of each cognitive factor on CVRC. Higher CVRC trended toward an association with better processing speed (b=0.03, p=0.06). This relationship was somewhat attenuated when controlled for age and blood pressure (b=0.02, p=0.11). No other cognitive domains were associated with CVRC. In contrast, greater global amyloid burden was associated with worse processing speed (b=-1.32, p=0.01), and memory (b=-2.31, p=0.008) and better language performance (b=1.26, p=0.044).

Given our findings we performed simple mediation analysis to see if CVRC was responsible for any part of the relationship of beta-amyloid and processing speed. In our sample, CVRC did not mediate the relationship of beta-amyloid and processing speed.

4.5. Discussion

The brain does not tolerate large increases in oxygen demand and blood flow delivery during exercise (194). However, the brain increases blood flow in response to the neuromuscular activation requirements of hand-grip exercise (195) and physical exercise (83, 169-176). Exercise-induced brain blood flow response is less understood in older adults who may be at risk for brain pathology. By conducting the present study, we aimed to understand whether the presence of beta-amyloid influences CVRC. Our primary finding was that lower CVRC was significantly associated with greater global amyloid burden in cognitively normal older adults.

Previous work in an animal model reported that mice (2-3 months old) with a genetic disposition for higher levels of amyloid precursor protein (APP) demonstrated disruption in cerebral autoregulation in response to pressure changes whereas wild-type showed no disruption

(180). Further, the APP+ mice with higher levels of beta-amyloid showed the greatest disruption in cerebrovascular regulation. Although this present study did not assess cerebrovascular autoregulation, we did examine changes in CVRC with concomitant changes in MAP from rest to moderate intensity exercise. Our findings in cognitively normal older adults demonstrate that lower CVRC was observed in those with higher levels of global beta-amyloid and are consistent with beta-amyloid-driven changes in cerebrovascular control seen in mice.

We divided the individuals into those considered beta-amyloid elevated and non-elevated to examine the CVRC response. Those in the elevated amyloid group (n = 14) exhibited a 36% lower CVRC response than those considered non-elevated amyloid (n = 42). We acknowledge that the non-elevated amyloid group had a significantly higher resting MAP but we report no differences between the groups for changes in MAP or $ETCO_2$ in response to exercise. Indeed, we would have expected to see greater CVRC of the elevated group as they increased their exercise MAP more over baseline than the non-elevated group. There may be other cardiovascular measures that could influence CVRC that we did not examine such as cardiac stroke volume, carotid-intima thickness, or arterial stiffness. We did capture cardiac risk and report similar representation across the 2 groups for moderate and high cardiovascular risk. Future work should include a comprehensive cardiac and vascular profile to extend upon this initial work. These findings suggest that greater level of global beta-amyloid may affect CVRC and have critical implications for cerebrovascular health in older adults.

A published review of the literature focused on cerebrovascular reserve and the potential implications of impaired cerebrovascular function in the progression to vascular dementia and Alzheimer's disease (168). In our study, we found that CVRC trended toward an association with better processing speed ($p=0.06$) but no other cognitive domains were associated with CVRC.

These results are encouraging since our older adults were considered cognitively normal. Individuals in the earliest stages of cognitive change, Mild Cognitive Impairment, may be a particularly interesting cohort to test for cerebrovascular associations with cognition. This would allow future work to determine whether CVRC could function as a surrogate marker of brain health or risk for converting to frank dementia.

The present study has several limitations. First, this study in humans did not focus on the mechanisms that may influence CVRC in those with elevated global amyloid. There may be other factors that influence cerebrovascular function such as cardiac function, hypertension and peripheral vascular dysfunction. Second, we did not use controlled methods for regulating changes in blood pressure or CO_2 when measuring MCAv mean across all subjects. Rather, we assessed each individual during moderate intensity exercise, which is influenced by individual responsiveness. However, we believe our methodology is a strength of the study as it presents more real-world challenges versus CO_2 gas inhalation. Finally, we did not account for individual *APOE* genotype. *APOE* is linked to higher risk of dementias and cerebrovascular diseases, and can therefore influence brain aging (196, 197). Thus, future investigations could determine how *APOE* impacts cerebrovascular responsiveness.

4.6. Conclusion

The findings of the present study demonstrate that lower CVRC in response to moderate intensity exercise is related to higher global amyloid burden. These results corroborate prior animal data whereby the cerebrovascular effects of beta-amyloid may have a role in the development of brain pathology.

Chapter 5

Discussion

5.1. Summary

Cerebrovascular function describes the interface of heart and brain functions. This body of work addresses important gaps regarding the role of cerebrovascular function in the aging brain. Cerebrovascular function is of tremendous interest to numerous fields, including, but not limited to, gerontology, physical therapy, rehabilitation medicine, psychology, neurology, and neurosurgery. The original aims of this work were to elucidate the links between resting cerebrovascular function and executive function; cerebrovascular function and brain structural integrity; and cerebrovascular function and a hallmark marker of Alzheimer's pathology, β-amyloid. However, secondary analyses of exercise-induced cerebrovascular functions revealed novel data which incrementally advances our understanding of cerebrovascular responsiveness and the links between cerebrovascular responsiveness, executive function, brain structural integrity, and β-amyloid. Hence, the findings are a prelude to further investigation into cerebrovascular responsiveness and its links to brain aging. Development of markers of cerebrovascular responsiveness may add supplemental information regarding brain aging. Long-term development of such markers may provide us with surrogate markers of brain pathological risk, i.e., a non-invasive marker of dementia risk. This could then serve as the basis for implementing brain-sparing interventions, particularly exercise interventions in high-risk populations, such as pre-clinical Alzheimer's individuals. Hence, the sum of this work lays the foundation for assessing whether longitudinal exercise-induced cerebrovascular functions could serve as potential indicators of brain aging.

5.1.1. Chapter 2: The partial relationships between cerebrovascular function at rest and during exercise in nondemented aging

The purpose of this initial experimental chapter was to establish a link between cerebrovascular function and one facet of cognition, executive function (EF). Both cerebrovascular function and EF decrease with age. Hence, we set out to establish correlations between greater cerebrovascular function, particularly hemodynamic measures, and better EF performance in a cohort of sedentary, nondemented older adults. To this end we obtained the following resting and exercise-induced cerebrovascular functions while participants were seated on a recumbent stepper: the resting mean middle cerebral artery velocity (MCAv mean); the resting cerebrovascular conductance index (CVCI); the exercise-induced percent change in MCAv mean, known as cerebrovascular reserve capacity (CVRC); and the exercise induced change in CVCI (CVCI percent change). We administered Trail Making Test Parts A and B (TMTA, TMTB) and calculated three iteration scores: age, sex, and education-normalized TMT B z-score (TMTB-Z); the delta, or difference, score between TMTA and TMTB (TMTD); and the ratio score of TMTB:TMTA (TMTR).

