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Cerebrovascular Reactivity and the fMRI-BOLD Response in Cardiorespiratory Fitness

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Cerebrovascular Reactivity and the fMRI-BOLD Response in Cardiorespiratory Fitness

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Dedication

To my husband, Christian Velasco, and my parents, Ronald and Cynthia Gonzales, for their boundless love, support, and encouragement.

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Cerebrovascular Reactivity and the fMRI-BOLD Response in Cardiorespiratory Fitness

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Accumulating evidence indicates that poorer vascular health accelerates cognitive decline and increases the likelihood of dementia in old age. Aerobic fitness, as a protective factor against vascular dysfunction, may thus serve to attenuate age-related cognitive pathology. The overarching aim of the current investigations was to determine the impact of cardiorespiratory fitness on cognition and its underlying neural substrates. Sedentary and endurance-trained middle-aged adults underwent general health assessment, neuropsychological testing, and functional magnetic resonance imaging (fMRI) during a working memory task and a hypercapnic (breath-hold) challenge. As compared with sedentary age-matched controls, the endurance-trained adults displayed a trend towards better executive function performance and faster reaction time on the working memory task, indicating enhanced speed of information processing. The neural substrates underlying fitness-related cognitive enhancement were explored by examining the blood oxygen level dependent (BOLD) response to a 2-Back working memory task. Additionally, breath-hold calibration of the working memory task was performed in order reduce vascular variance and provide a closer approximation of the neural contributions

to the BOLD signal. After breath-hold calibration, the endurance-trained adults displayed greater working memory-related activation in the right middle frontal gyrus, indicating that fitness likely benefits the neural processes underlying cognition over and above global fitness-related changes in cerebrovascular reactivity. Finally, endothelial function was examined as a potential mechanism underlying fitness-related differences in cerebrovascular reactivity. Peripheral endothelial function failed to predict the BOLD signal to hypercapnia, suggesting that the response may be governed by nonendothelial-dependendent vasoregulators. In summary, higher cardiorespiratory fitness at midlife may increase executive function abilities by enabling greater recruitment of neural resources during challenging cognitive tasks. Longitudinal studies will be instrumental in determining if these fitness-related changes are capable of modulating the trajectory of cognitive decline across the lifespan.

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Chapter 1: Introduction

OVERVIEW

Maintenance of cognitive abilities is crucial for preserving quality of life, functional ability, and independence in old age (1). However, more than 24 million adults suffer from dementia and the prevalence is expected to more than double over the course of the next 20 years (2). Less than 5% of dementia cases can be clearly linked to genetic causes (3), highlighting the importance of identifying modifiable causes and risk factors. Accumulating evidence indicates poorer vascular health accelerates cognitive decline and increases the likelihood of dementia in old age (4). Engagement in regular aerobic exercise greatly attenuates age-related declines in arterial health (5), suggesting that maintenance of an active lifestyle may have a preventive effect against cognitive decline. Nonetheless, little is known about how physical exercise may impact the brain and preserve cognitive function. Of particular interest is understanding how the interaction between vascular health and the central nervous system translates into functional outcomes.

VASCULAR HEALTH, COGNTION, AND BRAIN STRUCTURE IN THE ELDERLY

Large epidemiological studies have provided substantial evidence linking poorer vascular health with diminished cognitive outcomes in old age. In a longitudinal study of over 7,000 elderly adults, the Rotterdam Study prospectively identified numerous vascular risk factors that increase the likelihood of dementia including diabetes mellitus (6), high fibrinogen concentrations (7), elevated serum homocystine (8), atrial fibrillation (9), and smoking (10). Moreover, a direct link between arterial dysfunction and dementia

has been established. A community-based study of 308 elderly adults found increased arterial stiffness in individuals with mild cognitive impairment, Alzheimer's disease, and vascular dementia as compared to those without cognitive impairment, a finding that persisted even after adjustment for other cardiovascular risk factors (11). Similarly, diminished endothelial function has been reported in patients with Alzheimer's disease (12). Even in the absence of dementia, poorer arterial health predicts diminished cognitive ability. Haley et al. (2007) (13) found that increased atherosclerosis was associated with poorer executive function performance, suggesting a loss of integrity to the subcortical networks within this domain.

Neuroimaging studies have bolstered the evidence linking poorer vascular health with cognitive vulnerability. Cardiovascular burden has been consistently associated with the number and size of white matter hyperintensities. White matter hyperintensities are areas of high signal intensity on T2-weighted MR images that may reflect myelin pallor, tissue rarefaction, and mild gliosis (14). A study of 1,077 elderly adults found that hypertension was associated with higher burden of both periventricular and subcortical white matter hyperintensities (15). In particular, those who had been diagnosed with hypertension for over 20 years had a 24.3 increase in relative risk for subcortical white matter hyperintensities and a 15.8 increase in relative risk for periventricular white matter hyperintensities. Similarly, higher glycated hemoglobin values (16) and endothelial dysfunction (17) predict more pervasive white matter hyperintensities in the elderly. The presence of white matter hyperintensities is clinically significant. A recent meta-analysis of 46 longitudinal studies found that white matter hyperintensities are associated with increased risk of stroke, dementia, and mortality, as well as more precipitous declines in global cognitive performance, executive function, and processing speed (14).

Cardiovascular risk factors are also associated with cerebral atrophy in old age. In spontaneously hypertensive rats (SHRs) overall brain tissue volume and cortical thickness is reduced by 11 to 25% in comparison to Wistar-Kyoto controls (18). Similarly, Strassburger et al. (1997) (19) found that elderly hypertensive adults had significantly larger ventricles and smaller cerebral volumes than normotensive controls, indicating that vascular burden accelerates brain pathology.

VASCULAR HEALTH, COGNITION, AND BRAIN STRUCTURE IN MIDLIFE

Poor vascular health appears to exert its detrimental effects on cognition and cerebral health as early as midlife. The impact of midlife cardiovascular burden on cognition is illustrated by the Honolulu-Asia Aging Study, in which over 3,000 middle-aged men underwent cardiovascular assessment followed by cognitive testing 25 years later (20). The risk of impaired cognitive performance in old age increased by 5% with every 10 mmHg rise in systolic blood pressure at midlife even after controlling for age, education, and co-morbid disorders. Within the same cohort, higher cholesterol (21), body mass index (22), C-reactive protein (23) and smoking (24) at midlife have similarly predicted diminished cognitive outcomes in old age.

Longitudinal studies have also established a link between poor midlife vascular health and accelerated brain pathology. In particular, studies of identical twins are instrumental since they remove variance due to genetic risk. Utilizing this paradigm, Carmelli et al. (1999) (25) found that the twin with more severe vascular burden at midlife had a greater extent of white matter hyperintensities 35 years later as compared to their identical sibling. Moreover, the degree of white matter hyperintensities predicted

poorer cognitive abilities within the domains of memory, processing speed, and balance as compared to the co-twin, controlling for educational status.

The impact of poor vascular health on the brain at midlife has also been examined with neuroimaging. An MRI study of 134 middle-aged adults found that higher resting systolic blood pressured predicted lower grey matter volume in the supplementary motor cortex, the superior frontal gyrus, the right anterior cingulate cortex, and the left middle temporal gyrus (26). Similarly, a study of adults, ages 30-70 years, found that hypertensive subjects had smaller cortical volumes and poorer cognitive performance than age-matched controls (27).

While MRI enables structural brain changes to be observed, functional MRI (fMRI) provides an index of the cerebral hemodynamic response to cognition. During cognitive engagement, oxygenated hemoglobin is delivered to activated areas in excess of neuronal oxygen consumption, resulting in a detectable rise in the blood oxygen level dependent (BOLD) signal (28). FMRI has the advantage of being highly sensitive to early changes in the neural substrates supporting cognition and can detect alterations before clinically significant cognitive impairment is evident. A series of fMRI studies have documented that midlife cardiovascular risk factors are related to lower BOLD response during cognition (29–31). Gonzales et al. (2010) (30) found that poorer peripheral endothelial function was related to reduced task-related activation in the right superior parietal lobe. Similarly, Hoth et al. (2010) (31) reported that middle-aged participants with metabolic syndrome, a disorder reflecting a cluster of cardiovascular risk factors, displayed lower BOLD response in the right superior frontal, right superior parietal, and

inferior parietal lobes. In both studies, lower task-related activation was associated with poorer task performance, potentially indicating reduced ability to engage neural resources during cognitive challenges.

Poorer vascular health at midlife may be particularly problematic because it results in a longer cumulative lifetime exposure to the condition. Interventions at midlife are critical since they present an opportunity to intervene before clinically significant cognitive loss has ensued. Over the course of the lifespan, the arteries progressively harden (5), increasing vascular burden (32). However, engagement in regular physical activity attenuates age-related vascular dysfunction (5), suggesting that exercise may help preserve cognition and cerebral health throughout the lifespan.

CARDIORESPIRATORY FITNESS AND COGNITION

Large epidemiological studies have provided preliminary evidence that engagement in regular physical exercise can help maintain cognition across the lifespan. Larson et al. (2006) (33) collected self-report data from 1,740 elderly adults without cognitive impairment on the number of times per week that they engaged in physical activity for more than 15 minutes. Six years later, the individuals who exercised less than three times a week at baseline had a higher incidence of dementia (19.7 per 1,000 person year) compared to those who exercised more (13.0 per 1,000 person year). Additional evidence for the protective role of aerobic exercise comes from the Canadian Study of Health and Aging, which prospectively examined 4,615 cognitively-intact elderly individuals (34). Among women, self-reported engagement in moderate or high amounts of physical activity was associated with lower risk of cognitive impairment five years

later. In particular, women in the high physical activity group showed the greatest protection, displaying a 50% reduction in risk for cognitive impairment without dementia and a 60% reduced risk of dementia. However, no protective effects of physical activity were observed in men.

While these studies have the benefit of large sample sizes and prospective designs, they also have several limitations - the largest being the lack of objective measures of cardiorespiratory fitness. Self-reported physical activity may yield different results depending on the wording of the question, whether aerobic versus non-aerobic exercise is specified, and the amount of detail on duration, intensity, and frequency of exercise (35). Not surprisingly, self-reports of physical activity have only a moderate correlation with objective cardiorespiratory fitness tests (r=0.40-0.61) (36).

Fortunately, several smaller studies have conducted objective cardiorespiratory fitness tests, some utilizing a randomized trial design. In a sample of 349 adults over the age of 55 years, Barnes et al. (2003) (37) assessed cognitive status with the modified Mini Mental Status Exam (mMMSE) and cardiorespiratory fitness by calculating peak oxygen consumption (VO_2max) during a treadmill test. At the 6-year follow-up period, lower VO_2max at baseline predicted steeper declines on the mMMSE and poorer global cognitive and executive function performance.

The impact of fitness on cognition has also been examined with exercise intervention studies, which control for the possibility of pre-existing between active and sedentary individuals. A meta-analytical study by Colcombe and Kramer (2003) (38) revealed that exercise intervention had a positive moderate sized effect on cognition

(effect size = 0.48) with the largest gains in the executive function domain (effect size = 0.68). Additionally, brief aerobic exercise programs of 1-3 months were found to improve cognition as much of those lasting 4-6 months, suggesting that even short interventions are sufficient to observe cognitive benefits. Heyn et al. (2004) (39) conducted a meta-analysis to determine if exercise interventions could improve cognition in individuals with cognitive impairment and dementia. Similar to Colcombe and Kramer (2003), they found a moderate effect of physical fitness on cognition (effect size = 0.57), indicating that even individuals with neurodegenerative diseases may benefit from regular physical activity.

CARDIORESPIRATORY FITNESS AND BRAIN STRUCTURE AND FUNCTION

A small, yet growing area of research has revealed that cardiorespiratory fitness induces structural and functional changes in the brain. Colcombe et al. (2006) (40) randomly assigned sedentary elderly adults to a six-month walking or stretching control group. After the intervention, the participants in the control group displayed no changes in brain structure. However, individuals in the walking group showed significant increases in grey and white matter throughout the brain. In particular, regional brain volume increased in three primary areas; the dorsal anterior cingulate and supplementary motor cortex, the right inferior and middle frontal gyrus, and the left superior temporal lobe. Interestingly, these regions of the brain are most vulnerable to decline due to healthy aging and vascular burden (41), suggesting that physical exercise may attenuate age- and disease-related cerebral atrophy. Higher cardiorespiratory fitness level has also been associated with greater fractional aniosotrophy (FA) in the uncinate fasciculus and

the cingulum, indicating that fitness may enhance white matter integrity in tracts fundamental for higher cognitive functions through their connections with the prefrontal cortex (42).

FMRI studies have provided preliminary evidence that aerobic fitness may modulate the neural substrates underlying cognition. Colcombe et al. (2004) (43) randomly assigned older adults to an aerobic fitness or stretching control group. Before and after the intervention, participants underwent fMRI during completion of the Ericksen Flanker task (44), a paradigm that engages attentional control and inhibitory processes. After the intervention, those in the aerobic exercise group demonstrated a larger reduction in behavioral conflict than controls (11% vs 2%). Moreover, they displayed greater task-related activation in the middle frontal gyrus, the superior frontal gyrus, and the superior parietal lobe, areas necessary for higher cognitive processes especially attention (45). These results provide provocative evidence that fitness may alter the recruitment of neural resources during cognitive challenge, which may translate to better cognitive performance.

MECHANISMS UNDERLYING FITNESS-INDUCED CHANGES IN COGNITION AND CEREBRAL HEALTH

As reviewed above, empirical research indicates that higher cardiorespiratory fitness is associated with superior cognitive performance (33,37–39), greater brain volume (40), and changes in functional brain response to cognition (43). A comprehensive understanding of the mechanisms that govern the fitness-related changes is crucial for the development of targeted interventions to preserve cognitive function

across the lifespan. To date, the primary research on this topic has been conducted with animal studies due to the necessity of invasive produces. In general, the literature supports two primary mechanisms for fitness-related improvements; neurogensis and angiogenesis.

Neurogenesis, the birth of new neurons, has been demonstrated in response to aerobic exercise in the dentate gyrus (46–48), a sub-field of the hippocampus important for the formation of new memories. Van Praag et al. (2005) (46) gave aged mice (19 months) with and without running wheel access daily injections of bromodeoxyuridine to label the emergence of new cells. Histological examinations revealed that the active mice had more new neurons in the dentate gyrus. Furthermore, increased neurogenesis correlated with improved spatial navigation performance on the water maze task. Despite this compelling evidence, there is some controversy as to whether neurogenesis is sufficient to enhance cognition. For example, Rhodes et al. (2003) (49) found that exercise increased neurogenesis in mice without concurrent improvements in spatial navigation performance. Additional skepticism for the role of neurogenesis on cognition in humans comes from discrepant findings between animal and human studies. Animal studies have indicated that exercise-induced neurogenesis is specific to the hippocampus (46–49), whereas structural brain changes in humans have been observed primarily in the frontal and parietal regions (40). Furthermore, the most robust fitness-related cognitive benefits in humans occur in the executive function domain (38), which do not typically engage the hippocampus.

Angiogenesis, the formation of new blood vessels, is the other factor that is hypothesized to govern fitness-related cognitive enhancement. In rodents, aerobic exercise upregulates the production of cerebral insulin-like growth factor 1 (IGF-1) (50), a trophic factor that stimulates the development of new vasculature. Moreover, pharmacological inhibition of IGF-1 has been found to prevent exercise-related increases in c-FOS, a marker of neuronal activity (51). Exercise-induced angiogenesis has also been demonstrated in rodents with MRI. Rats that were given access to running wheels for 30 days showed reduced proton signal intensity in the motor cortex on T2*-weighted images, indicating increased capillary perfusion or enhanced cerebral blood volume (52). In the same study, flow-alternating inversion recovery (FAIR) fMRI was performed to measure blood flow. While basal cerebral blood flow remained unchanged as result of running, cerebral blood flow increased after exposure to high levels of carbon dioxide (CO_2), suggesting higher capillary reserve.

Similar cardiorespiratory fitness-related benefits in cerebral blood flow have been reported in humans. Rogers, Meyer, and Mortel (1990) (53) first demonstrated this in a four-year longitudinal study on recent retirees, discovering that the retirees who remained physically active did not display the declines in cerebral blood evidenced by the sedentary participants. A more recent study used transcranial Doppler ultrasound to measure middle cerebral artery blood flow in 307 normotensive men sedentary and endurance-trained men between the ages of 18-79 years (54). Middle cerebral artery velocity progressively declined with age regardless of fitness status. However, the endurance-trained men displayed an approximately 10-year reduction in age-related

decline. The impact of cardiorespiratory fitness on cerebral blood flow has also been assessed in women. A study of sedentary and endurance-trained post-menopausal women examined the relationship between fitness and cerebrovascular conductance (55), a measure of blood flow in the right middle artery after controlling for mean arterial pressure. Cerebrovascular conductance decreased with age, but correlated with higher cardiorespiratory fitness at rest and after exposure to CO_2. The results indicate that fitness may increase baseline cerebral blood flow, as well as enhance the ability to recruit capillary reserve under conditions of high demand. Increased cerebral blood flow may be a mechanism for exercise-related improvements in cognition as reductions in cerebral blood flow are associated with poorer cognitive function in healthy elderly (56) and those with Alzheimer's disease (57).