First, our results showed a direct relationship between resting MCAv mean and TMTR, contrary to our hypothesis, and thus indicates that greater resting brain blood flow velocity directly relates to poorer EF performance. However, further inspection of the cohort revealed that a) the MCAv mean was higher than normal compared to values previously reported in older adults; b) the cohort had smaller average white matter lesion burden compared to reported values in the literature, indicating less age-associated brain damage; and c) the cohort had better TMT performance compared to previously reported values in older adults. Thus, the cohort may have had relatively greater brain blood flow velocity and better EF performance compared to other

elderly cohorts. Second, we found a negative relationship between CVRC and TMTD. This supported our hypothesis and suggests greater cerebrovascular responsiveness is related to greater EF performance.

We report partial support for our hypotheses with these two findings as relationships were not found between all cerebrovascular measures and TMT iterations. Further, no relationships were found between resting and exercise-induced cerebrovascular functions and the TMTBZ, preventing us from generalizing the results to other nondemented, elderly populations. Nonetheless, this chapter lays the ground for further characterization of the relationships between cerebrovascular responsiveness and executive function. Ultimately, this may lead to a cerebrovascular marker sensitive to age-related executive decline, and this marker may be employed in identifying executive dysfunction during aging.

5.1.2. Chapter 3: Assessing white matter lesion burden relationships with cerebrovascular function in nondemented aging

White matter lesion (WML) burden is used as a marker for brain aging. There is contradictory evidence regarding the causative role of cerebrovascular dysfunction in WML burden development (145). Hence, the relationship between WML burden and cerebrovascular function is inconclusive. The purpose of the second experimental chapter was to assess relationships between resting and exercise-induced cerebrovascular function and WML burden using the same cohort of nondemented adults studied in Chapter 2. We calculated WML volume and WML count (quantity), and we conducted correlational analyses between these measures and the four cerebrovascular functions assessed in Chapter 2: MCAv mean, CVCI, CVRC, and

CVCI percent change. We expected WML burden to inversely relate to resting and exercise-induced cerebrovascular functions.

Our results did not demonstrate a relationship between WML burden and resting cerebrovascular function. Further, we did not find any relationships between WML volume and exercise-induced cerebrovascular function. However, we did find two relationships between WML count and exercise-induced cerebrovascular function. First, there was an inverse correlation between CVRC and WML count. Second, there was a direct correlation between the CVCI percent change decrease and WML count. This is the first report showing direct links between exercise-induced cerebrovascular function and WML burden in a cohort of healthy, older adults. Our results indicate that cerebrovascular responsiveness to exercise relates to WML burden quantity, thereby showing partial support for our hypotheses. The results are promising because this cohort had relatively lower than average WML burden and better than average cerebrovascular function. Thus, it is plausible this negative relationship would be greater in magnitude in clinical cohorts such as adults with mild cognitive impairment or dementia. Therefore, through further development, the CVRC and CVCI percent change could be used as markers of structural brain damage during the course of both healthy and pathological brain aging.

5.1.3. Chapter 4: Cerebrovascular reserve capacity is blunted during exercise in people with elevated β-amyloid

There is evidence suggesting that cerebrovascular function interacts with the β-amyloid, a protein hallmark of Alzheimer's disease pathology. However, little is known about how cerebral

β-amyloid levels affect cerebrovascular responsiveness. Therefore, in the third experimental chapter, we assessed exercised-induced cerebrovascular function in 56 nondemented older adults with and without elevated β-amyloid levels. We studied the MCAv at rest and during moderate intensity exercise, which allowed us to capture the cerebrovascular reserve capacity (CVRC). First, we expected that greater β-amyloid loads would associate with lower CVRC. Second, using the standard uptake value ratio (SUVR), a previously established criteria for quantifying β-amyloid load, we divided the older adults into 2 groups (elevated and non-elevated) to examine whether CVRC differed between groups. Lastly, we set out to explore the relationship of CVRC, β-amyloid burden, and cognition. We hypothesized that CVRC would mediate any relationship between β-amyloid burden and cognition in this cohort of older adults.

Our results showed that greater β-amyloid load was associated with worse CVRC, supporting our initial hypothesis. Second, there was a trend that the CVRC was lower in adults with elevated β-amyloid compared to those without elevated levels. Third, we found a trend between higher CVRC and faster processing speed, but this trend was dampened when the analysis was controlled for age and blood pressure. Fourth, we found that greater β-amyloid levels significantly related to slower processing speed, worse memory performance, and better language performance. Finally, contrary to our hypothesis, the CVRC did not mediate any relationships between β-amyloid and cognition. Thus we show that cerebrovascular responsiveness is related to β-amyloid. Therefore, further work could establish cerebrovascular responsiveness as a biomarker for dementia progression.

5.2. Discussion

5.2.1. Sample characteristics

By collaborating with the University of Kansas Alzheimer's Disease Center (KU ADC) we took advantage of the opportunity to study cerebrovascular function in a well-characterized cohort of non-demented older adults. These older adults were free of vascular disease, diabetes, orthopedic disability, and substance abuse. Although these adults were sedentary, they did not any have physician restriction against performing exercise. Hence, all the experimental chapters add to the literature regarding exercise responses in nondemented, sedentary older adults (198). Additionally, in Chapter 4, we further characterized the participants into one of two groups based on the level of an Alzheimer's pathological marker, β-amyloid. This effectively separated participants into a "preclinical" Alzheimer's group versus a lower Alzheimer's risk group. Thus, we were able to assess the impact of hallmark Alzheimer's pathology on cerebrovascular responsiveness, and we found a trend towards cerebrovascular responsiveness being lower in preclinical Alzheimer's (199). Hence, it is plausible that assessing older adults with mild cognitive impairment, vascular dementia, or dementia would reveal significantly diminished exercise-induced cerebrovascular responsiveness similar to the diminished CVRC seen using non-exercise stimuli (149, 200, 201).

5.2.3. Exercise Challenge

The exercise-induced increases in cerebrovascular function have been previously documented (8, 86, 146), but little is known regarding the extent exercise increases cerebrovascular function in older adults do to the relatively small samples of older adults studied.