NITRIC OXIDE AND REGULATION OF CEREBRAL BLOOD FLOW

In order to learn how exercise may increase cerebral blood flow, it is important to understand the mechanisms controlling cerebral vasoreactivity. In the periphery, arterial relaxation and constriction is regulated by the endothelium, a single layer of cells lining the lumen of vascular beds that separates the vessels from circulating blood. The endothelium governs vascular tone through the release of vasoconstrictive and vasoactive agents, one of the most important being nitric oxide (58). Nitric oxide synthases (NOS) uses L-arginine to intracellularly produce nitric oxide, where it can then bind and activate guanylate cyclase. This stimulates the release of cyclic guanine monophosphate (cGMP), initiating a molecular cascade that results in relaxation and vasodilation of smooth muscle cells (59). Similarly, in the central nervous system, NOS is released from the endothelium

and from cells in the neuronal parenchyma (60), where it can exert a powerful influence on cerebral vascular tone. Numerous in vitro and in vivo animal studies have demonstrated that inhibition of nitric oxide attenuates cerebral blood flow (61,62). White et al. (1998) (63) was the first to demonstrate this effect in humans. They found that exposure to NG-monomethyl-L-arginine (L-NMMA), a NOS inhibitor, resulted in a dose-dependent reduction in basal cerebral blood flow. Their findings demonstrate the importance of nitric oxide on resting cerebral blood flow in humans.

Nitric oxide is also involved in cerebral blood flow regulation, the ability of the vessels to maintain constant pressure under changing conditions. Mechanoregulation refers to the maintenance of near constant cerebral blood flow across a range of perfusion pressures, whereas chemoregulation refers to regulation during metabolic changes such as fluctuations in CO_2 levels (64). In healthy humans, exposure to high CO_2 levels (hypercapnia) increased cerebral blood flow by 50% and reductions in CO_2 levels (hypocapnia) induced a 35% decrease in cerebral blood flow (65). Nitric oxide appears to be particularly influential on chemoregulation. Lavi et al. (2003) (66) used transcranial Doppler ultrasound to measure cerebral blood flow velocity during hypercapnia and hypocapnia before and after infusion of the nitric oxide donor, sodium nitroprusside. Infusion of sodium nitroprusside enhanced the vasodilatory response to hypercapnia and blunted the vasoconstrictive effect of hypocapnia, indicating that nitric oxide modulates the cerebral blood flow response to metabolic changes.

This finding bears important implications for cognition since brain activation increases energy demand, necessitating a rise in regional cerebral blood flow (67). Poor

vascular health impairs cerebral autoregulation (68,69), which may induce cognitive deficits (70) and cerebral damage in the form of white matter hyperintensities (71). These consequences may be secondary to impairments in nitric-oxide vasodilation. A study comparing individuals with hypertension and/or diabetes mellitus to healthy controls found that those with cardiovascular risk factors had poorer peripheral endothelial function, as well as attenuated vasodilatory response to hypercapnia in the cerebrovasculature (72). The deficits in vasodilation could be reversed with sodium nitroprusside infusion, suggesting that reduced nitric oxide availability may be a primary mechanism for cerebral regulation impairments.

AEROBIC EXERCISE AND CHANGES IN NITRIC OXIDE

As summarized above, poor vascular health may be linked to reduced cerebral blood flow regulation and poorer cognition through its detrimental impact on nitric oxide availability. Substantial evidence indicates that poorer vascular health predicts diminished nitric oxide availability in the coronary, brachial, and cerebral arteries (72–74). In contrast, aerobic exercise enhances endothelial function (75) and stimulates nitric oxide production (76). For example, endurance-trained older adults do not display the typical age-related decrements in brachial endothelial function. Moreover, DeSouza et al. (2000) (75) reported that a three-month aerobic exercise intervention was sufficient to increase forearm blood flow response by 30% in previously sedentary middle-aged and older men, indicating that exercise can restore endothelial function. Fitness-related improvements in endothelial function are presumed to be secondary to nitric oxide release generated by increased blood flow during aerobic exercise. In support of this

hypothesis, a canine study found that aerobic exercise upregulated the expression of endothelial NOS mRNA, resulting in enhanced nitric oxide release in response to acetylcholine (76).

While the studies summarized above examined the peripheral arteries, evidence suggests that similar mechanisms occur in the cerebrovasculature. Strong correlations in endothelial function have been found in diverse vascular beds when similar assessments are used (77). Additional support comes from a transcranial Doppler study demonstrating that cardiorespiratory fitness level correlated with cerebrovascular conductance during hypercapnia (55), a mechanism regulated by nitric oxide (66). Thus, exercise-related improvements in endothelial function may be a viable mechanism for increasing cerebral blood and enhancing cognitive performance.

SUMMARY

In conclusion, cardiorespiratory fitness enhances cognitive performance (33,37–39) and attenuates age-related cerebral atrophy (40). The overarching aim of the current investigations is to determine the impact of cardiorespiratory on cognition and its underlying neural substrates. Of particular interest is understanding how the interaction between vascular health and the central nervous system translates into functional outcomes. To achieve this aim, sedentary and endurance-trained middle-aged adults will complete a general health assessment, neuropsychological testing, and functional magnetic resonance imaging (fMRI) during a working memory task and a hypercapnic (breath-hold) challenge. The neural substrates underlying fitness-related cognitive enhancement will be explored by examining the blood oxygen level dependent (BOLD)

to a working memory task. Moreover, a breath-hold calibration of the working memory task will be performed in order to reduce the variance of vascular factors on the BOLD signal and improve inference about the underlying neural response. Finally, peripheral endothelial function will be assessed as predictor of cerebrovascular reactivity as assessed by the BOLD response to hypercapnic challenge. The results from the studies are anticipated to elucidate the mechanisms underlying fitness-related cognitive enhancement, which may bear importance for the development of targeted interventions to preserve cognitive function across the lifespan.

Chapter 2: Methods

This chapter describes the methods and data collection procedures that were common to the three presented studies. All of the presented studies relied on the same pool of participants, a group of middle-aged adults between the ages of 40-65 years who were classified as either sedentary or endurance-trained. Participants underwent a streamlined sequence of three to four study visits, which are described in more detail below.

PARTICIPANTS

Sedentary and endurance-trained adults between the ages of 40-65 years were recruited from the greater Austin community with flyers and advertisements. Participants were initially classified into sedentary or endurance-trained groups based on their exercise behavior responses during the telephone screening. Participants were classified as endurance-trained if they reported engagement in cycling and/or running at a moderate or strenuous exercise intensity at least four days per week. Participants were classified as sedentary if they reported no regular engagement in physical exercise over the past two years. Aerobic fitness status was later verified with self-report on the International Physical Activity Questionnaire (IPAQ) Short Form (78), as well as objective assessment with a maximal oxygen consumption test (79). The final sample was composed of thirty-two endurance-trained participants and twenty-four sedentary participants. Participants self-identified as the following: 83.3% - Caucasian, 5.6% - African American, 3.7% - Hispanic, 1.8% - Asian and 5.6% - Other/Did Not Specify.

Inclusion/Exclusion Criteria

In addition to the age and fitness status requirements described above, enrolled participants were screened for eligibility on the following inclusion and exclusion criteria. The specific inclusion criteria were:

Right Hand Dominance: Only right-hand dominant individuals were included since neuroanatomical processing of language can be less accurately predicted in left-handed individuals (80). Given the verbal nature of the fMRI task, such variance would hinder group fMRI analysis. Hand dominance was assessed with observation and completion of a handedness questionnaire.

English Language Fluency: Participants were required to be fluent in English because the entire set of stimuli, instructions, and neuropsychological tests were written and standardized in English.

Specific exclusion criteria were:

Chronic diseases: Chronic diseases such as renal insufficiency, coronary artery disease, and diabetes are known to affect vascular response in the both the periphery and the brain (81–83), potential confounding the effects of cardiorespiratory fitness status. Medical conditions were assessed with telephone screening and via an in-depth medical history questionnaire. An objective assessment for the presence of diabetes (fasting glucose>126 mg/dl) was performed at the medical screening visit.

Current or past neurological and/or psychiatric conditions: Conditions such as stroke, seizure, attention deficit hyperactivity disorder, alcohol/drug abuse, and head injury with

loss of consciousness are known to affect brain structure and function (84–88). These conditions were assessed with telephone screening and via medical history questionnaire.

Current or recent history of smoking: As smoking has been demonstrated to affect the vasculature (89), participants were required to be abstinent from smoking for at least one year prior to recruitment. Smoking habits were assessed during the telephone screening and in the medical history questionnaire.

Current use of vaso-active medications: Due the importance of accurately assessing the impact of aerobic fitness level on vascular function, individuals on vasoactive medications such as anti-hypertensive medications and lipid-lowering drugs were excluded. Medication usage was assessed during the telephone screening and on the medical history questionnaire.

Contraindications for aerobic exercise: Since participants were required to complete a maximal aerobic fitness test, individuals who have medical restrictions on physical exercise or who have experienced potentially adverse events during exercise such as chest discomfort/pain, unusual shortness of breath or fatigue, and sensations of heart beat skipping/palpitations, were excluded. These symptoms were assessed during the telephone screening. Sedentary participants were also required to undergo a diagnostic electrocardiogram (ECG) while performing an incremental submaximal exercise test under physician supervision in order to ensure safety during the maximal aerobic fitness test.

MRI contraindications: In order to undergo MRI, participants were required to be free of MR contraindications including electronically or magnetically activated implants, any

type of ferromagnetic metal in the body, and claustrophobia. Participants were screened for MRI contraindications over the phone at initial contact and again prior to the scanning session.

Current or possible pregnancy: MRI is not currently performed on women who are pregnant, except in the case of medical emergency. Thus, women who were pregnant or potentially pregnant were excluded. Female participants were asked to provide the date of their last menstrual period in order to confirm their status.

EXPERIMENTAL PROCEDURES

The study was conducted in accordance with the Helsinki Declaration of 1975 and with approval from the local Institutional Review Board. Interested individuals contacted the lab and were administered a brief screening form over the phone. Individuals who met the eligibility criteria were enrolled in the study upon providing written informed consent. Enrolled participants then underwent a general health assessment, stress test (if sedentary), cardiorespiratory fitness assessment, and neuropsychological/brain imaging assessment (Figure 1). Assessments were conducted on separate days and participants completed the study within two months.

Figure 1

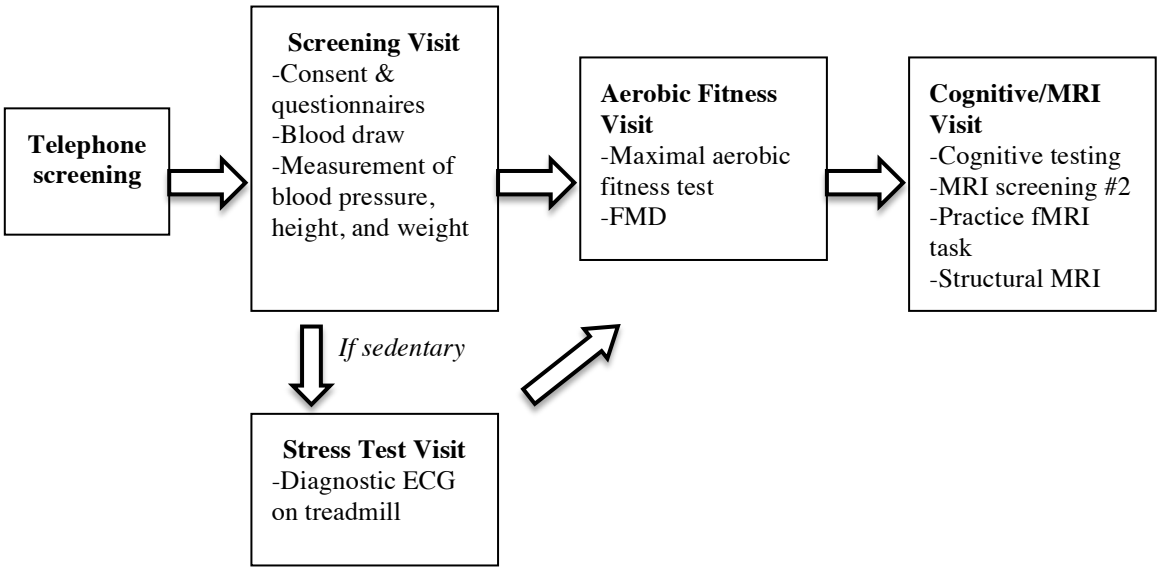

Figure 1. Experimental Overview

Telephone Screening

Individuals interested in the study contacted the lab and were administered a short screening form (Appendix A) over the telephone to determine eligibility. Questions on regular physical exercise, MR contradictions, handedness, smoking, current medications, medical history, age, weight and height were collected over the phone prior to study enrollment. Interested participants who were deemed eligible were mailed a packet with consent forms, maps, and the medical history questionnaire. Information from ineligible participants was shredded immediately unless interest in future studies was expressed.

Screening Visit

Participants were instructed to refrain from exercise and the consumption of caffeine and alcohol for twenty-four hours prior to their visit. They were also asked to fast for twelve hours prior to their scheduled appointment time. At this visit, participants completed a medical history questionnaire. Additionally, a fasting blood draw and measurements of blood pressure, height, and weight were obtained. In summary, participants completed the following assessments.

Medical History Questionnaire: This questionnaire (Appendix B) includes questions pertaining to years of education, occupation, ethnicity, substance use history, current medications, and history of certain health conditions (e.g., stroke, seizure, cancer, cardiac arrest). Supplemental components of this form collect information on physical exercise (78), diet, and sleep behaviors.

Fasting Lipids, Glucose, And Insulin Measurement: Approximately 3 milliliters of fasting blood was collected from the antecubital vein by venipucture. Plasma concentrations of cholesterol, triglycerides, and glucose were measured using standard enzymatic technique. For insulin measurement, plasma was separated and stored at -80°C until future analysis. Insulin levels were assessed from plasma with a radioimmunoassay kit (MP Biomedicals, Orangeburn, NY, USA). Insulin sensitivity was calculated using the quantitative insulin sensitivity check index (QUICKI), which is the inverse of the sum of logarithmically expressed values of fasting glucose and insulin.

Blood Pressure Recordings: After fifteen minutes of rest in a supine position, brachial blood pressure was measured using a semi-automated device (Omron Healthcare Inc,

Bannockburn, Illinois) Three separate recordings were obtained and averaged while participants sit upright in accordance with American Heart Association Guidelines.

Height and Weight Measurement: Body weight in kilograms and height in centimeters were measured on a beam-balance scale for the subsequent calculations of body mass index (BMI). BMI was calculated by dividing weight in kilograms by height in meters squared.

Stress Test Visit

Sedentary participants were required to undergo a stress test visit prior to the aerobic fitness test.

Electrocardiogram (ECG): During this visit, a diagnostic 12-lead ECG was performed at rest and during incremental submaximal exercise on a treadmill. The ECG was continuously monitored by a physician, and blood pressure and ratings of perceived exertion were recorded each minute. Participants who demonstrated potential signs or symptoms of chronic heart disease were not permitted to continue in the study unless clearance was obtained by a cardiologist.

Aerobic Fitness Visit

Participants were asked to refrain from exercise and the consumption of alcohol and caffeine for twenty-four hours prior to their visit. They were also asked to fast for four hours prior their visit. A test of maximal aerobic capacity test was performed to assess

cardiorespiratory fitness. In addition, peripheral endothelial function was measured with brachial flow-mediated dilation. In summary, the following assessments were obtained:

Maximal Aerobic Fitness Test: Maximal oxygen consumption (VO2max) was assessed with a graded treadmill exercise test during a modified Bruce protocol. Following a 5-minute warm up period, participants ran or walked as the treadmill slope was increased 2% every 2 minutes until volitional exhaustion. Oxygen consumption (indirect calorimetry via respiratory gas measurements; Physio-Dyne, Quogue, NY), heart rate, and ratings of perceived exertion (the original Borg scale (90)) were measured throughout the test. At the end of each stage, participants rated their perceived exertion using the Borg scale and heart rate was recorded. The gas analyzer was calibrated with standard gases of known concentrations before each trial.

Brachial Flow Mediated Dilation (FMD): Brachial artery diameter and blood flow velocity was obtained from a B-mode Doppler ultrasound machine (iE 33 Ultrasound System, Philips, Bothell, WA) with a customized transducer-holding device. Brachial artery images were obtained in a longitudinal orientation located 5-10 cm proximal to the antecubital fossa. After the acquisition of baseline measurements, a blood pressure cuff was placed on the ipsilateral forearm distal to the elbow and inflated to 100 mmHg above baseline systolic blood pressure for 5 minutes using a rapid cuff inflator (E20, Hokanson, Bellevue,WA).

Cognitive/MRI Visit

Upon arrival to this visit, participants completed questionnaires and a brief battery of standardized neuropsychological tests assessing general cognition, memory, and

executive function. Following cognitive testing, participants were screened for MRI safety and given the opportunity to practice the experimental fMRI tasks on a laptop. The MRI session consisted of structural and functional sequences.