Additionally, little is known regarding the cerebrovasculature response to exercise in individuals who have higher risk for brain pathology, such as individuals with preclinical AD. Cerebrovascular regulation is an intricate, integrative process that is driven by carbon dioxide, mean arterial pressure (MAP), neurovascular coupling, and autonomic nervous system functioning (33). All of these factors are profoundly changed by exercise challenge (83). Thus, employing the cerebrovascular response to an exercise challenge provides investigators the opportunity to study cerebrovascular responsiveness, at a higher temporal resolution than neuroimaging, and without the contraindications associated with neuroimaging and gas exposure (33, 61). A review by Querido & Sheel suggests that the exercise-induced increases in cerebrovascular function, measured via TCD, are dependent on intensity (83). We had the older adults perform one session of moderate intensity exercise on a recumbent stepper. Our results are in line with previous reports of exercise-induced CVRC values between 15-20% in nondemented older adults (84, 85). We are the first to show exercise-induced cerebrovascular responsiveness on a recumbent stepper related to executive function, brain structural integrity, and a β-amyloid. These links were found in a cohort of nondemented older adults; therefore, this work lays the foundation for future investigations to assess these relationships in not just nondemented older adults, but also older adults with stroke, mild cognitive impairment, and other dementias.

5.2.4. Cerebrovascular Function and Executive Function

Executive function (EF) is an umbrella term which encompasses various cognitive skills, such as task switching, planning, inhibition, working memory, sustained attention, and selective attention (97). Executive function is a major concern during aging because decreased EF is linked to decreased functional status in retired adults (202), and in some adults, executive

dysfunction precedes memory deficits prior to the diagnosis of AD (203). There is evidence of lesions in the frontal cortex, particularly in the frontal-striatal tracts underlying executive function, developing alongside medial temporal atrophy in areas underlying memory (102). Hence, capturing the early decline of executive function is important in identifying the time window of burgeoning memory impairment in adults who will first develop non-amnestic mild cognitive impairment, which could develop into dementia. However, there is no consensus regarding the biomarker which best captures the nascent executive dysfunction preceding the development of dementia.

At the advent of this project, our discussion focused on the use of cerebrovascular markers as biomarkers for EF. Decreased brain hemodynamics has been implicated in the development of executive dysfunction (92, 117). For example, Bailey and colleagues showed that adults with chronic traumatic brain injury had impaired cerebrovascular function, specifically a reduced CVRC after exposure to hypercapnic gas (92), and these individuals had diminished performance across the Trail Making Tests, which are widely-used measures of EF (143). Specifically, there was a 38% slower completion time in the TMTA and a 43% slower completion time in the TMTB, compared to controls (92).

In Chapter 2, we reported on the links between TMT performance and resting and exercise-induced cerebrovascular function. Originally, we proposed to use one iteration of the TMTB, specifically a z-score normalized for age, sex, and gender. The purpose of this was two-fold. First, we aimed to generalize the results with regards to older adults, but raw TMT scores vary from study to study and between education levels (133, 143). Hence, accounting for the influence of age, sex, and education affords the opportunity to generalize EF performance (119). Second, and related to the first justification, we recruited all the participants from one of the

NIH-designated Alzheimer's Disease Centers. The normative calculator was designed for use by these centers and for other studies utilizing any aspect of the Uniform Data Set (UDS) cognitive battery, which includes the TMT. The UDS designates the TMTBZ as the measure of EF. Therefore, by using the TMTBZ, we aimed to contribute data to a larger normative data set sensitive to demographic characteristics among the centers' participants. However, our TMTBZ results did not support our hypotheses, which thus limits the generalizability of the significant relationships we found between cerebrovascular function and EF.

The significant relationships we found were based on raw TMT data. Hence, we have partial evidence that the Trail Making Test is related to resting and exercise-induced cerebrovascular function, and the relationships between cerebrovascular function and EF is conditional upon the cerebrovasculature being at rest or being stressed via exercise. When at rest, greater cerebrovascular function relates to poorer EF performance, but greater exercise-induced cerebrovascular function relates to better EF performance. It is possible we did not find significant relationships between all cerebrovascular functions and all TMT scores due to greater resting brain blood flow velocity, better executive function/ higher raw TMT scores, and potential limited impact of white matter lesions observed in our cohort compared to previous reports. Nonetheless, by showing relationships between cerebrovascular function and EF in a cohort of relatively healthy older adults, we argue that cerebrovascular function markers may be useful in identifying burgeoning executive dysfunction in older adults.

5.2.5. Cerebrovascular Function and Brain Structural Integrity

In Chapter 3, we sought to elucidate the relationships between resting and exercise-induced cerebrovascular function and WML burden. The importance of this work is rooted in the lack of understanding how cerebrovascular function and WML burden interact with or cause one another (107, 145). Hence, by studying the relationship between exercise-induced cerebrovascular function and WML burden, we hoped to further our understanding by using exercise-induced measures of cerebrovascular responsiveness as new and potential markers of WML burden. We failed to reproduce prior work which showed inverse relationships between WML burden and resting cerebrovascular function (107, 150, 157). We suspect this is due to our cohort having lower than average WML volume compared to previous research using the Lesion Segmentation Toolbox software in older adults (131, 132). Further, factoring in the WML volume standard deviation would still indicate our cohort had smaller than average WML volume. Furthermore, our cohort had greater resting MCAv compared to previously published data (8, 204). Factoring the general health of the cohort (no vascular disease; control of hypertension with medication), it is reasonable to conclude the greater than average resting MCAv is indicative of healthier than average cerebrovascular aging. Having less WML burden and greater resting cerebrovascular function could preclude finding significant relationships, as the correlations would be based on a narrow distribution of both factors. Thus, our significant findings pertaining to exercise-induced cerebrovascular function and WML count is promising because we essentially show a link between more WML burden and worse cerebrovascular responsiveness in healthy older adults. Thus, future investigations can determine whether cerebrovascular responsiveness is more strongly related to WML burden in clinical populations.

5.2.6. Cerebrovascular Function and Amyloid Burden

The Amyloid Cascade Hypothesis (205) posits that increases in β-amyloid deposition is the crucial pathophysiological step leading to amyloid plaque creation, tau neurofibrillary tangle formation, neuronal death, and ultimately, dementia. This hypothesis has been the focal point of AD research for over 25 years (206), yet the failure of recent anti-β-amyloid drug trials (207, 208) suggests our understanding of the role of β-amyloid in brain aging is still unclear. Although individuals with elevated levels of β-amyloid are classified as having preclinical AD (199), elevated levels of β-amyloid does not necessarily precede the development of AD. For instance, post-mortem brain studies of cognitively-healthy older adults showed substantial levels of β-amyloid (209) and PET studies revealed up to 30% of cognitively normal older adults have substantial levels of β- amyloid (210, 211). However, β-amyloid does place individuals at higher risk for AD (212), and increased levels of cerebral β-amyloid in older adults are linked to decreased cognition (213). The purported role of the blood brain barrier in β-amyloid clearance (214), the binding of hemoglobin to β-amyloid (215), the role of β-amyloid in fibrinogen oligomerization (216), and the mediation of endothelial function (217, 218) by β-amyloid in animal models suggest an interaction between β-amyloid and vascular function. However, the human literature concerning this interaction is not fully understood, and little is known about how β-amyloid load directly impacts cerebrovascular function. Thus in Chapter 4, we sought to characterize the relationship between β-amyloid load and cerebrovascular responsiveness.