Neuropsychological Battery

Participants completed an assessment battery including standard clinical neuropsychological instruments with established reliability and validity. The tests were administered and scored using standard administration and scoring criteria. The battery included the following tasks:

Mini Mental Status Exam (MMSE) (91): a 30-item test of general mental status including arithmetic, memory, and orientation.

Wechsler Test of Adult Reading (WTAR) (92): provides an estimate of general intelligence since unlike many intellectual abilities, reading recognition is relatively stable in the presence of cognitive declines associated with normal aging or brain injury. The test consists of reading aloud from a list of 50 words that have atypical grapheme to phoneme translations. There is a maximum of 50 raw points, which can be transformed to age-normed T-scores and an IQ composite score with a mean of 100 and a standard deviation of 15.

California Verbal Learning Test-II (CVLT-II) (93): a measure of verbal learning and memory. A 16-item word list is presented five times and participants must recall as many words as they can after each presentation. Following the presentation of a 16-word distracter list, participants are asked to recall the original list with and without semantic cuing. After a 20-mintute delay, participants recall the original list with and without

semantic cuing and complete a yes/no item recognition test. Free recall trials have a maximum of 16 points with 1-point awarded for each correctly recalled item from the list. A recognition discriminability index (d') is calculated from the yes/no item recognition section. The calculation of d' is based on the actual number of hits, possible number of total hits, actual number of false-positives, and possible number of total false-positives, yielding a maximum score of 4.0.

Wechsler Adult Intelligence Scale-III (WAIS-III) Digit Span Subtest (94): a measure of apprehension span and working memory. On Digits Forward, participants must repeat sequences of verbally presented numbers. On Digits Backwards, participants must recall the numbers in reverse sequence. 1-point is awarded for each correctly sequenced item on Digits Forward and Backwards with their composite sums creating a Digits Total Score.

Controlled Oral Word Association Test (COWAT) (95): a measure of verbal fluency according to phonemic rule. Participants must name as many words as they can beginning with a specified letter within a 1-minute time frame.

Trail Making Test A & B (96): a measure of visual attention and processing speed. For part A, participants draw a line connecting 25 randomly dispersed numbers in numerical order. On part B, participants must alternate between connecting randomly dispersed numbers and letters in sequence (1-A-2-B-3-C). Time to completion is recorded for both parts.

Beck Depression Inventory-II (BDI-II) (97): a 21-item self-report questionnaire assessing depression. The maximum scores is 63, with scores greater than 14 suggesting clinically significant depression symptoms

Spielberger Trait Anxiety Inventory (STAI-T) (98): a 20-item self-report questionnaire on trait anxiety with higher scores representing greater anxiety.

MRI Session Procedures

MRI Safety Screen Form: Before the MRI, participants completed an MRI safety screening form (Appendix C) to further ensure that they were free of MRI contraindications. This form has been approved by both the Imaging Research Center and the Institutional Review Board.

3-Tesla GE Signa Excite MRI Scanner: Scanning was performed on The University of Texas at Austin Imaging Research Center's whole body 3T GE MRI scanner with an 8-channel phase array head coil. The magnetic fields, at the strengths used (3T main field, 40 mT/m gradient fields), are considered to be without harm and the proposed MRI scanning procedures fall within the FDA guidelines for radiofrequency electromagnetic field exposure.

Structural Image Acquisition: T_1-weighted anatomical scans of the entire brain in the sagittal plane were collected using a high-resolution Spoiled Gradient Echo (SPGR) sequence (256 x 256 matrix, FOV=24 x 24 cm^2, 1 mm slice thickness, 0 gap). The scanning duration was approximately ten and a half minutes.

Functional Image Acquisition Functional imaging was performed using a whole brain echo-planar imaging (EPI) sequence (TR=3000 ms, TE=30 ms, FOV=24 x 24 cm^2, 64 x 64 matrix, 42 axial slices, 3 mm slice thickness, 0.3 mm gap). Imaging was performed while participants completed the tasks. The tasks were projected onto a screen positioned

behind the participant's head, and subsequently viewed through a double-mirror attached to the head coil.

2-Back Verbal Working Memory Task Paradigm: Participants completed a verbal working memory n-Back task (99,100), composed of alternating blocks of 0-Back, 1-Back, and 2-Back conditions (Figure 2). Within each block, a series of fifteen individual consonants were displayed for 500 ms each followed by a 2500 ms inter-stimulus interval. For each consonant presentation, participants made a response using a two-button MR-compatible response box to indicate whether or not the letter was a target (33% in each block). The target for the 0-Back condition was pre-specified letter (h). The target for the 1-Back and 2-Back conditions was any letter identical to the one displayed one or two stimuli before, respectively. Participants completed two consecutive seven-minute runs, each consisting of three blocks of alternating 0-Back, 1-Back, and 2-Back conditions.

FIGURE 2

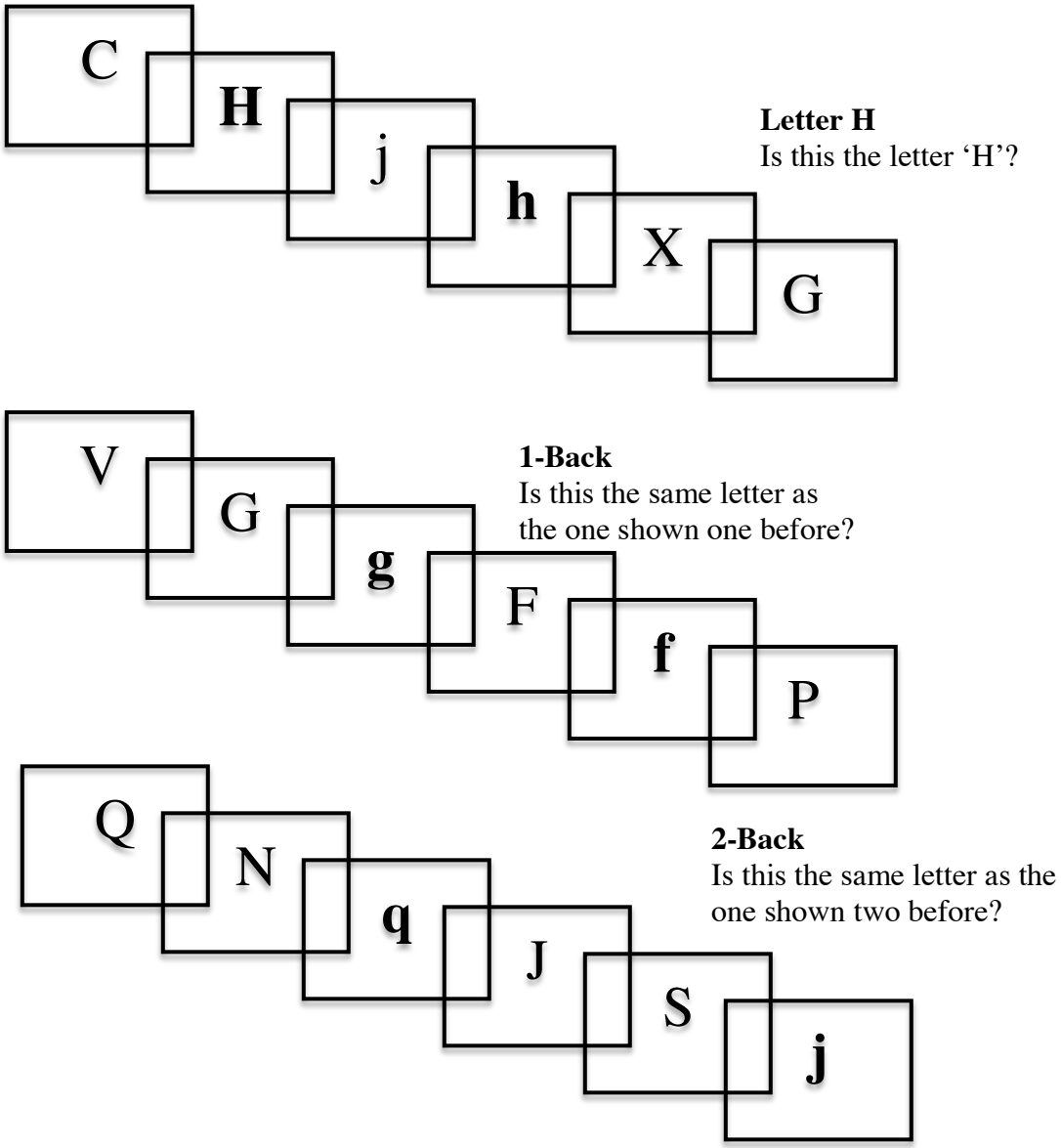

Figure 2. Schematic of the 2-Back Task

Hypercapnia Task Paradigm: Breath-hold induced hypercapnia was used to invoke a global change in the BOLD response in the absence of a cognitive challenge. Participants were cued by colored squares to breathe normally (green square, 13.5 seconds), exhale (yellow square, 3 seconds), and hold their breath (red square, 13.5 seconds) (101) (Figure 3). This sequence was repeated 7 times within a single imaging run with a duration of approximately 4 minutes.

Figure 3

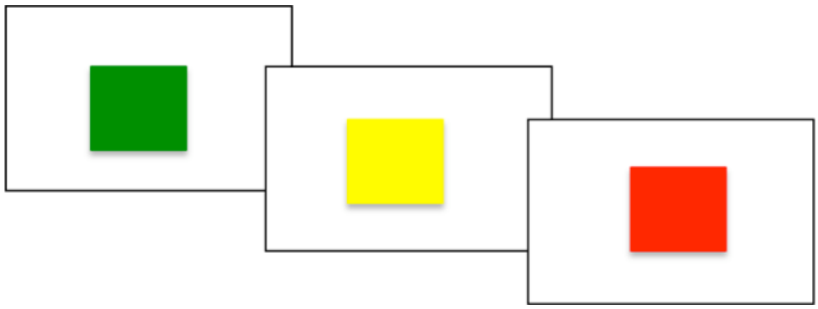

Figure 3. Schematic of the Hypercapnia Task

PARTICIPANT CHARACTERISTICS

A total of thirty-two endurance-trained and twenty-four sedentary adults completed the study and were included in the present analyses. Means and standard deviations of the demographic and physiological variables for each group are reported in Table 1. Group comparisons were completed with non-parametric chi-square or Mann-Whitney U Tests. As expected, the two groups differed significantly for VO_2max (Table 1). The groups also differed in BMI, triglycerides, and insulin sensitivity. However, there were no significant group differences in gender (sex) distribution, age, years of education, systolic and diastolic blood pressure, LDL-cholesterol, HDL-cholesterol, and fasting blood glucose concentration (Table 1). Overall, the two groups were equivalent across most demographic

and physiological indices. Raw means and standard deviations for cognitive tests for each group are reported in Table 2. Overall, the sample displayed a high level of cognitive ability.

Table 1. Participant characteristics

	Sedentary	Endurance Trained	P-value
Age (years)	53.2±5.3	51.4±5.8	0.245
Sex (Male/Female)	5/19	13/19	0.117
Education (years)	16.6±1.8	17.3±2.2	0.367
Systolic Blood Pressure (mmHg)	118.2±7.8	119.4±13.2	0.993
Diastolic Blood Pressure (mmHg)	71.8±6.5	70.3±7.4	0.461
Body Mass Index (kg/m^2)	27.0±6.5	23.2±2.3	0.042*
LDL-Cholesterol (mg/dl)	120.0±28.5	110.9±31.4	0.298
HDL-Cholesterol (mg/dl)	63.3±20.7	72.7±21.1	0.110
Triglycerides (mg/dl)	93.8±5.1	68.0±20.7	0.023*
Fasting Glucose (mg/dl)	91.2±8.3	89.2±8.1	0.384
Insulin Sensitivity (QUICKI)	0.31±0.019	0.33±0.015	0.002*
V02max	25.3±5.1	44.3±8.8	<0.001*

*$p<0.05$

Table 2. Raw neuropsychological test scores in the sedentary and endurance-trained groups

Test Measures By Domain	Sedentary	Endurance-Trained	Total Possible Score
Global Cognitive Functioning			
Mini Mental Status Exam	28.8±1.3	29.0±1.0	30
Wechsler Test of Adult Reading, Raw	45.4±4.8	44.4±4.4	50
Wechsler Test of Adult Reading, FSIQ	113.3±5.2	112.3±5.0	125
Memory			
California Verbal Learning Test II			
Immediate Recall	11.0±3.9	11.2±2.5	16
Delayed Recall	11.5±3.7	12.0±2.5	16
Recognition (Yes/No)	2.9±0.9	3.0±0.6	4.0
Executive Function			
Trail Making Test A, sec	32.2±9.6	28.3±7.4	300 second limit
Trail Making Test B, sec	62.5±16.9	55.7±19.0	300 second limit
Controlled Oral Word Test	44.8±9.4	44.8±11.4	3 minute limit
WAIS-III Digit Span	17.9±3.4	19.2±3.8	30

Chapter 3: Fitness-Related Changes in Cognition and the BOLD Response to Working Memory

INTRODUCTION

Across the lifespan, cognitive abilities steadily decline with the most precipitous decreases occurring in the working memory, processing speed, and selective attention domains (102). Decrements in cognition constitute a critical public health concern as they predict loss of independence, reduced functional ability, and mortality (103). Despite the inevitably of age-related cognitive decline, the trajectory of towards cognitive decline is highly variable across individuals (104), indicating that certain genetic and lifestyle factors may modulate the rate of cognitive aging. In particular, engagement in regular aerobic exercise is a well-established protective factor (33,37–39), particularly for the cognitive domains most vulnerable to the effects of aging.

A comprehensive understanding of the mechanisms that govern fitness-related cognitive enhancement may provide insight on neural plasticity in the adult brain that can be extrapolated to benefit many populations. The animal literature has identified several pathways through which exercise may benefit cognition such as neurogenesis (46–49), increased vascularization (50,52,105), upregulation of neurotransmitters (106–108), and attenuated inflammation (109). However, until recently, translational studies on humans were limited due the necessity of invasive procedures. Fortunately, the advent of neuroimaging has enabled researchers to safely examine the neural underpinnings of fitness-related cognitive gains. For example, Colcombe et al. (2006) (40) reported that older adults with higher cardiorespiratory fitness display greater grey matter volumetry in

the frontal, parietal, and temporal cortices. Similarly, fMRI has been utilized to examine how fitness may affect brain activation during challenging cognitive tasks. In older adults, higher aerobic fitness levels are associated with increased BOLD response in the frontal and parietal lobes (43,110). Even at rest, higher cardiorespiratory fitness predicts greater functional connectivity between the posterior cingulate cortex and the middle frontal gyrus (111), interconnections that are critical for executive function performance.

To date, the majority of work on fitness and cognition has been conducted on sedentary older adults. However, studies on middle-aged adults are necessary since midlife may represent a critical period for initiating interventions that prevent the progression to clinically significant cognitive decline in older age. Accordingly, the goal of the current project was to explore the impact of cardiorespiratory fitness on cognition and its underlying neural substrates in middle-aged adults. Utilizing a cross-sectional design, neuropsychological test performance and the BOLD response to a 2-Back working memory task were compared between sedentary and endurance-trained adults. The 2-Back task was selected because it engages executive function (99,100), the cognitive domain that displays the more robust fitness-related improvements (38). Moreover, prior studies have demonstrated fitness-related enhancement of the BOLD response during executively-mediated inhibition tasks (43,110), but working memory has yet to be examined. Based on prior literature on fitness-related gains in older adults (38,43,110), it was hypothesized that the endurance-trained group would display superior executive function performance on neuropsychological tests and higher BOLD signal

during the working memory task, particularly in the middle frontal gyrus and superior parietal lobe.

METHODS

Study methods were described in Chapter 2.

STATISTICAL ANALYSES

Neuropsychological Test Performance

Participants completed a battery of clinical neuropsychology tests with established reliability and validity (112) as described in Chapter 2. Individual cognitive tests were catergorized into one of three composite domain scores (global cognition, memory, or executive function) in effort to reduce the number of statistical comparisons. To create the domain scores, raw tests scores were converted to standardized z-scores based on the study sample's mean and standard deviations. For timed tests, raw scores were multiplied by -1 so that higher scores were indicative of superior performance. Within each domain, participants' z-scores were averaged as follows: 1) *global*: Mini Mental Status Exam (MMSE) (91) and Wechsler Test of Adult Reading (WTAR) (92); 2) *memory*: California Verbal Learning Test II (CVLT-II) immediate recall, delayed recall, and recognition discrimination (93); 3) *executive*: Trail Making Test Parts A and B time to completion (96), Controlled Oral Word Associations Test (COWAT) (95), and Wechsler Adult Intelligence Scale III (WAIS-III) Digit Span Subtest (94). A trained research assistant administered and scored the neuropsychological test battery in

accordance with standardardized administration and scoring criteria. Group differences in the cognitive domain scores were assessed with independent sample t-tests.

2-Back Task Performance

Behavioral performance on the 2-Back task was assessed with accuracy and reaction time. Accuracy was calculated as the number of correct responses for each condition over the total number of responses for each condition, multiplied by 100. Reaction time was computed by averaging response time in milliseconds for correct trials in each condition. Group differences in 2-Back performance were explored using independent samples t-tests.

2-Back Task Whole Brain fMRI Analysis

FSL v 4.1.2 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) was used to conduct fMRI analyses. Images first underwent motion correction using MCFLIRT (113) and removal of non-brain structures using BET (114). Next, preprocessing consisting of FILM prewhitening, high-pass filtering with a cut-off 100 seconds, and spatial smoothing with a 5 mm full width half maximum Gaussian kernel was completed. Each participant's functional images were aligned to his/her high-resolution anatomical images using a 7-parameter affine transformation with FSL's registration tool, FLIRT (113). A 12-parameter affine transformation was then used to align these images to standard stereotaxic space (MNI 152) (113).

First level analyses were performed with FSL's FEAT (FMRI Expert Analysis Tool, version 5.98) using the general linear model (GLM). Within each voxel, the time

series was modeled with the regressors for the block events (1-Back, 2-Back) against a baseline condition (0-Back), after convolution with a double-gamma hemodynamic response function. The GLM also included reaction time, missed trials, motion parameters, and the temporal derivatives of all regressors as additional covariates. Three linear contrasts (1Back > 0Back, 2Back > 0Back, 2Back > 0Back) were conducted to examine changes in the BOLD response with increasing task difficulty.

A second-level fixed effects analysis was performed to combine the parameter estimates across task runs. To complete the group analyses, a higher-level mixed effects design was implemented with FMRIB's Local Analysis of Mixed Effects (FLAME) (115). Whole brain Z (Gaussianized T/F) statistical images were thresholded by retaining clusters corresponding to Z>2.3 and applying a familywise error corrected cluster significance of $p<0.05$.

2-Back Task ROI Analysis

The BOLD signal for the three contrasts of interest (1Back > 0Back, 2Back > 0Back, and 2Back > 0Back) was extracted from a set of eight *a priori* regions of interest (ROIs). The *a priori* ROIs were selected from published coordinates that were empirically derived from a verbal 2-Back task within an independent sample and have established sensitivity for populations with vascular risk factors (116). GingerAle 2.0 (www.brainmap.org) was used to convert the Talairach coordinates reported in Haley et al. 2007 into MNI space for the current analyses (117). 5mm spheres were created around these MNI coordinates and were then binarized in a mask. Within each ROI, BOLD

signal for each of the three contrasts (1Back > 0Back, 2Back > 0Back, and 2Back > 0Back) was extracted and then converted to percent signal change, as described in detail in http://mumford.bol.ucla.edu/perchange_guide.pdf. Briefly, percent signal change was computed by multiplying the parameter estimate of interest by a reference regressor height. This value was then divided by the mean activation across the entire time series and converted to percentage by multiplying by 100.

Group differences in the *a priori* ROIs were assessed using non-parametric permutation tests on the maximal T statistic. This method does not necessitate assumptions about the shape of the underlying distribution and moreover, preserves the type 1 error rate across multiple comparisons by applying the critical value for the maximum statistic (118). The null distribution was created by shuffling group labels and performing 5,000 permutations of the Welch t-test. P-values were derived from the max T distribution.

RESULTS

Neuropsychological Test Performance

As seen in Table 3 and Figure 4, no group differences were observed for any of the cognitive domain scores. However, there was a modest trend towards better executive function performance in the endurance-trained group as compared to the sedentary group.

Table 3. Cognitive domains z-scores

	Sedentary	Endurance-Trained	t(54)	P-value
Global Cognition	-0.007±0.83	0.006±0.67	0.065	0.948
Memory	-0.069±1.11	0.052±0.72	0.494	0.624
Executive Function	-0.164±0.61	0.123±0.64	1.702	0.095

Figure 4

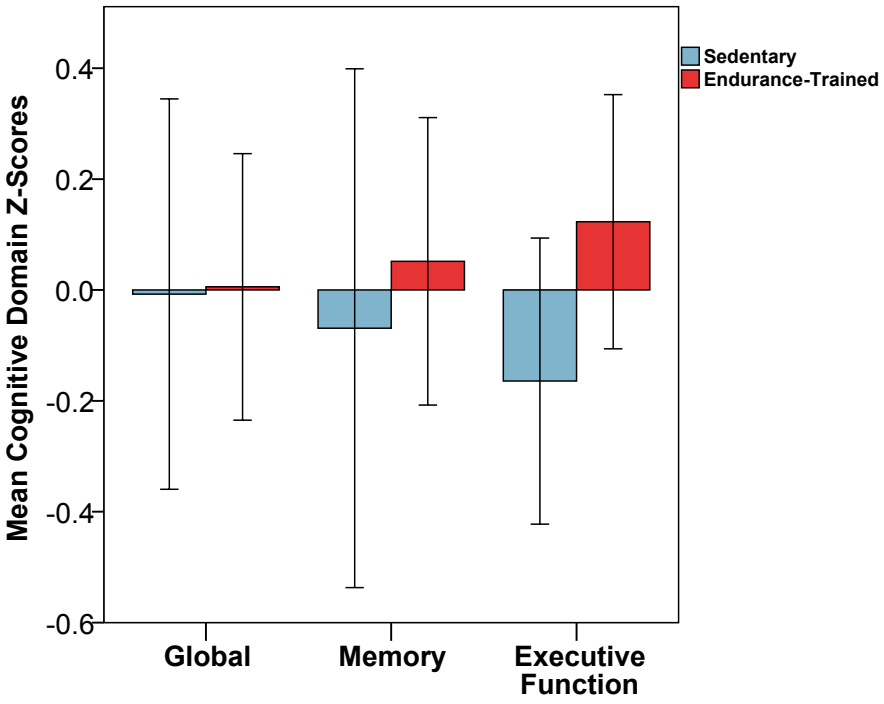

Figure 4. Cognitive Domain Z-scores

Cognitive domain z-scores for the sedentary and endurance-trained groups. Error bars represent 95% confidence intervals.

2-Back Task Performance

Performance accuracy for all conditions was equivalent between the two groups (Table 4). The endurance-trained group displayed faster reaction time for the 1-Back condition, with a strong trend towards faster reaction time for the 0-Back condition (Table 4, Figure 5). Reaction time for the 2-Back condition did not differ between groups.

Table 4. 2-Back task performance

	Sedentary	Endurance-Trained	t(54)	P-value
0-Back Accuracy	96.1±3.9	97.0±4.9	0.722	0.474
1-Back Accuracy	88.0±10.1	91.7±7.1	1.594	0.117
2-Back Accuracy	74.8±8.7	77.8±12.5	1.004	0.320
0-Back Reaction Time	784.9±128.2	716.4±127.4	-1.986	0.052
1-Back Reaction Time	990.2±208.0	876.5±190.5	-2.125	0.038*
2-Back Reaction Time	1197.7±228.7	1132.3±241.4	-1.025	0.310

Figure 5

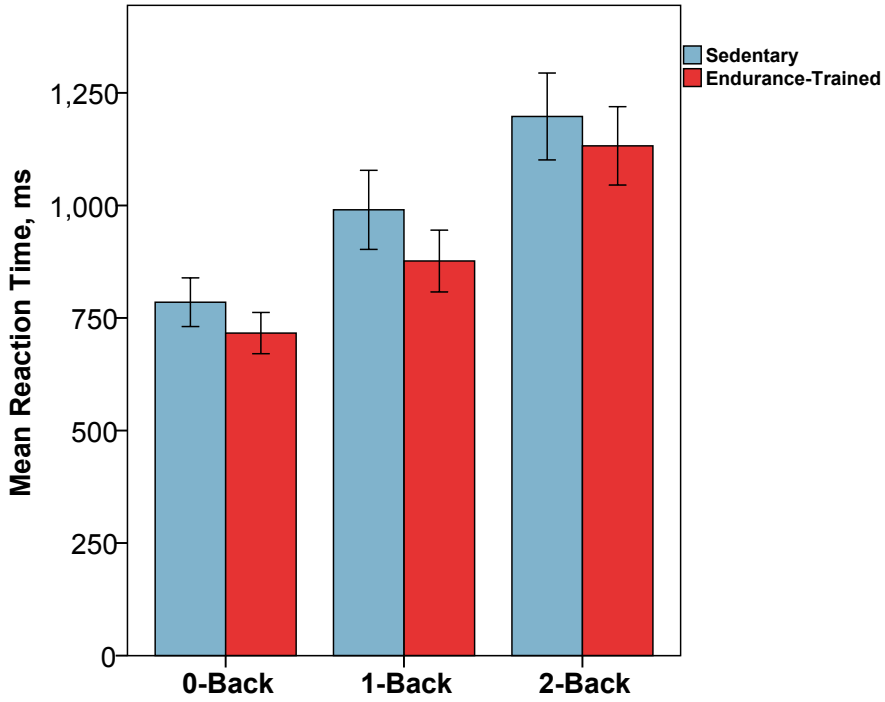

Figure 5. Faster 2-Back Task Reaction Time in the Endurance-Trained Group

Mean 2-Back task reaction time (ms) in the sedentary and endurance-trained groups. Error bars represent 95% confidence intervals.

2-Back Task Whole Brain Analysis

1-Back>0Back: As seen in Table 5 and Figure 6, whole brain analysis of the 1-Back>0-Back contrast revealed increased signal in expected areas including the left middle frontal gyrus, left inferior frontal gyrus, and bilateral parietal lobes (99,100). In comparison to the sedentary group, the endurance-trained group displayed higher activation in cerebellum bilaterally (Table 6 and Figure 7). There were no regions of increased activation in the sedentary group.

Table 5. Clusters of 1-Back>0-Back Activation

Region	Max Z-stat	MNI Coordinates (x,y,z)
Left Middle Frontal Gyrus	8.76	-30, 0, 52
Right Inferior Parietal Lobe	8.71	42, -50, 34
Left Inferior Frontal Gyrus	8.55	-44, 6, 20
Left Inferior Parietal Lobe	8.50	-32, -58, 36

Max Z-stat: maximum z statistic for each cluster; MNI Coordinates (x, y, z) indicate the the peak of each cluster

Figure 6

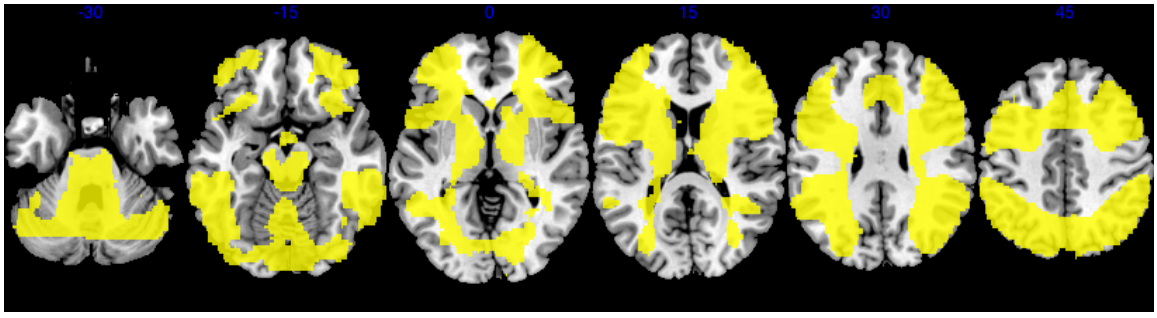

Figure 6. Whole Brain1-Back>0-Back Activation

Table 6. Clusters of greater 1-Back>0-Back activation in the endurance-trained group

Region	Max Z-stat	MNI Coordinates (x,y,x)
Left Cerebellum	4.33	-2, -80, -26
Right Cerebellum	3.42	34, -52, -34

Max Z-stat: maximum z statistic for each cluster; MNI Coordinates (x, y, z) indicate the peak of each cluster

Figure 7

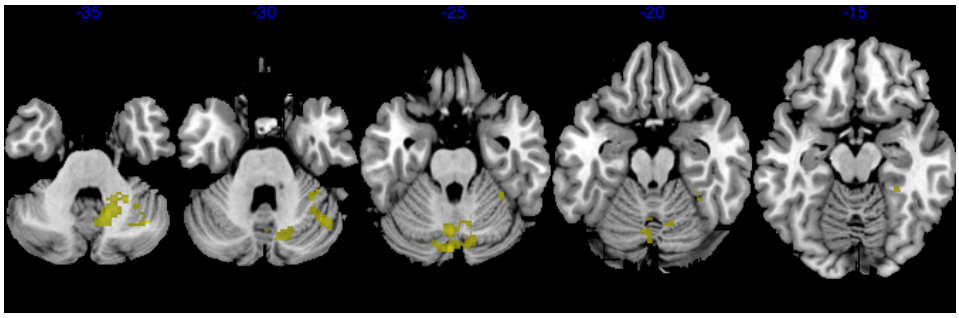

Figure 7. Greater 1-Back>0-Back Activation in the Endurance-Trained Group

2-Back>0Back: Whole brain analysis of the 2-Back>0-Back contrast revealed similar areas of activation as the 1-Back>0-Back contrast including the left middle frontal gyrus, left inferior frontal gyrus, and bilateral inferior parietal lobes (Table 7 and Figure 8). In correspondence with the literature (99), the 2-Back>0-Back contrast induced a larger change in signal magnitude than the 1-Back>0-Back contrast, as well as increased bilateral frontal activation. There were no significant differences in task-related activation between the sedentary and endurance-trained groups.

Table 7. Clusters of 2-Back>0-Back Activation

Region	Max Z-stat	MNI Coordinates (x,y.z)
Right Inferior Parietal Lobe	9.71	40, -50, 34
Left Inferior Frontal Gyrus	9.53	-44, -6, 20
Left Middle Frontal Gyrus	9.34	-30, 2, 52
Left Insula	9.02	-30, 20, -6
Left Inferior Parietal Lobe	8.93	-36, -54, 36
Right Middle Frontal Gyrus	8.82	30, 14, 46

Max Z-stat: maximum z statistic for each cluster; MNI Coordinates (x, y, z) indicate the peak of each cluster

Figure 8

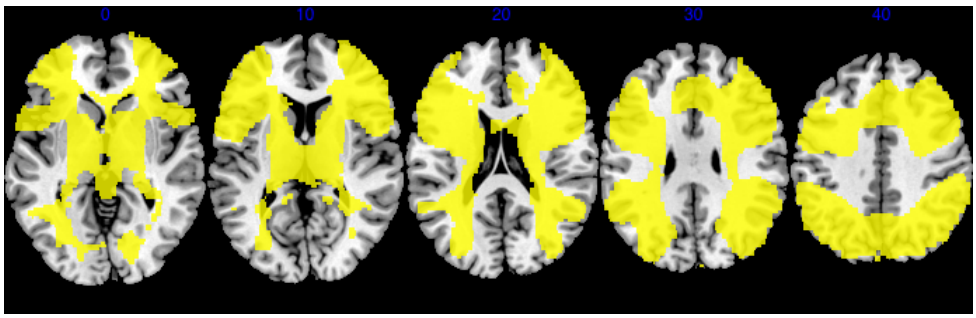

Figure 8. Whole Brain2-Back>0-Back Activation

2-Back>1Back: Changes in the BOLD response with increasing task difficulty were further compared with 2-Back>1-Back contrast. The 2-Back>1-Back analysis revealed several areas of increased signal activation in the frontal and parietal lobes, as well as the anterior cingulate gyrus (Table 8 and Figure 9). There were no significant differences in 2-Back>1-Back BOLD response between the sedentary and endurance-trained groups.

Table 8. Clusters of 2-Back>1-Back Activation

Region	Max Z-stat	MNI Coordinates (x,y,z)
Left Middle Frontal Gyrus	7.03	-30, 4, 50
Left Anterior Cingulate Gyrus	6.96	-8, 28, 32
Right Inferior Parietal Lobe	6.61	52, -38, 36
Right Middle Frontal Gyrus	6.57	28, 10, 46
Right Superior Frontal Gyrus	6.55	22, 18, 48
Left Inferior Parietal Lobe	6.03	-40, -60, 34
Left Occipital Lobe	5.97	-24, -62, 30
Right Occipital Lobe	5.85	38, -66, 38

Max Z-stat: maximum z statistic for each cluster; MNI Coordinates (x, y, z) indicate the peak of each cluster

Figure 9

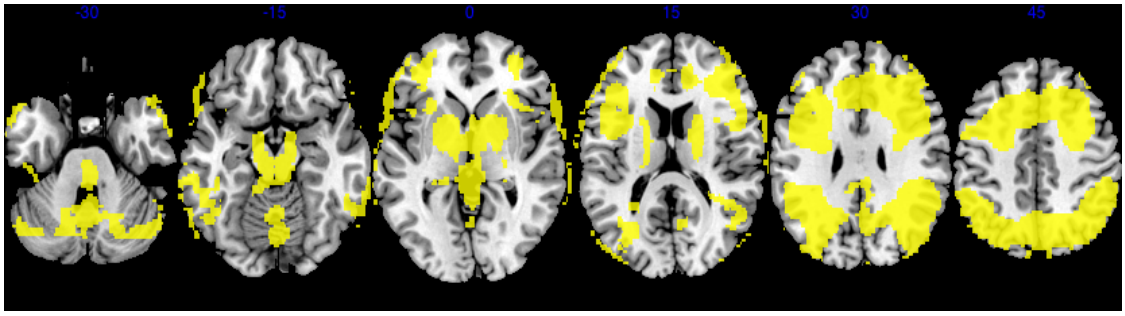

Figure 9. Whole Brain 2-Back>1-Back Activation

2-Back Task ROI Analysis

1-Back>0Back: Based on the permutation based T-test results, there were no significant differences between the sedentary and endurance-trained groups in any of the examined ROIs (Table 9).