We leveraged the KU Alzheimer's Disease Center's (KU ADC) Florbetapir β-amyloid imaging. The Florbetapir tracer selectively binds to cerebral amyloid, thus allowing us to further characterize older adults into a non-elevated or elevated/preclinical AD group. At this dissertation's outset, we had originally proposed to study 17 older adults with elevated β-

amyloid compared to non-elevated controls matched for age (± 3 years), sex, and cardiovascular risk factors. The KU ADC had projected to annually screen and recruit 400 older adults, 100 of whom were expected to display elevated levels of β-amyloid. However, the recruitment efforts did not meet these projections. In two-and-half years, we have recruited 14 individuals with elevated amyloid. Thus, Chapter 4 presents the evolution of our original aim. Instead of studying 17 matched pairs, we looked at 56 older adults, and 14 had elevated amyloid levels. Our results suggest greater β-amyloid load relates with worse cerebrovascular responsiveness. Further, a trend showing lower CVRC in adults with elevated β-amyloid and a trend showing higher CVRC linked to faster processing speed provide further basis for investigating the impact of amyloid load on cognitive performance and cerebrovascular function.

5.3. Limitations

This body of work is not without limitations. First, most of our analyses looked at relationships between cerebrovascular measures and other indicators of brain aging. Hence, we cannot deduce possible causative mechanisms. Second, we used a cross-sectional design, which also prevents us from inferring mechanisms of causation. Nonetheless, the underlying theme of our aims was to establish relationships between resting and exercise-induced cerebrovascular function and not to establish causative relationships. By demonstrating relationships between cerebrovascular function and other indicators of brain aging, we set the rationale for longitudinal investigations, which could theoretically determine causative relationships. Third, we did not capture a wide range of brain aging. Our cohort was primarily made of adults in their mid-60s and early 70s, and only 9% of our cohort was over age 80. Hence, we captured a limited spectrum of nondemented aging. Further, brain hemodynamics begin to decline in the third

decade of life (30), so determination of cerebrovascular function being a long-term determinant of diminished brain aging requires investigations which look at cerebrovascular function decades before retirement age. Therefore, to adequately use cerebrovascular responsiveness as a marker of aging, we would need to recapitulate our findings in cohorts younger and older than our cohort.

5.4. Future Directions

5.3.1. Develop Exercise-induced Indices as Markers of Cerebrovascular Function

This work lays the foundation for using cerebrovascular responsiveness as markers of brain aging. Further use of exercise-induced cerebrovascular markers could provide additional knowledge regarding the aging brain. For instance, use of pulsatility index (PI) and resistive index (RI) percent changes could show how downstream vascular resistance is impacted by exercise (160, 219). The PI is calculated as the difference between peak systolic and diastolic velocities over the mean blood flow velocity, and the resistive index (RI) is the difference between peak systolic and diastolic velocity over the peak systolic velocity (219). Because downstream cerebrovascular resistance is normally low in order to maintain brain blood flow (219), knowing how exercise impacts these indices may help distinguish between healthy and pathological brain aging.

5.3.2. Characterize Executive Function in Relation to Cerebrovascular Responsiveness

As previously mentioned, we used a single, widely-used test to measure executive function, the Trail Making Test. However, it does not capture all facets of executive function,

such as attention. Within the Uniform Data Set, the attention factor score can be calculated using Digit Forward, Digit Forward Length, Digit Backward, and Digit Backward length scores (119). The Stroop task assesses inhibition, an executive function profoundly affected by frontal lobe damage (220). The KU ADC collects the Digits test and Stroop task performance. Thus, future cross-sectional and longitudinal investigations could employ the Trail Making Test, the Digit tests, and the Stroop task to capture more comprehensive details of executive function than this body of work.

5.3.3. Characterize Regional White Matter Lesion Burden and Cerebrovascular Responsiveness

Although we are the first to report relationships between exercise-induced cerebrovascular function and WML quantity, we cannot draw inferences as to where these WMLs occur. Specifically, periventricular and deep white matter lesions may influence cognitive decline differently in nondemented and demented aging (44). Thus, future investigations could determine if cerebrovascular responsiveness mediates the relationship between WML burden and cognitive aging.

5.4. Clinical Implications

This body of work has potential clinical implications regarding cerebrovascular health and brain aging markers. Particularly, stroke and dementias are conditions in which compromised cerebrovascular function are implicated in the decline of cognitive function (221-223). Studying exercise-induced cerebrovascular reserve could be of use to physical activity or exercise regimens aimed at staving off age-related cognitive and neural declines. Specifically,

the methodology we present here affords the ability to look at real-time cerebrovascular responses to exercise, and the MCAv-centered measures could be used as biomarkers of cognitive aging through further development. Thus, the cerebrovascular functions presented in this work could be used to assess how cerebrovascular responsiveness mediates the relationship between cognition and fitness (168, 224), giving investigators, and potentially, clinicians, cerebrovascular "targets" for which interventions could work through in order to ultimately preserve brain and cognitive function. For instance, further development of the exercise-induced CVRC may lead to its use as a non-invasive marker of dementia risk in adults physically able to perform exercise. Exercise interventions could then be aimed at high-risk populations, such as pre-clinical Alzheimer's individuals, and could be designed to enhance or preserve CVRC, in efforts to preserve brain and cognitive function. Hence, the sum of this work lays the foundation for using exercise-induced cerebrovascular functions as potential non-invasive indicators of brain aging.

5.5. Conclusion

In conclusion, the totality of this work addressed important gaps regarding cerebrovascular responsiveness in aging, and it laid the foundation for further development of exercise-induced cerebrovascular function. Such development could have implications in stroke and dementia research and treatment, impacting the rehabilitation, cognitive, and neurological fields. This work advances our understanding of cerebrovascular responsiveness and its links to executive function, brain structural integrity, and β-amyloid. Long-term development of the markers may provide us with surrogate markers of pathological brain aging. The findings are a

prelude to further investigation into cerebrovascular responsiveness and its links to and possible roles in the development of both healthy and pathological brain aging.

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Appendix

Dynamics of Cerebrovascular Function During Exercise

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Abstract

The dynamic response to a stimulus such as exercise can reveal valuable insights into systems control in health and disease that is not evident from the steady-state perturbation. However, the dynamic response profile and kinetics of cerebrovascular function have not been determined to date. We tested the hypotheses that bilateral middle cerebral artery blood flow mean velocity (MCA_V) increases exponentially following the onset of moderate intensity exercise in ten healthy young subjects. The MCA_V response profiles were well fit to a delay (TD) + exponential (time constant, τ) model with substantial agreement for baseline (L: 69, R: 64 $cm \cdot s^{-1}$, coefficient of variation, c.v. 11%), response amplitude (L: 16, R: 13 $cm \cdot s^{-1}$, c.v. 23%), TD (L: 54, R: 52 s, c.v. 9%), τ (L: 30, R: 30 s, c.v. 22%), and mean response time (MRT) (L: 83, R: 82 s, c.v. 8%) between left and right MCA_V as supported by the high correlations (e.g., MRT $r=0.82$, $P<0.05$) and low c.v.'s. Test retest reliability was high with coefficient of variations for the baseline, amplitude, and MRT of 3, 14, and 12%, respectively. These responses contrasted markedly with that of three healthy older subjects in whom the MCA_V baseline and exercise response amplitude were far lower and the kinetics slowed. A single older stroke patient showed baseline ipsilateral MCA_V that was lower still and devoid of any exercise response whatsoever. We conclude that kinetics analysis of MCA_V during exercise has significant potential to unveil novel aspects of cerebrovascular function in health and disease.

New & Noteworthy

Resolution of the dynamic stimulus-response profile provides greater understanding of underlying physiological control processes than steady-state measurements alone. We report a novel method of measuring cerebrovascular blood velocity (MCAv) kinetics under ecologically

valid conditions from rest to moderate intensity exercise. This technique reveals that brain blood flow increases exponentially following the onset of exercise with: 1. a strong bilateral coherence in young healthy individuals, and 2. reveals potential for unique age and disease specific profiles.

Introduction

Vascular control across different organs subserves a range of primary requirements from thermoregulation in the skin to blood filtration in the kidney and support of cellular energetics in the heart, skeletal muscle and brain. In each organ blood flow control must be regulated in accordance with the required function within an error tolerance presumably dictated by the extent of damage incurred to the organ or organism by hypoperfusion. The brain does not endure the up-to-100 fold increases in oxygen demand ($\dot{V}O_2$) incurred by vigorously contracting muscle(s). However, it supports extremely high oxidative function at rest and this increases in response to the neuromuscular activation requirements of physical exercise (83, 169-176).

Unlike skeletal muscle the brain is notably lacking in energy storage mechanisms (21, 225, 226) and increases its fractional oxygen extraction to ~50% rather than the 80-90% found in skeletal muscle (176, 227). Thus, once thought to be constant (228), there is now substantial evidence that cerebrovascular blood flow increases during exercise (83, 169-173, 175, 176, 229-231). Moreover that increase is highest, and laterally symmetrical, for cycling at moderate exercise intensities but reduced for vigorous and maximal intensities where hyperventilation and hypocapnia induce cerebral arteriolar vasoconstriction (83, 170, 174-176, 232, 233). Given the brain's lack of O_2 stores, and intolerance to anoxia, that elevated blood flow and oxygen delivery must be rapidly and precisely matched to the brain's metabolic requirements.

Whereas quantitative steady state responses of blood flow, ventilation or $\dot{V}O_2$ can provide valuable information, resolution of the response kinetics to a change in demand from rest to exercise, for example, can uncover sentinel features of the underlying control mechanisms. Such analyses are technically and mathematically challenging but also extremely rewarding. For instance, kinetics analyses of pulmonary and muscle $\dot{V}O_2$ have demonstrated that, in health but not disease (e.g., heart failure, Type II diabetes, COPD), the speed of the kinetics response at exercise onset is limited by mitochondrial function and not O_2 delivery (227, 234, 235). At the arteriolar level kinetics analyses of vasodilation (and vasoconstriction) have extended these findings and explain how O_2 delivery temporally and quantitatively matches oxidative demands in healthy young but not aged muscles (236-239). Similar analyses for ventilation have unveiled how the carotid body facilitates ventilatory dynamics and blood-gas and acid-base regulation across metabolic transitions (240). It is surprising therefore that cerebrovascular kinetics have not been studied to date.

The exercise hyperemia of the brain has been demonstrated with anterior and middle cerebral artery blood velocity (MCA_V) (169, 171-173, 175), and "steady-state" cerebral blood flow increasing by 10-30% following the onset of low and moderate-intensity exercise (171, 241, 242). It is also thought that life-long physical activity results in greater cerebral blood flow to support brain tissue, possibly through angiogenesis and improved cerebrovascular responsiveness to demand (243, 244). In this regard cerebral endothelial nitric oxide synthase levels are increased following an exercise training program in rodents (245, 246) supporting that chronic exercise elevates cerebrovascular control as seen for the coronary and skeletal muscle

vasculatures (247). It is not known whether such training improves either the speed or amplitude of the hyperemic response of brain blood flow in response to physical exercise or alternatively whether that response is altered or compromised by age and or diseases such as Alzheimer's, stroke, diabetes or heart failure, in part, because kinetics analyses of cerebrovascular blood flow have not been conducted.

The MCA was chosen as the vessel of interest for assessing the kinetics of cerebrovascular regulation since previous work has focused on the MCA during exercise (171, 241-243, 248, 249). Here we report the use of a novel analysis method for assessing the kinetics of cerebrovascular regulation to provide the first characterization of the dynamic cerebrovascular (specifically the middle cerebral artery blood mean velocity, mean MCA_V, (termed MCAv throughout) response to exercise. Specifically, we tested the hypotheses that: 1) following the onset of exercise MCA_V increases exponentially, 2) the parameters of this response (amplitude, time constant, mean response time) are not systematically different between right and left MCA, and 3) the kinetics profile and kinetics parameters may be substantially altered by age and disease (stroke). Specifically, we demonstrate the putative application of this technique for characterizing differences across age and disease states with the future purpose of identifying and testing therapeutic strategies for better preserving, or recovering, cerebrovascular and, potentially, brain function.

Materials and Methods

Ten healthy young adults with low cardiac risk (250) and three older adults without cardiovascular disease but considered high cardiac risk, in part, because of their age (250) were

recruited to participate in the study. One additional individual, 88 days post-ischemic stroke, was recruited as a case example of known cerebrovascular injury. No individuals were considered to be competitive athletes (Table 1 contains participant demographics). KU Medical Center Human Subjects Committee approved all experimental procedures, which complied with the *Declaration of Helsinki*. Institutionally approved written informed consent was obtained from each individual prior to participation in the study. We did not directly assess hormone level but pre-menopausal females exercised around the early follicular phase of the menstrual cycle (241, 251). Female participants over 65 were considered post-menopausal.