Table 9. 1-Back>0-Back activation in the ROIs

Brain Region & MNI Coordinates (X, Y, Z)	Sedentary	Endurance-Trained	p-values
Left Middle Frontal Gyrus -33, 8, 58	0.41±0.28	0.42±0.41	0.952
Left Medial Frontal Gyrus -4, 24, 43	0.25±0.21	0.19±0.16	0.486
Right Superior Parietal Lobe 40, -62, 59	0.53±0.46	0.42±0.70	0.713
Left Inferior Parietal Lobe -50, -51, 49	0.36±0.33	0.43±0.33	0.667
Left Middle Frontal Gyrus -45, 48, 8	0.42±0.55	0.38±0.35	0.859
Right Superior Frontal Gyrus 36, 52, 9	0.37±0.25	0.24±0.27	0.181
Right Middle Frontal Gyrus 35, 10, 56	0.40±0.23	0.61±0.59	0.194
Right Inferior Frontal Gyrus 51, 16, -2	0.18±0.21	0.14±0.23	0.712

2-Back>0Back: As seen in Table 10, there were no group differences in any of the ROIs.

Table 10. 2-Back>0-Back activation in the ROIs

Brain Region & MNI Coordinates (X, Y, Z)	Sedentary	Endurance-Trained	p-values
Left Middle Frontal Gyrus -33, 8, 58	0.56±0.33	0.59±0.50	0.952
Left Medial Frontal Gyrus -4, 24, 43	0.40±0.29	0.34±0.23	0.762
Right Superior Parietal Lobe 40, -62, 59	0.74±0.69	0.56±0.82	0.724
Left Inferior Parietal Lobe -50, -51, 49	0.41±0.39	0.63±0.48	0.231
Left Middle Frontal Gyrus -45, 48, 8	0.53±0.45	0.46±0.57	0.875
Right Superior Frontal Gyrus 36, 52, 9	0.46±0.35	0.31±0.34	0.352
Right Middle Frontal Gyrus 35, 10, 56	0.47±0.40	0.75±0.67	0.194
Right Inferior Frontal Gyrus 51, 16, -2	0.25±0.28	0.17±0.27	0.614

2-Back>1Back: As compared to the sedentary group, the endurance-trained group displayed higher BOLD response in the left inferior parietal lobe (Figure 10). 2-Back>1-Back activation did not differ between the groups in any other examined *a priori* ROIs (Table 11).

Table 11. 2-Back>1-Back activation in the ROIs

Brain Region & MNI Coordinates (X, Y, Z)	Sedentary	Endurance-Trained	p-values
Left Middle Frontal Gyrus -33, 8, 58	0.14±0.26	0.16±0.23	0.878
Left Medial Frontal Gyrus -4, 24, 43	0.14±0.19	0.14±0.14	0.967
Right Superior Parietal Lobe 40, -62, 59	0.19±0.39	0.12±0.33	0.739
Left Inferior Parietal Lobe -50, -51, 49	0.04±0.20	0.20±0.24	0.036*
Left Middle Frontal Gyrus -45, 48, 8	0.10±0.35	0.09±0.35	0.962
Right Superior Frontal Gyrus 36, 52, 9	0.08±0.31	0.08±0.22	0.999
Right Middle Frontal Gyrus 35, 10, 56	0.06±0.24	0.13±0.29	0.584
Right Inferior Frontal Gyrus 51, 16, -2	0.06±0.27	0.02±0.21	0.822

*$p<0.05$

Figure 10

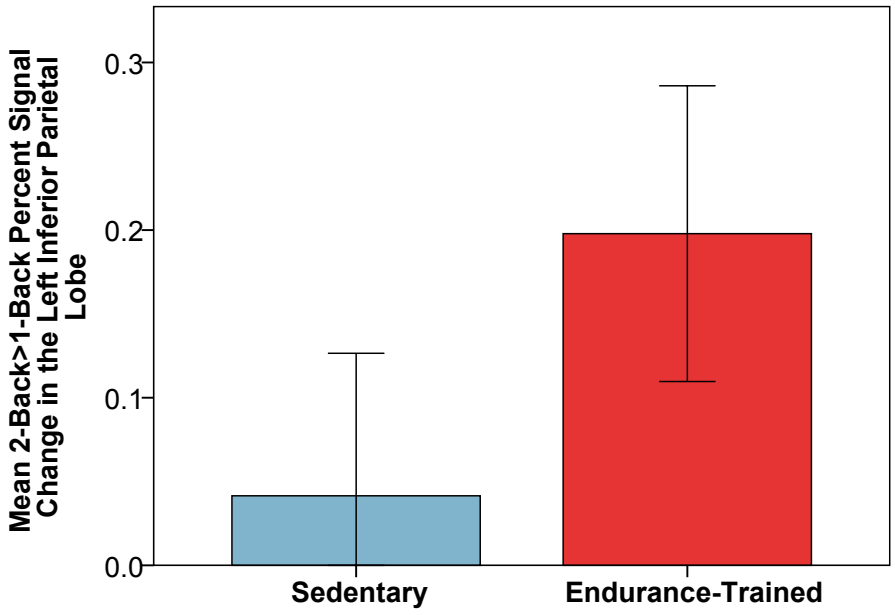

Figure 10. Greater Left Inferior Parietal 2-Back>1-Back Activation in the Endurance-Trained Group

Mean left inferior parietal lobe 2-Back>-1-Back percent signal change in the sedentary and endurance-trained groups. Error bars represent 95% confidence intervals.

DISCUSSION

A primary goal of the current project was to examine the impact of cardiorespiratory fitness on cognition in middle-aged adults. In contrast to previous studies demonstrating fitness-related cognitive enhancement (33,37–39), neuropsychological test performance in the domains of global cognition and memory did not significantly differ between the sedentary and endurance-trained groups. However, there was a modest trend towards better executive performance in the endurance-trained group. Meta-analytical studies on older adults have similarly reported the most robust

fitness-related benefits in the executive function domain (38). Commensurate with the trend towards better executive function performance on the neuropsychological measures, endurance-trained adults also displayed faster reaction time during the 0-Back (trend) and 1-Back conditions of the 2-Back task. Task accuracy did not differ between the two groups, indicating that enhanced speed of information processing abilities enabled the endurance-trained group to execute quicker responses while maintaining a high level of performance.

The current study also aimed to examine fitness-related changes in the BOLD response to a verbal 2-Back working memory task. Whole brain fMRI analyses of the 2-Back task revealed no significant differences in the BOLD response between the sedentary and endurance-trained groups for the 2-Back>0-Back and 2-Back>1-Back conditions. However, as compared to the sedentary group, the endurance-trained group displayed greater bilateral cerebellum activation in the 1-Back>0-Back condition.

Traditionally, the role of the cerebellum was considered restricted to gait, posture, and motor control (119). However, studies on patients with cerebellar lesions and neuroimaging studies on healthy volunteers have revealed that the cerebellum is critical for executive function tasks such as planning, monitoring, and temporal sequencing (120–123). Given that the n-Back task requires sustained attention, task management, and updating and monitoring of working memory (99,100), it is not surprising that lateral and medial cerebellum activation is a well-established finding in the literature (122,124). Through its diffuse afferent connections with the prefrontal and parietal cortices, the cerebellum optimizes and fine-tunes higher executive function processes required for

successful n-Back completion (122). In the current study, increased task-related cerebellar activation in the endurance-trained group may indicate enhanced neural efficiency that may serve to facilitate executive function performance. Interestingly, aerobic exercise has been found to stimulate angiogenesis (125) and attenuate age-related atrophy in the cerebellum (126). Thus, increased cerebellar activation in the endurance-trained group may be secondary to exercise's beneficial effects on perfusion and/or neuronal integrity in this region.

In addition to the whole brain fMRI analyses of the 2-Back task, we performed *a priori* ROI analyses. The ROIs were derived from an independent sample (116) and included regions consistently identified for successful n-Back task completion such as the bilateral middle frontal gyrus and parietal lobes (99,100,124). 1-Back>0-Back and 2-Back>0-Back BOLD response did not significantly differ between the sedentary and endurance trained groups. However, the endurance-trained group displayed greater BOLD signal in the left inferior parietal lobe for the 2-Back>1-Back contrast.

Successful n-Back task completion requires the ability to maintain task-relevant stimuli in working memory, as well as the use of executive function skills to manipulate the stored information (99,124). The 2-Back>1-Back contrast reduces some of the shared variance in maintenance demands, allowing for a closer examination of changes in activation due to increased executive function demands (127). The inferior parietal lobe has been consistently identified amongst a network of regions critical for facilitating memory load during the n-Back task (45,99,127,128). During working memory tasks, the inferior parietal cortex is recruited by the fronto-parietal circuit to govern rapid

attentional shifting and task preparation (45). Our finding of increased parietal activation during a challenging executive function task within the endurance-trained group is consistent with prior research (43,110). Moreover, higher cardiorespiratory fitness has been associated with greater grey matter volumetry in the parietal cortex (40). Thus, regular engagement in aerobic exercise may enhance neuronal viability within the parietal region, enabling greater recruitment of the attentional control network under conditions of high task demand.

The limitations of the current study must be considered when interpreting the results. Previous research has reported that aerobic fitness has a large to moderate effect on cognitive performance (38). In contrast, the current study identified only a modest trend towards improved executive function performance. It is possible that our study lacked the power to detect significant effects. A prior meta-analysis revealed greater fitness-associated cognitive gains in mid-old adults (66-70 years) when compared to young-old adults (55-65 years) (38), indicating that a larger sample size may be necessary to detect cognitive benefits when the age range is restricted to middle-aged adults. Another contributing factor could be that our sample consisted of highly educated adults (mean education = 17.0 years). Higher educational attainment has been consistently associated with cognitive reserve (129), a factor that has been found to moderate the relationship between the degree of cerebral damage and cognitive performance (130). Thus, age-related cognitive decline may have been attenuated by education in our sample, creating a ceiling effect for observing fitness-related enhancement. Finally, the results may be less robust in the current study due to other sample selection factors. Our study

exerted careful control for cardiovascular disease burden by excluding the use vascular-acting medications and ensuring that our groups were equivalent on blood pressure, total cholesterol, and plasma glucose levels. Unfortunately, few studies on aerobic fitness have reported data on cardiovascular risk factors. Failure to control for cardiovascular risk factors may confound results since they are associated with poorer cardiorespiratory fitness (131), as well as impaired cognitive performance (6–10) and alterations in the BOLD response (29–31). Future studies should employ larger, more heterogeneous samples so that the interactions between cardiorespiratory fitness, cardiovascular risk factors, and cognitive performance can be more fully explored.

In summary, our results indicate a modest trend towards better executive function performance in endurance-trained adults as compared to age-matched sedentary controls. Moreover, endurance-trained adults displayed faster reaction time on the 0-Back (trend) and 1-Back conditions of the 2-Back task, indicating enhanced speed of information processing. On the verbal working memory task, the endurance-trained group displayed higher 1-Back-related activation bilaterally in the cerebellum, as well as increased left inferior parietal lobe activation during higher memory load conditions. Thus, cardiorespiratory fitness appeared to enhance recruitment of the attentional control network. Future studies examining the components of the BOLD response across tasks and larger, more heterogeneous samples will be necessary to fully characterize the impact of cardiorespiratory fitness on cognition and neural plasticity in middle-aged adults.

Chapter 4: Fitness-Related Changes in Cerebrovascular Reactivity

INTRODUCTION

A growing body of literature indicates that high cardiorespiratory fitness attenuates age-related cognitive decline and is a protective factor for dementia (33,37,38). Thus, a comprehensive understanding of the physiological mechanisms that govern fitness-related cognitive enhancement may yield insights on factors that modulate the rate of cognitive decline, leading to the development of new interventions to preserve cognitive functioning across the lifespan.

Recently, fMRI has emerged as a popular method for assessing the neural underpinnings of these fitness-related changes in humans. In these studies, higher cardiorespiratory fitness has been linked to greater BOLD response in the prefrontal and parietal cortices during inhibitory control tasks (43,110). The findings undoubtedly provide provocative evidence that fitness may improve cognitive performance by enhancing neural recruitment during challenging cognitive tasks. However, interpretations of neural activation from the BOLD signal may be obscured by fitness-related differences in cerebral vascular reactivity, which can modulate the BOLD signal independently of cognition.

During cognition, regional neural activation increases, enhancing the rate of cerebral oxygen metabolism. Through a process referred to as neurovascular coupling, neural activation induces concomitant rises in cerebral blood volume and cerebral flow. Thus, the observable BOLD signal reflects the composite changes of cerebral metabolic rate, cerebral blood volume, and cerebral blood flow (132). Within young, healthy

populations, neural activation has been found to closely correlate with the BOLD signal due to intact neurovascular coupling (133). However, in populations with alterations in vascular reactivity, neurovascular coupling may be impaired, causing the BOLD signal to be a less reliable indicator of neuronal activation (132,134). For this reason, fMRI findings may be limited in studies on cardiorespiratory fitness as aerobic capacity has a well-established impact on vascular reactivity and compliance in both the peripheral and central nervous system (5,75,135). Interpretations from fMRI studies of cardiorespiratroy fitness may be greatly improved by employing methods designed to reduce to intra-individual differences in vascular function.

Prior studies have reported that the vascular variance in the BOLD signal can be reduced with a hypercapnia (breath-hold) calibration (101,134,136,137). Hypercapnia increases carbon dioxide accumulation, resulting in vessel dilation and enhancement of cerebral blood flow. Thus, breath-hold induces a global rise in the BOLD signal on the magnitude of 3-10%, despite minimal cognitive engagement (138,139). Prior studies have reported a strong correlation between BOLD signal magnitude and extent of activation between breath-hold and cognitive tasks (R^2 = .25 to .57) (134,140,141), which enables a voxel-wise calibration approach to be utilized. Employing such a method, Thomason et al. (2007) (137) reported an approximately 25% decrease in intersubject variability in the BOLD response during a working memory task. Similarly, Murphy et al (2011) (142) reported that breath-hold calibration reduced model variability and increased the number of significantly activated voxels during task completion. Given the well-documented differences in vascular reactivity between sedentary and endurance-

trained adults (5,75,135), hypercapnia calibration may reduce vascular variability in the BOLD response and enhance sensitivity for detecting fitness-related differences in the underlying neural response.

Accordingly, the aim of the current study was to examine fitness-related changes in the BOLD response during a working memory task before and after hypercapnia calibration. A cross-sectional design was employed to compare the BOLD response in sedentary and endurance-trained middle-aged adults. During fMRI, participants completed a 2-Back working memory task and a hypercapnic challenge with breath-hold. The hypercapnia fMRI data were modeled with a sine-cosine function rather than the traditional GLM as the BOLD response from tasks with short breath-hold durations (5-30 seconds) tends to be sinusoidal in nature (101,142). Moreover, the hypercapnic-induced peak in the BOLD response is typically delayed by 10-15 seconds due to the accumulation of carbon dioxide over time and changes intrathoracic pressure (142–144). Sine-cosine models enable the peak of the BOLD response to be flexibly captured regardless of onset and have been validated to provide a superior model fit than traditional GLM approaches (142). Based prior reports of enhanced cerebral blood flow in association with aerobic fitness (52–55), it was hypothesized that endurance-trained adults would demonstrate greater BOLD response during the hypercapniac challenge. Moreover, hypercapnic calibration of the working memory task was expected to enhance sensitivity for detecting cognitive-related BOLD differences between sedentary and endurance-trained adults.

METHODS

Please refer to Chapter 2 for study methods.

STATISTICAL ANALYSES

Hypercapnia Task Whole Brain fMRI Analysis

FSL v 4.1.2 (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) was used to conduct fMRI analyses. Images first underwent motion correction using MCFLIRT (113) and removal of non-brain structures using BET (114). Next, preprocessing consisting of FILM prewhitening, high-pass filtering with a cut-off 100 seconds, and spatial smoothing with a 5 mm full width half maximum Gaussian kernel was completed. Each participant's functional images were aligned to his/her high-resolution anatomical images using a 7-parameter affine transformation with FSL's registration tool, FLIRT (113). A 12-parameter affine transformation was then used to align these images to standard stereotaxic space (MNI 152) (113).

First level hypercapnia data were modeled within FSL's FEAT using sine and cosine waveforms with a duration equal to one cycle of the task (30 seconds). Additional regressors included in the model were motion parameters and their temporal derivatives. Within each voxel, the predicted time course was obtained by multiplying the sine wave regressors by their corresponding parameter estimates ((sine wave parameter estimate * sine wave time course) + (cosine wave parameter estimate * cosine wave time course)), Peak-to-peak estimates of model fit were obtained by calculating the difference between the maximum and minimum t-statistic across the time course.

For group analyses, each participant's peak-to-peak map was concatenated together in time. FSL's Randomise tool was employed to complete non-parametric

permutation testing. Briefly, this procedure reorders the rows of the design matrix and retains the maximum statistic across the brain for each sample over 5,000 iterations (118) Threshold-Free Cluster Enhancement (145) was implemented to define clusters. Correction for multiple comparisons were employed with a familywise error corrected cluster significance of $p<0.01$.

Breath-hold Calibration of the 2-Back Task

2-Back task fMRI data were processed and analyzed as described under Study 1. To perform the calibration for each contrast of interest (1-Back>0-Back, 2-Back>0-Back, and 2-Back>1-Back), three separate group level mixed effects designs were employed. For each analysis, the absolute range of the peak-to-peak hypercapnia-induced BOLD response was included as a voxel-wise regressor and the group-level 2-Back task contrast of interest was included as the dependent variable. BOLD signal within the *a priori* ROIs was then extracted from the residuals. Thus, the calibrated 2-Back data represented the BOLD response for the task after statistically removing the variance accounted for by the breath-hold task.

Hypercapnia and Calibrated 2-Back Task ROI Analysis

In order to build upon our prior research, we examined the same set of independent *a priori* ROIs used in Study 1 (116). Peak-to-peak hypercapnic and calibrated 2-Back response from each contrast of interest were extracted from each ROI. As in Study 1, group differences in the *a priori* ROIs were assessed using non-parametric permutation tests on the maximal T statistic. Briefly, this method reorders the group

labels and creates a null hypothesis distribution by performing 5,000 permutations of the Welch t-test. P-values were then derived from the max T distribution.

Exploratory Analyses

Multiple linear regression was used to examine the association between cardiorespiratory fitness (VO_2max) and calibrated 2-Back-related activation within the one ROI with significant group effects, statistically adjusting for age and sex. Additionally, calibrated 2-Back-related activation in this region was correlated with mean task accuracy and reaction time.

RESULTS

Hypercapnia Task Whole Brain Analysis

Whole brain analysis revealed that the hypercapnia task induced a strong increase in the BOLD signal globally throughout the brain. Moreover, the sedentary group displayed significantly greater hypercapnia-induced activation in multiple clusters throughout the brain including left medial prefrontal cortex (x = -6, y = 30, z = 34), right medial prefrontal cortex (x = 6, y = 26, z = 34), left middle frontal gyrus (x = -34, y = 26, z = 32), left thalamus (x = -10, y = -20, z = 10), and right thalamus (x = 12, y = -10, z = 12) (Figure 11).

Figure 11

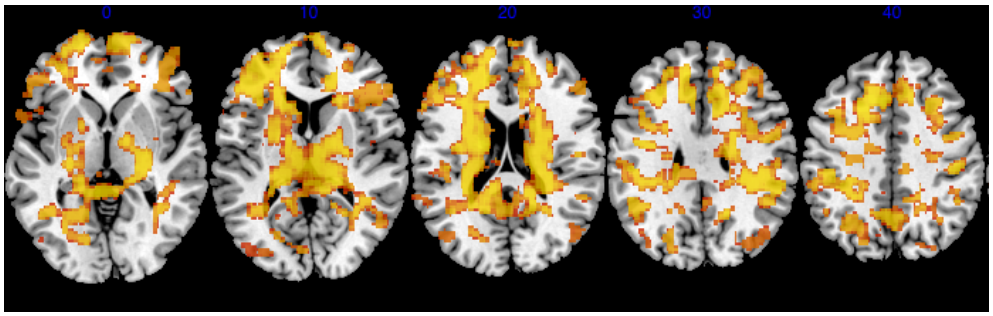

Figure 11. Greater Hypercapnia-Induced Activation in the Sedentary Group

Hypercapnia Task ROI Analysis

As seen in Table 12, the hypercapnia task induced a large rise in the BOLD signal across ROIs. In comparison to the endurance-trained group, the sedentary group demonstrated a significantly higher peak-to-peak response in the left medial frontal gyrus, left middle frontal gyrus, and the right superior frontal gyrus (Figure 12).

Table 12. Hypercapnia-induced activation in the ROIs

Brain Region & MNI Coordinates (X, Y, Z)	Sedentary	Endurance-Trained	p-values
Left Middle Frontal Gyrus -33, 8, 58	178.4±116.4	132.4±80.4	0.220
Left Medial Frontal Gyrus -4, 24, 43	209.5±77.3	142.2±53.7	0.001*
Right Superior Parietal Lobe 40, -62, 59	137.5±108.3	119.2±157.2	0.783
Left Inferior Parietal Lobe -50, -51, 49	151.4±69.4	175.0±97.0	0.498
Left Middle Frontal Gyrus -45, 48, 8	227.7±96.6	148.6±67.3	0.002*
Right Superior Frontal Gyrus 36, 52, 9	258.0±125.4	184.7±89.1	0.040*
Right Middle Frontal Gyrus 35, 10, 56	171.6±100.9	141.0±73.5	0.392
Right Inferior Frontal Gyrus 51, 16, -2	198.0±69.2	164.8±77.9	0.210

*$p<0.05$ as derived from permutations tests

Figure 12

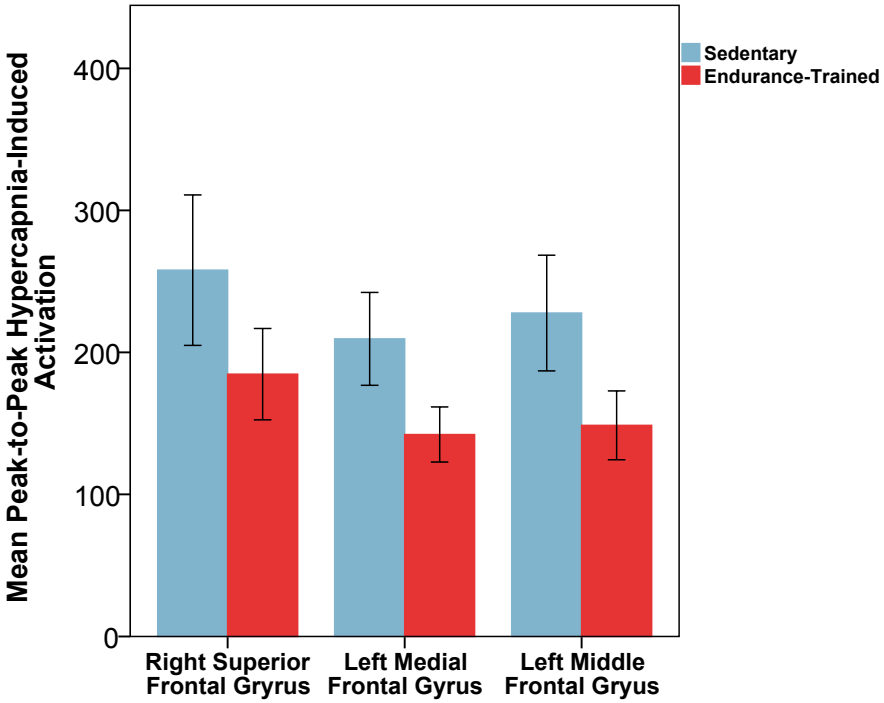

Figure 12. Bar Graph of Greater Hypercapnia-Induced Activation in the Sedentary Group

Mean peak-to-peak hypercapnia-induced activation in the sedentary and endurance-trained groups. Error bars represent 95% confidence intervals.

Calibrated 2-Back Task ROI Analysis

1-Back>0-Back: As compared to the sedentary group, the endurance-trained group displayed a trend towards higher task-related activation in the right middle frontal gyurs (Table 13). No significant group differences were observed for any of the other *a priori* ROIs after breath-hold calibration.

Table 13. Calibrated 1-Back>0-Back activation in the ROIs

Brain Region & MNI Coordinates (X, Y, Z)	Sedentary	Endurance-Trained	**p-values**
Left Middle Frontal Gyrus -33, 8, 58	-2.80±20.37	3.56±26.89	0.541
Left Medial Frontal Gyrus -4, 24, 43	3.10±23.60	-0.28±18.67	0.738
Right Superior Parietal Lobe 40, -62, 59	4.42±23.21	1.77±41.32	0.867
Left Inferior Parietal Lobe -50, -51, 49	-1.65±28.27	4.03±24.66	0.647
Left Middle Frontal Gyrus -45, 48, 8	2.74±36.27	1.55±24.55	0.937
Right Superior Frontal Gyrus 36, 52, 9	8.43±28.75	-2.80±31.50	0.344
Right Middle Frontal Gyrus 35, 10, 56	-8.43±16.43	9.05±38.00	0.061
Right Inferior Frontal Gyrus 51, 16, -2	2.79±19.46	1.17±20.24	0.868

2-Back>0-Back: As shown in Figure 13, the endurance-trained group displayed significantly higher 2-Back-related BOLD response in the right middle frontal gyrus after breath-hold calibration. No significant group differences were observed in any other *a priori* ROIs after breath-hold calibration (Table 14).

Table 14. Calibrated 2-Back>0-Back activation in the ROIs

Brain Region & MNI Coordinates (X, Y, Z)	Sedentary	Endurance-Trained	p-values
Left Middle Frontal Gyrus -33, 8, 58	-4.95±19.63	4.38±31.19	0.492
Left Medial Frontal Gyrus -4, 24, 43	1.26±33.84	1.11±25.82	0.995
Right Superior Parietal Lobe 40, -62, 59	5.38±33.69	1.20±48.29	0.911
Left Inferior Parietal Lobe -50, -51, 49	-7.10±33.64	7.88±33.50	0.349
Left Middle Frontal Gyrus -45, 48, 8	1.83±33.14	-0.13±42.88	0.960
Right Superior Frontal Gyrus 36, 52, 9	5.72±39.32	-4.09±39.29	0.719
Right Middle Frontal Gyrus 35, 10, 56	-13.64±26.62	11.37±41.99	0.039*
Right Inferior Frontal Gyrus 51, 16, -2	4.83±27.23	-1.80±25.22	0.787

*$p<0.05$ as derived from permutation tests

Figure 13

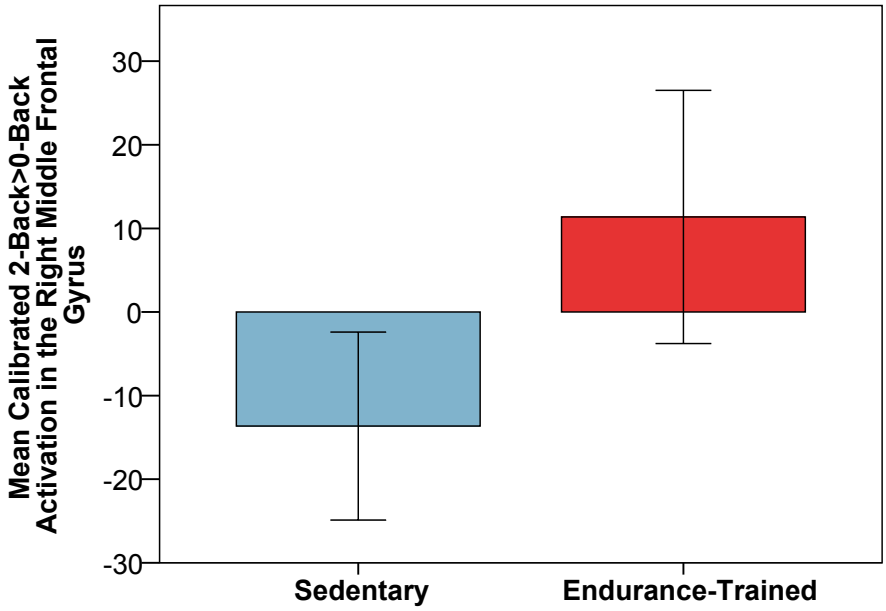

Figure 13. Greater Right Middle Frontal Gyrus Calibrated 2-Back>0-Back Activation in the in the Sedentary Group

Mean right middle frontal gyrus calibrated 2-Back>0-Back activation in the sedentary and endurance-trained groups. Error bars represent 95% confidence intervals.

2-Back>1-Back: As shown in Table 15, no significant group differences in the BOLD response were observed after breath-hold calibration. There was a modest trend towards greater left inferior parietal activation in the endurance-trained group as compared with the sedentary group.

Table 15. Calibrated 2-Back>1-Back activation in the ROIs

Brain Region & MNI Coordinates (X, Y, Z)	Sedentary	Endurance-Trained	p-values
Left Middle Frontal Gyrus -33, 8, 58	-2.44±16.21	0.86±16.28	0.683
Left Medial Frontal Gyrus -4, 24, 43	-1.12±21.72	2.27±15.54	0.738
Right Superior Parietal Lobe 40, -62, 59	1.39±20.79	0.49±18.19	0.937
Left Inferior Parietal Lobe -50, -51, 49	-5.81±15.71	4.39±19.10	0.095
Left Middle Frontal Gyrus -45, 48, 8	-1.66±24.94	-1.23±26.66	0.977
Right Superior Frontal Gyrus 36, 52, 9	-3.59±34.58	-0.43±24.29	0.857
Right Middle Frontal Gyrus 35, 10, 56	-5.30±17.69	2.27±20.33	0.324
Right Inferior Frontal Gyrus 51, 16, -2	2.17±25.29	-2.31±19.33	0.700

Exploratory Analyses

The association between breath-hold calibrated 2-Back>0-Back-related BOLD response in the right middle frontal gyrus and cardiorespiratory fitness was examined with multiple liner regression after statistical adjustment for age and sex. The overall model was significant ($p = 0.002$) with significant main effects for both age (ß=-0.314, $p=0.027$, 95% CI: -4.043 – -0.251) and VO_2max (ß=0.363, $p=0.024$, 95% CI: 0.164 – 2.195). The main effect for sex did not reach statistical significance (ß=-0.156, $p=0.336$, 95% CI: -38.940 – 13.551). As seen in Figure 14, older age was associated with reduced 2-Back-related activation in the right middle frontal gyrus, whereas higher VO_2max predicted increased 2-Back-related activation in this region (Figure 15). Greater calibrated 2-Back>0-Back activation in the right middle frontal gyrus correlated with faster 0-Back reaction time ($r=-0.274$, $p=0.041$). Calibrated 2-Back>0-Back activation in

this region also correlated with better 2-Back task accuracy (r=0.280, p=0.036) and faster reaction time (r=-0.258, p=0.055), but the significance of these results were attenuated after removal of an outlier (accuracy r=0.235, p=0.084, reaction time: r=-0.239, p=0.078). The association with 0-Back accuracy was not statistically significant (r=-0.188, p=0.164).