Participants were screened either over the phone or in person. Inclusion criteria were: 1) 20-85 years of age, 2) ability to perform repeated bouts of moderate intensity exercise, and 3) transportation to KU Medical Center for testing. Exclusion criteria were: 1) inability of study staff to acquire signal of the MCA using transcranial Doppler ultrasound, 2) inability to perform the alternating leg movements on the seated recumbent stepper (T5XR NuStep, Inc. Ann Arbor, MI), 3) diagnosis of Parkinson's disease, mild cognitive impairment, Alzheimer's disease or multiple sclerosis, and 4) pulmonary disease or dependency on supplemental oxygen. Before reporting to the laboratory at KU Medical Center, participants were asked to abstain from the following prior to testing: food for two hours prior to testing (241), caffeine for at least six hours, and vigorous exercise for twelve hours. The laboratory room for the experimental session was dimly lit, quiet and temperature maintained between 22-24 degrees Celsius (183, 184). External stimuli were kept to a minimum.

After written informed consent was obtained, resting heart rate (HR) was taken by a hand-held device (Tuffsat Ohmeda, GE Healthcare, Chicago, IL). Then the participant was familiarized with the equipment and procedures. All participants were instructed to breathe only through their nose during the experiments. A nasal cannula was placed in the participants' nares and if needed, adjustments to the position of the nasal cannula were made to ensure optimal end-tidal carbon dioxide ($P_{ET}CO_2$ in mmHg) reading (BCI Capnocheck Sleep 9004 Smiths Medical, Dublin, OH). The nasal cannula remained in place at rest and during exercise familiarization to allow the individual to practice breathing through their nose. During testing, participants were observed closely to ensure breathing was exclusively through their nose.

Participants practiced the reciprocal motion of the recumbent stepper at the prescribed rate of 120 steps per min. The recumbent stepper was used for this study since it is the modality of choice for older adults (252) and is often used with those after stroke due to motor and balance impairments (188, 253-255). Next, a target work rate was identified by setting the resistance to 40 watts and then increasing at a rate of 10 watts every 30 s until their target heart rate for moderate intensity exercise was achieved and maintained for one min. Moderate intensity exercise was defined as 45-55% of HR reserve calculated using the Karvonen formula and age predicted maximum heart rate (APMHR) of 220-age (250). Work rates estimated to be in the upper region of the moderate intensity domain were specifically selected because we wished to evoke the greatest increase in MCA_V (83, 170, 174-176, 232, 233). The lactic acidosis and consequent hyperventilation associated with vigorous and maximal intensity exercise were avoided because hypocapnia induces cerebral vasoconstriction and reduced cerebral blood flow (233). After the target work rate was found, study staff then completed ACSM cardiac risk stratification (250), non-exercise $\dot{V}O_2$max estimate questionnaire (256), participant

demographics and information pertaining to past medical history and physical activity participation (~20 min) allowing the HR returned to within 5 beats of resting.

Three laboratory members conducted the experimental sessions. One individual read instructions to the participant using a standardized script, the second team member monitored beat-to-beat blood pressure (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) and $P_{ET}CO_2$ and the third team member set up and monitored the transcranial Doppler ultrasound (TCD) (Multigon Industries Inc. Yonkers, NY), electrocardiogram (ECG) (Cardiocard, Nasiff Associates, Central Square, NY) and data acquired through an analog to digital data acquisition unit (NI-USB-6212, National Instruments) and custom written software operating in MATLAB (v2014a, The Mathworks Inc. Natick, MA).

Experimental Protocol

Set up

Participants were seated in the semi-recumbent stepper. Heart rate was monitored continuously using V5 on the ECG.

Blood Pressure. The left arm was placed on a padded table and was adjusted to ensure the arm remained at the level of the right atrium (241) and continuously monitored to ensure minimal movement during the experiment. Beat-to-beat BP was acquired from the left middle finger using a finger photoplethysmograph (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). Right arm brachial artery BP was assessed with the arm at heart level using an automated sphygmomanometer with microphone (Tango M2, Suntech, Inc. Morrisville, NC). This

allowed for comparison between devices to ensure accurate BP measures prior to data collection (241).

Middle Cerebral Artery Velocity (MCA_V). MCA_V was measured using TCD at rest and during exercise. Using an adjustable headband, 2MHz probes with ultrasonic gel were placed over the cranial temporal bone window (257). The MCA was accurately identified using practice standards for probe positioning and orientation, depth selection and flow direction (257). Depth and gain settings were adjusted to ensure optimal signal strength and then the probes were fixed in place.

Experiment

During the initial set up, the participants sat quietly for 20 min and were reminded to keep their arms and hands relaxed, breathe through their nose and to face forward. The recording period started with 90 s of rest. After 60 s of rest, the participant was informed that exercise would begin shortly. At 84 s, a visual countdown was provided for the participant to begin exercise. We chose to standardize exercise initiation while minimizing the ramp-up time to target intensity. All subjects began exercising at 60% of their target work rate for the subsequent moderate intensity exercise. We increased the watts at 10-s intervals using 1/3 the difference of the starting and target watts until the target power was achieved resulting in attainment of the target work rate 30 s into the transition. Subjects maintained this work rate for 6 min and then cooled down for 2 min. The participant rested quietly until HR reached +/-5 beats of their resting HR before subsequent exercise bouts. Subjects completed three rest-to-exercise transitions, which were temporally aligned to the start of exercise and then averaged. This procedure improves the signal-to-noise ratio and thus better reveals the underlying physiological response (258).

Test re-test reliability for the baseline (BL) and criterion kinetics measurements: response amplitude (AMP), time delay (TD), time constant (τ) and mean response time (MRT, TD + τ) were established for all eight younger participants across the three transitions.

Data Acquisition

All variables were sampled at 500 Hz. To analyze, the data were divided by R-to-R cardiac interval. For each cardiac cycle, mean finger arterial pressure calculated as area under the pressure curve (MAP in mmHg), mean left and right MCA_V ($cm \cdot s^{-1}$), $P_{ET}CO_2$, and HR (beats per min) were calculated. Data with R-to-R intervals greater than 5 Hz or changes in peak blood flow velocity greater than 10 $cm \cdot s^{-1}$ in a single cardiac cycle were considered not physiologically real and censored. Acquisitions with more than 15% of data points censored were discarded. MCA_V, MAP and $P_{ET}CO_2$ were then interpolated to 0.5 Hz using shape-preserving, piecewise cubic interpolation.

MCA_V Model Fitting

Kinetics analyses were conducted for the left and right MCA during exercise using 3 s time-binned mean values over the entire exercise bout with a mono-exponential model:

$$MCA_V(t) = BL + A\text{mp}(1 - e^{-(t - TD)/\tau})$$

where $MCA_V(t)$ is the MCA_V at any point in time, BL is the baseline before the onset of exercise, Amp is the peak amplitude of the response, TD is the time delay proceeding the increase in MCA_V, and τ is the time constant. Mean response time (MRT) was calculated as the sum of the model derived τ and TD. The total exercising MCA_V response (Tot) was calculated as the sum of BL and Amp. Time-to-63% of the steady-state response was assessed as a model-independent measure of the response. Specifically, this measurement provides a non-biased

check of the model fitting without making any assumptions regarding the temporal profile of change.

Statistical analysis

All curve fitting and statistical analyses were performed using a commercially available software package (SigmaPlot 12.5, Systat Software, San Jose, CA, USA). Differences in resting values, exercise responses, and kinetic parameters were analyzed using Student's paired t-tests. Differences between younger and older participants were analyzed using Student's unpaired t-tests. Normality was verified via the Shapiro-Wilk normality test. Differences were considered significant when $p < 0.05$. The limits of agreement between left and right MCA_V variables were investigated using the Bland-Altman method (259). Data are presented as mean ± standard error unless otherwise noted.

Results

Young healthy participants

Pertinent cardiorespiratory variables. Eight healthy young participants (6 female, 2 male) were included in the data analysis. Two subjects were excluded from kinetics analysis because a valid MCA_V signal was not acquired from both the left and right MCA. The work rate for the exercise transitions was 104 ± 5 (range: 85 - 125) Watts. Consistent with moderate intensity exercise (45-55% of HR reserve) from rest to exercise, HR increased from 86 ± 5 to 129 ± 6 beats·min^{-1} ($p<0.01$) and MAP increased 8 ± 4 mmHg ($p=0.08$). $P_{ET}CO_2$ was increased from rest 36 ± 1 to exercise 42 ± 2 mmHg ($p<0.01$).

The test retest reliability (n=8) was high with coefficients of variation for the BL, AMP, and MRT of 3, 14, and 12%, respectively.

Mid cerebral artery blood velocity (MCA_V)

Overall response profile. MCA_V increased from rest to exercise in all young subjects with a mean Amp of 15.5 ± 3.1 (range: 9.0 - 35.7) $cm \cdot s^{-1}$ for the left and 13.4 ± 2.1 (range: 7.4 - 25.6) $cm \cdot s^{-1}$ for the right MCA (p=0.21) (Table 2). As seen from the exemplar presented in Figure 1, MCA_V increased in a close-to-exponential pattern being well-fit by a delay + exponential function. This notion was supported by inspection of each individual response, analysis of the residuals (Figure 1, line at bottom of panel) and the high r^2 of the fits themselves: left MCA_V 0.85 ± 0.03 (range: 0.73 - 0.97); right MCA_V 0.82 ± 0.04 (range: 0.6 - 0.95). In addition, the coefficient of variation of the actual time-to-63% of the steady-state response with the MRT calculated by the model was a mere 5 and 11%, for the left (82.7 ± 4.1 vs 83.3 ± 5.2 s; p=0.78) and right MCA (81.9 ± 6.0 vs 82.1 ± 6.5; p=0.98), respectively, again indicating good model fit to the response. The delay was highly variable among subjects being 21.0 - 76.6 s for the left and 12.1 - 64.1 s for the right MCA (Table 2). Similarly τ varied from 13.6 - 52.8 s for the left and 12.8 - 53.8 s for the right MCA. No MCA_V parameter (i.e., BL, Amp, MRT, etc.) were significantly correlated with work rate or $\Delta P_{ET}CO_2$ (data not shown).

Figure 2 presents the best (top) and worst (bottom) model fits from our group of young healthy subjects to emphasize that, despite some variability among subjects, the technique is tenable.

Is there systematic lateral heterogeneity? As evident in Table 2 there was a close correspondence of the mean values between the left and right MCA for BL, Amp, TD, τ, or MRT although the coefficient of variation did differ among the parameters (i.e., BL 10.8%, Amp 22.8%, τ 22.1%, TD 8.7%, MRT 7.6%). Figure 3 demonstrates the close correlation for the overall response (MRT, $r = 0.82$, $P<0.05$) with this conclusion supported by the Bland-Altman plot (Figure 3, bottom panel). In addition when the absolute exercising total MCA_V was examined during exercise there was no difference between left (84.8 ± 4.8 cm·s^{-1}) and right (77.8 ± 4.7 cm·s^{-1}, $p=0.07$) with a coefficient of variation of 11.1%.

Older adult participants and post-ischemic stroke patient

Older adult participants (2 female, 1 male) were considered high cardiovascular risk due to reporting existing signs/symptoms of cardiac disease (ankle edema (n=2), orthopnea (n = 1)) and the following risk factors: age, sedentary lifestyle, hypertension and dyslipidemia(250). The work rate for the exercise transitions was 88 ± 7 (range: 70 - 90) Watts and trended towards being significantly lower than younger participants ($p=0.06$). In stark contrast to the young healthy participants, the older adults had a lower MCA_V BL in both the left (43.1 ± 5.2 cm·s^{-1}, $p=0.002$) and right MCA (44.7 ± 7.0 cm·s^{-1}, $p=0.03$) as well as a markedly slowed MRT for the left (117.9 ± 10.3 s, $p=0.009$) but not the right MCA (99.4 ± 7.1, $p=0.17$). Despite the lower work rate, BL, and slowed kinetics for these three older subjects, the AMP was not reduced significantly in either the left (10.4 ± 2.6 cm·s^{-1}, $p=0.37$) or right MCA (11.8 ± 2.4 cm·s^{-1}, $p=0.67$) compared to the younger participants. An older subject is represented by the open circles in Figure 4.

The patient had a right ischemic stroke in the MCA territory. A dramatic response for the right MCA_V was the extremely low BL (37.9 $cm \cdot s^{-1}$) and absence of any increase in MCA_V with exercise (Fig. 4). The signal for the left MCA was not attainable.