Figure 14

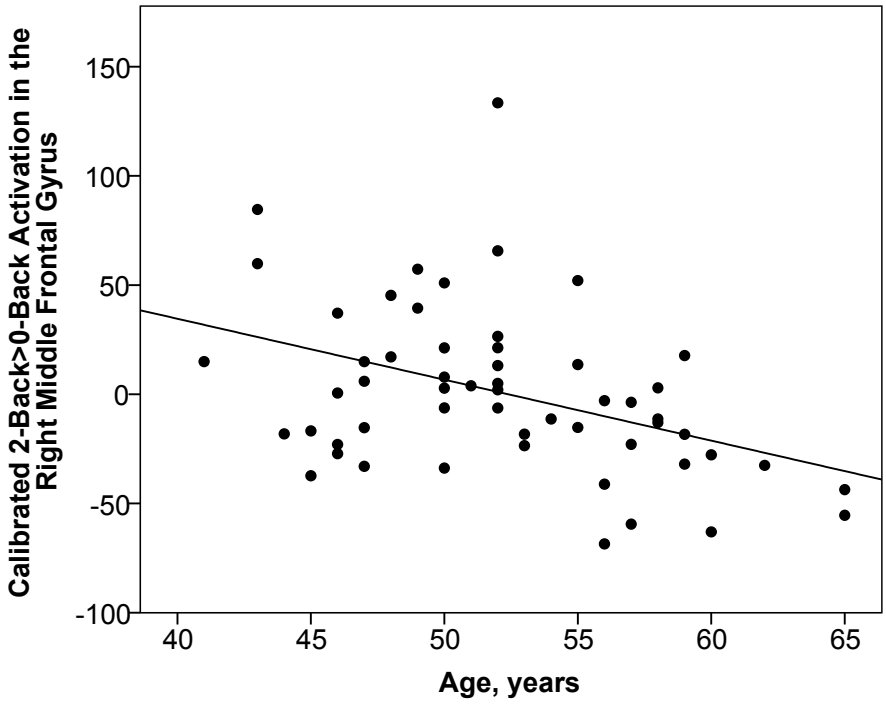

Figure 14. Scatterplot between Age and Calibrated 2-Back>0-Back Activation in the Right Middle Frontal Gyrus

Figure 15

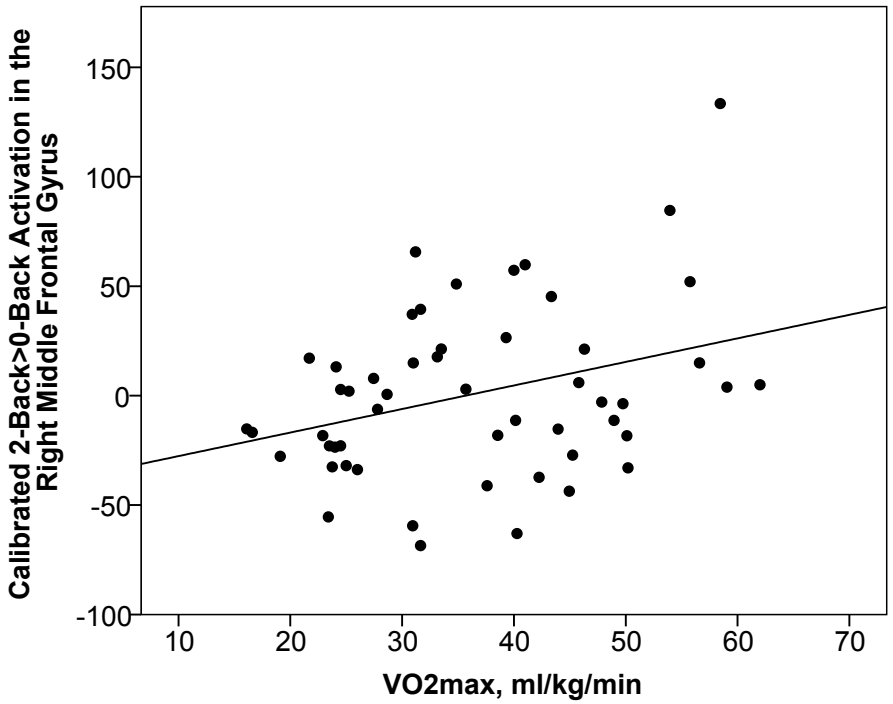

Figure 15. Scatterplot between VO_2max and Calibrated 2-Back>0-Back Activation in the Right Middle Frontal Gyrus

DISCUSSION

The primary aim of the present investigation was to examine fitness-related differences in the BOLD response to working memory after breath-hold calibration. To our knowledge, this is the first study on aerobic fitness to implement breath-hold calibration despite the well-documented effects of fitness on arterial compliance (5,75,135). In the current study, the endurance-trained group displayed greater breath-hold calibrated 2-Back>0-Back activation in the right middle frontal gyrus as compared with age-matched sedentary controls. These results provide preliminary evidence that

fitness-related differences in the neural response to a cognitive challenge may exist over and above global differences in vascular function. Moreover, exploratory analyses revealed that older age was associated with diminished calibrated 2-Back response in the right middle frontal gyrus. However, even after controlling for age, cardiorespiratory fitness was related to higher BOLD response in this region, indicating that fitness may serve to attenuate the effects of aging on the brain.

In the current study, endurance-trained adults displayed a diminished BOLD response to hypercapnia as compared to age-matched sedentary controls. This result was unanticipated as prior studies have reported increased cerebral blood flow at rest and during hypercapnia in association with aerobic fitness (52,55). Several factors may explain the discrepancy in findings. Reduced BOLD signal in the endurance-trained group may indicate a lower ratio of oxygenated-to-deoxygenated hemoglobin secondary to increased cerebral metabolism. Animal studies have reported that aerobic exercise upregulates cerebral markers of mitochondrial biogenesis (146) and enhances mitochondrial enzymes that catalyze cellular oxygen uptake in the brain (109). Increased oxygen consumption in the endurance-trained group would serve to reduce the oxygenated to deoxygenated hemoglobin ratio, attenuating the BOLD signal despite equivalent or elevated cerebral blood flow delivery. Alternatively, aerobic fitness has been shown to improve autonomic regulation (147,148), which may cause the endurance-trained group to display a comparatively modest change in cerebral blood flow and/or velocity. While determination of the mechanisms that underlie group differences in the BOLD response to hypercapnia are beyond the scope of the current study, the results

indicate that fitness-related alterations in the BOLD signal exist independently of cognition. Thus, hypercapnic calibration may be instrumental in decreasing variability in the BOLD signal that may obscure detection of cognitive-related BOLD differences. Accordingly, 2-Back>0-Back fitness-related differences in the BOLD signal were only observed after the breath-hold calibration.

Following breath-hold calibration, the endurance-trained group displayed greater BOLD response in the right middle frontal gyrus in conjuction with superior reaction time performance. Given that fitness-related differences in the BOLD response were observed after removing variance due to reaction time and global vascular reactivity, the results indicate that fitness may have beneficial effects on the neural processes underlying cognition. The finding of increased right middle frontal gyrus activation in endurance-trained adults is consistent with the well-documented phenomenon of "hemispheric asymmetry reduction in older adults (HAROLD)" (149). On verbal tasks, young adults tend to display strongly left lateralized activation, whereas high-performing middle-aged and older adults display increased bilateral activation (149). This hemispheric asymmetry reduction appears to compensate for age-related reductions in neural and metabolic efficiency, thus enabling middle-aged and older adults to maintain successful task performance (149–151). Employing a verbal n-back task similar to the one in the current study, Mattay et al. (2006) (150) reported that older adults displayed increased right prefrontal activation only during conditions in which they were able to perform as successfully as younger adults. Similarly, in the current study, higher BOLD response in the right middle frontal gyrus correlated with faster reaction time in the 0-Back condition.

Moreover, previous studies on aerobic fitness have also reported that higher cardiorespiratory fitness is associated with greater frontal and parietal lobe activation during challenging executive function tasks (43,110). Overall, the results indicate that aerobic fitness may enhance cognitive performance by promoting flexible engagement of cognitive resources (43).

Interpretation of the study results must be considered within the context of its limitations. First, the study employed a cross-sectional design, which precludes determination of causation. Individuals who elect to engage in regular aerobic exercise may have pre-existing differences, which could account for the alterations in the BOLD response and the trends towards better executive function performance. However, this possibility is reduced by the equivalence of the endurance-trained and sedentary groups in regards to age, education, and global cognitive ability. Additionally, while the results are intriguing, it must be acknowledged that the effect of the breath-hold calibration was relatively small and the significance was restricted to one region of interest. Another potential limitation is the short duration of the breath-hold task, which may induce a less robust hypercapnic response than a task with a longer breath-hold period. Moreover, the breath-hold method of hypercapnia itself has limitations. Breath-hold requires a limited amount of cognitive engagement and cannot guarantee a standard dosing of carbon dioxide across all participants. Fortunately, breath-hold induced hypercapnia has been shown to produce similar results to 5% carbon dioxide inhalation (138). Moreover, the breath-hold hypercapnia method is advantageous in the sense that it does not necessitate extra equipment and has superior spatial resolution than transcranial doppler and arterial

spin labeling (138). Nonetheless, future studies would benefit from the incorporation of multiple indices of hypercapnic-induced cerebral blood flow.

In summary, a simple breath-hold task improved detection of 2-Back>0-Back BOLD differences during cognitive challenge in sedentary and endurance-trained adults. After breath-hold calibration, endurance-trained adults displayed higher right middle frontal gyrus activation during working memory. Moreover, older age was predictive of lower BOLD response in this region, whereas cardiorespiratory fitness was associated with greater signal even statistically adjusting for age. Thus, fitness appears to attenuate age-related changes in the BOLD response during working memory in the right middle frontal gyrus. While longitudinal studies are crucial, the results indicate that cardiorespiratory fitness may enhance the underlying neural response to cognition, which may serve to slow the trajectory of age-related cognitive decline.

Chapter 5: Cardiorespiratory Fitness, Endothelial Function, and Cerebrovascular Reactivity

INTRODUCTION

A growing body of literature indicates that aerobic exercise attenuates age-related cognitive decline (37,38) and cerebral atrophy (40). While the underlying mechanisms for these changes have not been formally established, one of the most well supported hypothesis is angiogenesis, the development of new blood vessels. Animal studies have demonstrated that aerobic exercise stimulates the release of trophic factors necessary for the development of new vasculature (50,51) and enhances cerebral blood flow during hypercapnia (52). Similarly in humans, cardiorespiratory fitness has been associated with enhanced cerebral blood flow (53–55). The ability to increase cerebral blood flow in response to demand is critical for cognition, as sustained cognitive activity requires increased blood flow to engaged brain regions (67).

Aerobic exercise may increase cerebral blood flow during cognition by enhancing nitric oxide, a potent vasodilator. In the central nervous system, nitric oxide synthases is released from the endothelium of the vessels, as well as from cells in the neuronal parenchyma (60). Nitric oxide synthase upregulates the production of nitric oxide, which initiates a molecular cascade resulting in relaxation and dilation of the smooth muscle cells in the vessel wall (59). Numerous human and animal studies have demonstrated that nitric oxide is critical for maintaining basal cerebral blood flow, as well as enhancing cerebral blood flow in response to hypercapnic demands (63,72,152,153). Aerobic exercise may facilitate nitric oxide release (75,76,154), enhancing cerebral blood and attenuating age-related cerebral atrophy and cognitive decline.

Nitric oxide release in the cerebrovasculature is difficult to measure in humans. However, it can be easily examined in the periphery using brachial flow mediated

dilation (FMD). During brachial FMD, forearm occlusion induces sheer stress, which generates nitric oxide release and subsequent vasodilation in healthy adults (155). In older adults, vasodilation in response to nitric oxide is diminished or even becomes paradoxical (156). Assessment of brachial FMD may be a viable proxy for cerebrovascular endothelial function as strong correlations in endothelial-dependent vasodilation are obtained across diverse vascular beds (77). Moreover, peripheral endothelial dysfunction has been associated with diminished cognitive ability (12) and a greater extent of white matter hyperintensities in older adults (17), indicating the peripheral measure's relevance to the brain.

The aim of the current study was to examine peripheral endothelial function and its relation to cerebrovascular reactivity in sedentary and endurance-trained middle-aged adults. Peripheral endothelial function was non-invasively measured with brachial FMD. Cerebrovascular reactivity was assessed with fMRI by examining the BOLD response to hypercapnic challenge. Based on prior literature (75), endurance-trained adults were predicted to display enhanced endothelial function. Moreover, better peripheral endothelial function was anticipated to predict increased cerebrovascular reactivity.

METHODS

Brachial FMD measurements were not obtained from one sedentary and two endurance-trained participants. The sample for the current study consisted of twenty-three sedentary and thirty endurance-trained participants. Please refer to Chapter 2 for additional study methods.

STATISTICAL ANALYSES

Brachial Flow-Mediated Dilation

Brachial FMD was collected as described in Chapter 2. For data analysis, ultrasound-derived blood velocity and diameter data were assessed using image analysis software (Vascular Research Tool Brachial Analyzer, Medical Imaging Applications, Coralville, IA). FMD was expressed as the percent change in brachial artery diameters recorded during the pre and post occlusion phases using the equation: (maximum diameter – baseline diameter)/baseline diameter × 100. The average of 10 end-diastolic brachial artery diameters before blood flow occlusion were used for baseline diameters and the average of 3 peak end-diastolic diameters during the reperfusion phase were used for maximum brachial artery diameter. Group differences in brachial FMD were assessed with an independent samples t-test.

Brachial Flow-Mediated Dilation & Hypercapnia Task Whole Brain Response

Hypercapnia data was preprocessed and analyzed at the individual participant level as described in Study 2. The association between brachial FMD and the BOLD response to hypercapnia was examined at the group-level using non-parametric permutation testing with the FSL Randomise tool. Mean-centered age, sex, systolic blood pressure, and brachial FMD were included as regressors in the model. The rows of the design matrix were reordered 5,000 times and the maximum statistic across the brain for each sample was retained. Clusters were identified using Threshold-Free Cluster Enhancement (145) and applying a familywise error corrected cluster significance of p=0.01.

Brachial Flow-Mediated Dilation & Hypercapnia Task ROI Response

The BOLD response to hypercapnia within *a priori* ROIs was computed as described in Chapter 4. The relationship between brachial FMD and mean percent BOLD signal change during hypercapnia in *a priori* ROIs was modeled using hierarchical linear regression. Covariates (age, sex, systolic blood pressure) were added in the first step of the analysis. Brachial FMD was added to the second step in order to quantify the amount of variance explained by its inclusion.

RESULTS

Brachial Flow-Mediated Dilation

As displayed in Table 16, brachial FMD did not significantly differ between the endurance-trained and sedentary groups.

Table 16. Mean brachial FMD in the sedentary and endurance-trained groups

	Sedentary	Endurance-Trained	t(51)	P-value
Brachial FMD	5.35±3.19	6.35±3.21	1.133	0.262

Brachial Flow-Mediated Dilation & Hypercapnia Task Whole Brain Response

Brachial FMD did not predict hypercapnia-induced BOLD response. Age was the only covariate in the model that had a significant main effect. Older age predicted lower hypercapnia-induced activation in the left occipital lobe (x = -26, y = -96, z = 24), right occipital lobe (x = 36, y = -94, z = 14), and right thalamus (x = -16, y = -12, z = -2).

Brachial Flow-Mediated Dilation & Hypercapnia Task ROI Response

There were no significant findings for the fully adjusted models across all *a priori* ROIs (Table 17).

Table 17. Linear regression models exploring the association between brachial FMD and Hypercapnia Task ROI response

	Model 1		Model 2	
Model Terms	Estimate (SE)	p-values	Estimate (SE)	p-values
Left Middle Frontal Gyrus		0.477		0.271
Age	3.12 (2.65)	0.245	5.44 (2.96)	0.072
Sex	25.48 (30.67)	0.410	31.97 (30.40)	0.298
Systolic blood pressure	-0.161 (1.27)	0.900	0.03 (1.26)	0.983
Brachial FMD			8.30 (5.04)	0.106
Left Medial Frontal Gyrus		0.493		0.591
Age	2.55 (1.93)	0.193	3.23 (2.21)	0.150
Sex	-21.06 (22.35)	0.351	-19.16 (22.67)	0.402
Systolic blood pressure	-0.38 (0.93)	0.680	-0.33 (0.94)	0.727
Brachial FMD			2.43 (3.76)	0.520
Right Superior Parietal Lobe		0.193		0.320
Age	4.59 (3.64)	0.214	4.80 (4.18)	0.257
Sex	52.82 (42.14)	0.216	53.42 (49.93)	0.219
Systolic blood pressure	1.09 (1.75)	0.536	1.10 (1.78)	0.535
Brachial FMD			0.77 (7.11)	0.915
Left Inferior Parietal Lobe		0.194		0.317
Age	-2.61 (2.28)	0.259	-2.32 (2.62)	0.381
Sex	-19.13 (26.41)	0.472	-18.32 (26.89)	0.499
Systolic blood pressure	2.08 (1.10)	0.064	2.10 (1.11)	0.065
Brachial FMD			1.04 (4.46)	0.816
Left Middle Frontal Gyrus		0.141		0.233
Age	5.04 (2.30)	0.033	5.54 (2.63)	0.041
Sex	-31.62 (26.57)	0.240	-30.21(27.03)	0.269
Systolic blood pressure	0.45 (1.10)	0.687	0.49 (1.12)	0.664
Brachial FMD			1.80 (4.48)	0.690

Table 17 (continued)

Right Superior Frontal Gyrus		0.758		0.644
Age	2.44 (3.00)	0.421	4.31 (3.40)	0.212
Sex	-16.56 (34.73)	0.636	-11.35 (34.91)	0.746
Systolic blood pressure	0.87 (1.44)	0.548	1.02 (1.45)	0.482
Brachial FMD			6.66 (5.78)	0.255
Right Middle Frontal Gyrus		0.912		0.958
Age	0.70 (2.35)	0.768	0.27 (2.70)	0.921
Sex	15.71 (27.19)	0.566	14.51 (27.68)	0.603
Systolic blood pressure	0.3 (1.13)	0.978	-0.00 (1.15)	0.997
Brachial FMD			-1.54 (4.59)	0.739
Right Inferior Frontal Gyrus		0.678		0.515
Age	2.41 (2.05)	0.245	3.87 (2.31)	0.101
Sex	-6.84 (23.70)	0.774	-2.78 (23.72)	0.907
Systolic blood pressure	0.25 (0.99)	0.799	0.37 (0.98)	0.708
Brachial FMD			5.20 (3.93)	0.192

DISCUSSION

The aim of the current investigation was to explore the impact of cardiorespiratory fitness on peripheral endothelial function. Moreover, we sought to examine the efficacy of peripheral endothelial function for predicting cerebrovascular reactivity as derived from the BOLD response to hypercapnia.

In the current study, peripheral endothelial function as assessed by brachial FMD did not differ between the sedentary and endurance-trained groups. This result was surprising given that aerobic exercise stimulates the production of nitric oxide synthases mRNA (76). Additionally, prior studies have reported that highly aerobically fit older adults display attenuated age-related declines in endothelial function when compared to age-matched sedentary controls (75). It is possible that the current study lacked the power necessary for detecting significant effects as fitness-related improvements in endothelial

function are more evident in older adults (75). Moreover, an overall strength of the current study was that the endurance-trained and sedentary groups were well matched in terms of cardiovascular risk factors such as blood pressure, total cholesterol, and glucose levels. Fitness may preserve endothelial function in older age at least in part by reducing the prevalence of cardiovascular risk factors, which are known to diminish nitric oxide production (72,157). Thus, fitness-related differences in endothelial function may be less evident in our well-controlled sample as compared to the general population.