Discussion

The principal novel findings of the present investigation are aligned closely with our hypotheses. Namely, following the onset of moderate intensity exercise MCA_V in young healthy individuals increased, after a time delay, in a close-to-exponential profile to achieve an elevated steady-state within 2-3 min. The MCA_V values for BL, the response parameters (Amp, τ, MRT, TD and Tot) and exercising steady-state were not systematically different between right and left MCA with coefficients of variation between 8 and 23% and high correlation coefficients (up to 0.82 for MRT). The three older healthy individuals exhibited lower baseline and total amplitude of MCA_V response following the onset of exercise and notably slower MCA_V kinetics, with τ and MRT being well outside the range of their younger healthy counterparts (i.e., 2-4 standard deviations longer than the young average, Table 2). In marked contrast to the healthy individuals the stroke patient exhibited a baseline that was markedly lower than the healthy young or aged subjects and displayed absolutely no increase in MCA_V during exercise.

Given the current emphasis on cerebrovascular health in brain aging and disease, understanding the speed of these kinetics responses may have important clinical implications. There is mounting evidence that vascular disease, poor cerebral endothelial function and cardiac risk factors (hypertension, hyperlipidemia) contribute to vascular dementia and Alzheimer's disease (19, 260-265). We may find that resolution of the kinetics response to a change in physical

demand such as from rest to exercise can help clarify the interconnection between cerebrovascular health and brain function/dysfunction.

The present investigation suggests that there are not systematic differences in response kinetics or Amp between right and left MCA in healthy adults. This finding supports that either the left or right MCA can be used as an indicator of the kinetics response in both arteries during bilateral lower extremity exercise although this notion should be tested in larger and possibly more diverse populations. However, we do not know whether systematic lateral differences exist in the presence of neurologic injury such as stroke. Understanding whether true differences exist in the kinetics response between the ipsi- and contralateral vessels is an important future direction.

Experimental Considerations

Herein, we present what we hope will prove to be a sensitive technique for assessing the kinetics of cerebrovascular regulation with exercise (or other interventions). It is anticipated that determination of MCA_V kinetics will be especially valuable for investigating cerebrovascular function in those at risk for, or suffering from, neurologic disease. Detecting improvements in cerebrovascular function following therapeutic or exercise interventions especially as these relate to response kinetics as well as amplitude would be a powerful capability for refining/assessing such interventions objectively. That said we recognize important limitations that must be considered:

First, we are unable to measure changes in MCA diameter. The assumption of constant MCA diameter is critical for MCA_V to be used as a direct proxy for cerebral blood flow in the absence of direct diameter measures. Prior work has not permitted consensus as to whether MCA diameter changes with exercise or not (266, 267). Whereas there are some reports that MCA diameter is invariant under hypercapnic, hypocapnic and orthostatic challenges (72, 195, 249, 268-271) there is also evidence for MCA diameter changing dynamically with motor activity (272) and visual stimulation (268, 273), though fluctuations may be very modest in larger vessels such as the MCA (268, 273). It is also thought that cerebral vessels may undergo regular oscillation at rest (274). If so, a dynamic challenge such as exercise might be important for improving the signal-to-noise ratio for assessment of cerebrovascular function.

Second, the values presented in this investigation represent a small population and therefore should not be considered to span the normal range for cerebrovascular responses in young healthy adults. We provide cardiovascular risk and estimated $\dot{V}O_2max$ as descriptive measures of general cardiovascular health. A much broader range of ages and diseases must be characterized with this technique to approach generalizability, as appropriate. Additionally, the inability to demonstrate significantly lower response AMP in the older subject cohort was undoubtedly due to the small subject number and consequently low statistical power.

Third, the kinetics parameters reported here must, to some degree, be dependent upon the extant experimental conditions and not necessarily indicative of the capacity of the system to adapt to all moderate-intensity exercise challenges. For example, in order to minimize movement artifact we chose to increase work rate over 30 s rather than measuring the response to an

immediate step-increase to the intended work rate. It is likely that our kinetics parameters may have been different had we chosen a different forcing function. However, we specifically selected an exercise modality and test that does not compromise cranial stability and thus signal fidelity making them suitable for young healthy subjects as well as their older counterparts, and, crucially, many stroke patients.

Fourth, changes in MAP and also the partial pressure of arterial CO_2 (PCO_2) can impact MCA_V via alterations of driving pressure and also downstream vascular (arteriolar) resistance, respectively. For the moderate-intensity exercise used herein MAP increased 8 mmHg (P=0.08) during exercise. The correlation between ΔMCA_V and ΔMAP was not significant with <40% of the MCA_V variance being potentially explained by MAP. With respect to $P_{ET}CO_2$ it is important to note that $P_{ET}CO_2$ is not the same as arterial PCO_2 and the increase from 36 to 42 mmHg (P<0.01) is expected to arise from the altered breathing pattern and rate of CO_2 evolution rather than altered arterial PCO_2. Indeed, for moderate intensity exercise a substantial literature supports that humans regulate their arterial PCO_2 ~40 mmHg (275). As for MAP there was no significant correlation between changes in $P_{ET}CO_2$ and MCA_V. For our experimental purposes it was important to track $P_{ET}CO_2$ to ensure that the subjects did not hyperventilate (i.e., drive down $P_{ET}CO_2$) due to excitement, nervousness or some other non-specific ventilatory responses, which would have induced cerebral arteriolar vasoconstriction. As the $P_{ET}CO_2$ - arterial PCO_2 difference is inversely related to breathing frequency and directly related to tidal volume and CO_2 output, for the exercise intensity herein the work of Jones and colleagues (26) supports that an increase in $P_{ET}CO_2$ of 3-5 mmHg would be expected in the absence of altered arterial CO_2.

Perspectives

Current work in healthy adults considers that moderate intensity exercise may be beneficial for motor learning (276) while other studies report high or vigorous intensity results in improved performance (277, 278). If the premise for improving motor learning is related to increased cerebral blood flow, we need to consider what exercise intensity, duration and frequency evokes the greatest increase in cerebral blood flow. As mentioned previously, there is evidence that moderate exercise intensities do increase cerebral blood flow whereas cerebral arteriolar vasoconstriction reduces blood flow at higher exercise intensities due to the arterial acidemia and consequent peripheral chemoreceptor-mediated hyperventilation and hypocapnia (83, 170, 174-176, 232, 233). Future work should include the MCA_V kinetics response in addition to Amp and consider how they relate across specific age groups and chronic health conditions and whether there might be gender-related differences.

In conclusion, we have described a method for assessing the kinetics of cerebrovascular response to exercise. We present evidence for symmetrical vascular responses in young healthy subjects and preliminary data supporting notable differences in the MCA_V response kinetics parameters related to age and cerebrovascular damage. With the growing interest in exercise benefits for the brain ecologically valid methods for measuring cerebrovascular response kinetics across the transition from rest to exercise may become an established investigative technique. Future work should explore the reliability, sensitivity and specificity of this technique across populations of interest with a view to determining the potential efficacy of therapeutic interventions designed to improve cerebrovascular health and neurological function.

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