The second aim of the current study was to examine if cerebrovascular reactivity could be predicted by peripheral endothelial function. Our study failed to find an association between peripheral endothelial function and cerebrovascular reactivity as assessed by the BOLD response to hypercapnia. Our study paradigm utilized a short breath-hold duration (13.5 seconds), which may not have been sufficient to elicit endothelial-dependent vasodilation. Future studies should examine the association between brachial FMD and hypercapnia-induced BOLD response using longer breath-hold durations. Alternatively, it is possible that the hypercapnia-induced BOLD response may not sufficiently reflect cerebrovascular reactivity. The BOLD signal is a composite measure of cerebral blood flow, cerebral blood flow, and cerebral metabolic rate (67). Thus, contributions of non-endothelial-dependent factors may obscure the association between the BOLD response and endothelial function. Transcranial Doppler imaging, which provides a more direct assessment of vascular reactivity, may provide a closer correlate to endothelial function. In congruence with this hypothesis, poorer peripheral

endothelial function has been associated with attenuated middle cerebral artery vasodilation in response to hypercapnia (72).

In summary, peripheral endothelial function did not significantly differ between the sedentary and endurance-trained groups. Our study may have lacked power for detecting differences given that the participants were middle-aged and had few cardiovascular risk factors. We also failed to find an association between peripheral endothelial function and cerebrovascular reactivity as assessed by the BOLD response to hypercapnia. Future studies will benefit from the inclusion of larger, more diverse samples as well as the implementation of multiple indices of cerebrovascular reactivity.

Chapter 6: General Discussion

Accumulating evidence indicates that poorer vascular health accelerates cognitive decline and increases the likelihood of dementia in old age (6–10). Aerobic fitness, as a protective factor against vascular dysfunction (5), may thus serve to attenuate age-related cognitive pathology. Indeed, older adults with higher cardiorespiratory have been found to display superior cognitive performance (37,38) and increased frontal and parietal lobe engagement during challenging cognitive tasks (43,110). The overarching goal of the current investigations was to expand the current literature by examining fitness-related changes in cognition and its underlying neural substrates in middle-aged adults. Moreover, cerebrovascular reactivity and endothelial function were examined as potential determinants of fitness-related cognitive enhancement. Successful identification of the factors underlying the beneficial effects of fitness is tantamount to the development of interventions to preserve cognitive function across the lifespan.

In the first study, a cross-sectional design was employed to compare cognitive abilities in sedentary and endurance-trained middle-aged adults. Standard neuropsychological measures were utilized to assess cognition within the domains of global cognition, memory, and executive function. The main finding was a modest trend towards better executive function in the endurance-trained group as compared to age-matched sedentary controls. Similarly, studies on older adults have reported the greatest fitness-related cognitive gains in the executive function domain (38). In congruence with the trend towards enhanced executive function, the endurance-trained group also displayed faster reaction time on the 0-Back (trend) and 1-Back conditions of the 2-Back

task. Given that task accuracy was equivalent between the two groups, faster reaction time in the endurance-trained group may indicate increased speed of processing, a component of executive functioning. Overall, our findings suggest that higher cardiorespiratory fitness at midlife may be associated with mildly enhanced executive function performance. The extension of exercise-related cognitive benefits to middle-aged adults is important since midlife may represent a critical period for implementing interventions to preserve cognitive function. However, longitudinal studies will be necessary to fully evaluate the impact of midlife aerobic fitness on individual cognitive trajectories.

An additional goal of the first study was to compare the BOLD response to a challenging cognitive task between the sedentary and endurance-trained groups. Animal studies have reported that neurogenesis (46–49), angiogenesis (50,51,105), and upregulation of neurotransmitters (106–108) may be mechanisms underlying fitness-related cognitive enhancement. However, it is unclear on how accurately these findings translate to human models, particularly given the complexity of human thought and brain organization. fMRI provides a non-invasive method for studying the brain in humans and has demonstrated efficacy for detecting phenotypic expressions of cognitive vulnerability and protection (43,110,116,158). Thus, it may have useful applications for studies of aerobic fitness.

In Study 1, the sedentary and endurance-trained groups completed a 2-Back working memory task during fMRI. The 2-Back task was selected since it engages working memory (99,100), an executive function skill that has not yet been evaluated

with fMRI in fitness studies. The main findings were that the endurance-trained group displayed increased bilateral cerebellum activation during the 1-Back>0-Back condition and higher left inferior parietal lobe activation during the 2-Back>1-Back condition. The cerebellum and parietal lobe are both instrumental for successful executive function performance (45,99,122,124). Along with the frontal lobes, the cerebellum and inferior parietal lobes facilitate 2-Back task performance by increasing sustained attention, improving task management, and regulating the updating and monitoring of contents in working memory (45,122). Thus, higher bilateral cerebellum and left inferior parietal lobe activation in the endurance-trained group may indicate greater flexibility for increasing neural recruitment during cognitive challenges. Interestingly, aerobic exercise stimulates angiogenesis in the cerebellum (125) and attenuates age-related atrophy in the both the cerebellum and the parietal lobe (40,126). Thus, increased cerebellar and inferior parietal activation in the endurance-trained group may be secondary to exercise's beneficial effects on perfusion and/or neuronal integrity.

The second study extended the findings from Study 1 by attempting to disentangle the vascular and neural components of the fMRI BOLD signal. The BOLD signal elicited during cognition reflects composite changes in cerebral blood flow, cerebral blood volume, and cerebral oxygen metabolism (132). Thus, the utility of fMRI for detecting the underlying neural response critically depends on a close coupling between cerebral oxygen metabolism and the neurovasculature. Importantly, this property may be violated by a host of vascular factors including baseline cerebral blood flow, hemocrit concentrations, atherosclerosis, and responsivity to vasoactive substances (134).

Uncoupling is a concern for studies on fitness since greater aerobic capacity has been linked to changes in vascular reactivity and compliance (5,75). Thus, neural inferences on fitness-related changes in the BOLD signal may be confounded by differences in the local vasculature. Fortunately, recent studies have reported that hypercapnia, secondary to a simple breath-task, can improve interpretation by reducing variability due to intra-individual differences in vascular function (134,136,137). Accordingly, the aim of the second study was to reassess fitness-related changes in the BOLD response to working memory after employing a breath-hold calibration.

One of the primary findings in the second study was that the sedentary group displayed greater hypercapnia-induced BOLD signal. This outcome was unexpected based on prior reports of enhanced cerebral blood flow in association with aerobic fitness (52,54). Several mechanisms, requiring further study, may account for the unanticipated results. One possibility is that endurance-trained group may have higher baseline cerebral metabolism, which would serve to lower the ratio of oxygenated-to-deoxygenated hemoglobin and attenuate the BOLD signal. Animal studies have provided evidence that aerobic exercise training upregulates brain markers of mitochondrial biogenesis (146) and stimulates the production of mitochondrial enzymes that catalyze cellular oxygen uptake in the brain (109). Thus, the endurance-trained group may have a lower oxygenated to deoxygenated hemoglobin ratio, attenuating the BOLD signal despite equivalent or elevated cerebral blood flow delivery. Alternatively, superior autonomic regulation in the endurance-trained group (147,148) may yield a comparatively modest change in cerebral blood flow and/or velocity. Determination of

the mechanisms that underlie group differences in the BOLD response to hypercapnia are beyond of the scope of the study. However, they indicate the presence of significant fitness-related alterations in the BOLD signal that operate independently of cognition. Thus, hypercapnic calibration may be instrumental in decreasing variability in the BOLD signal that may obscure detection of cognitive-related BOLD differences.

In accordance, the main finding of study 2 was the observation of increased working memory-related BOLD signal in the right middle frontal gyrus in the endurance-trained group *after* breath-hold calibration. Given that group differences were detected after calibration for global differences in vascular function, it appears that fitness may induce alterations in the underling neural response to a cognitive challenge. Moreover, exploratory analyses revealed that older age was associated with diminished calibrated 2-Back response in the right middle frontal gyrus. However, even after statistically adjusting for age, cardiorespiratory fitness was predictive of greater activation in this region, indicating that fitness may serve to attenuate the effects of aging on the brain.

The third study attempted to extend Study 2 by examining endothelial function as a mechanism underlying fitness-related differences in cerebrovascular reactivity. The rationale for the study was based on prior research displaying a positive association between cerebral blood flow and cardiorespiratory fitness (52,54). Enhanced endothelial function, secondary to the upregulation of the vasodilator nitric oxide, was predicted to explain the link between fitness and elevated cerebral blood flow. Nitric oxide has a critical role in the regulation of cerebral blood flow both at rest and under conditions of hypercapnic challenge (63,72,152,153). Interestingly, aerobic fitness has been found to

stimulate nitric oxide mRNA production (76) and improve peripheral endothelial function (75). Thus, the goal of Study 3 was to determine if peripheral endothelial function, as assessed by brachial flow-mediated dilation (FMD), predicted the BOLD response to hypercapnia.

In contrast to the original hypothesis, there was no significant difference in peripheral endothelial function between the two groups. Additionally, the results from Study 3 failed to find an association between peripheral endothelial function and the BOLD response to hypercapnia. Thus, endothelial function may not be a critical factor governing the BOLD response to hypercapnia. These results are supported by the finding of enhanced hypercapnia-induced BOLD response in the sedentary group as compared to the endurance-trained group despite equivalent endothelial function. However, it is possible that the breath-hold duration (13.5 seconds) utilized in the current study was not sufficient to induce nitric oxide release. Future studies should use longer breath-hold durations and incorporate multiple indices of cerebrovascular reactivity, particularly transcranial Doppler imaging, which provides a more direct assessment of cerebrovascular function.

Overall, the three studies collectively indicate that higher cardiorespiratory fitness may modestly improve executive function performance by enhancing the underlying neural response to cognition. Unfortunately, Study 3 failed to establish endothelial function as a predictor of fitness-related changes in the BOLD response. Thus, the underlying mechanisms governing fitness-related increases in neural flexibility are still unclear. Nonetheless, it is interesting to speculate about potential directions to explore in

future research. In particular, studies on aging have indicated that functional connectivity may be an important mechanism underlying increased neural engagement during cognitive tasks (159).

Studies of functional connectivity frequently examine the default mode network (DMN), a synergistic collection of brain regions that activate during rest and are hypothesized to represent self-referential and free associative thought (160,161). In addition to the important role of the DMN at rest, this network also governs successful performance during cognitive tasks (162,163). For example, Sambataro et al. (2010) reported that weaker functional connectivity between the posterior cingulate cortex and the medial prefrontal cortex predicts poorer working memory task performance in older adults. Interestingly, higher levels of aerobic fitness have been found to enhance functional connectivity between the posterior cingulate cortex and medial prefrontal cortex (111), which may facilitate sustained attention and thus improve task performance in older adults.

On a molecular level, increases in functional connectivity are hypothesized to be secondary to enhanced dopaminergic modulation of GABA inhibitory neurons in the prefrontal cortex (159). With advancing aging, dopamine D2 receptor availability declines, predicting poorer performance on frontally-mediated tasks (164). In contrast to the detrimental effects of aging on D2 receptors, aerobic exercise enhances the survival and function of dopaminergic neurons through stimulation of trophic factors such as brain derived neurotrophic factor (BDNF) (108,165). Thus, exercise-induced stimulation of trophic factors may serve to reduce age-related disruptions in dopamingeric function,

enhancing the prefrontal cortex's ability to maintain representations in working memory and thus, improving executive function in older adults (166). Future work on dopaminergic function in relation to cardiorespiratory fitness will be necessary to fully explore this hypothesis.

The limitations of the studies must be contemplated when interpreting the results. It is important to highlight that the fitness-related changes in cognition and the BOLD response were relatively modest. These results contrast to prior studies in older adults, which have reported moderate to large effect sizes (38). It is possible that the current study lacked sufficient power to detect significant findings. Prior research has reported smaller fitness-related cognitive benefits in young-old (55-65 years) versus mid-old adults (66-70 years) (38). Given that the current sample consisted of adults between the ages of 45-65 years, a larger sample size may be necessary. Additionally, the current sample was relatively homogenous in terms of education. Higher educational attainment has been consistently associated with cognitive reserve (129), a factor that moderates the relationship between the degree of cerebral pathology and manifested cognitive ability (130). Thus, age-related cognitive decline may have been attenuated by the high level of educational attainment in our sample, creating a ceiling effect for observing fitness-related enhancement. Finally, the current findings may less robust than prior research due to our careful control of cardiovascular risk factors. Poorer cardiorespiratory fitness in the general population is associated with increased cardiovascular burden (131). Similar to low levels of aerobic fitness, cardiovascular risk predicts poorer cognitive function (6–10) and alterations in the BOLD response (29–31). Unfortunately, few studies have

controlled for cardiovascular risk factors, which may confound results and lead to an overestimation of the benefits of aerobic fitness per se.

The interpretations of the findings are also limited by other methodological constraints. The current studies utilized a cross-sectional design, which precludes inferences of causation. Individuals who elect to engage in regular aerobic exercise may have pre-existing differences that account for the observed findings. An additional methodological limitation is the relatively short duration of the hypercapnic challenge as it may induce less robust changes than tasks with longer breath-hold periods. Moreover, the breath-hold method of hypercapnia itself has disadvantages. Successful completion of breath-hold requires a limited amount of cognitive engagement and does not guarantee a standard dosing of carbon dioxide across all participants. Future studies should employ a longitudinal study design including larger, more heterogeneous samples and multiple indices of hypercapnia so that the interactions between cognition, cardiorespiratory fitness and cerebrovascular reactivity can be more fully understood.

In summary, the current series of studies indicate that higher cardiorespiratory fitness is associated with a modest trend towards better executive function performance in healthy middle-aged adults. A closer analysis of fitness-related effects on executive function was completed with functional neuroimaging. fMRI revealed that the endurance-trained group displayed greater working memory-related BOLD response in the cerebellum and inferior parietal lobe, as well as superior task performance. The impact of fitness on the underlying neural substrates of cognition was further examined after breath-hold calibration, a technique utilized to reduce vascular variance and provide a

closer approximation of the neural contributions to the BOLD signal. The results revealed that after breath-hold calibration the endurance-trained group displayed greater working memory-related activation in the right middle frontal gyrus, indicating that fitness-related BOLD changes can be detected over and above global differences in vascular function. Moreover, older age was predictive of lower BOLD response in this region, whereas cardiorespiratory fitness was associated with greater signal even after statistically adjusting for age. Thus, fitness appears to attenuate age-related changes in the BOLD response during working memory in the right middle frontal gyrus. While longitudinal studies are crucial, the results indicate that cardiorespiratory fitness may enhance the underlying neural response to cognition, which may ultimately serve to slow the trajectory of age-related cognitive decline.

Appendix A: Telephone Screening Form

Telephone Screening
Physical Fitness, Cardiovascular and Brain Health Study

Date: _____

Background Information

Name:
Address:
Phone/Email:
Age: **Height:** **Weight:** **BMI :**

Basic Screening Questions

❒ **Yes** ❒ **No** Do you smoke? – *If does now or quit in past 6mths, reject*
❒ **Yes** ❒ **No** Are you right-handed? – *If no, reject*
❒ **Yes** ❒ **No** **BP**_____/_____ Do you have high blood pressure?
❒ **Yes** ❒ **No** Are you currently taking any medication to control your blood pressure?
❒ **0** ❒ **1** ❒ **2** ❒ **3 or more** In the past year, over an average week, how many days did you engage in moderate or vigorous physical activity such as biking, running, or playing soccer?

If currently exercises:

- What type of exercise do you do? (i.e. aerobic/weightlifting)? _____
- Have your ever participated in any local or national races?
- ❒ **No** ❒ **Yes, date of last race** _____

❒ **Yes** ❒ **No** Have you ever been told by a doctor that you shouldn't exercise? – *If yes, reject*
❒ **Yes** ❒ **No** Do you have type of pain or discomfort that prevents you from exercising ? – *If yes, reject*
❒ **Yes** ❒ **No** Have you experienced unusual pain or discomfort in your chest, neck, jaw, arms or other areas that may be due to heart problems? – *If yes, reject*
❒ **Yes** ❒ **No** Have you experienced unusual fatigue or shortness of breath at rest, during usual activities, or during mild-to-moderate exercise (e.g., climbing stairs, carrying groceries, brisk walking, cycling)? – *If yes, reject*
❒ **Yes** ❒ **No** When you stand up, or sometimes during the night while you are sleeping, do you have difficulty breathing? – *If yes, reject*

❒ **Yes** ❒ **No** Do you lose your balance because of dizziness or do you ever lose consciousness – *If yes, reject*

❒ **Yes** ❒ **No** Have you experienced an unusual and rapid throbbing or fluttering of the heart? – *If yes, reject*

❒ **Yes** ❒ **No** Have you undergone a barium test within the last week or an injection with X-ray dye or nuclear medicine scan within the last two weeks?

If yes – date of procedure: ___/___/___ **must postpone DEXA scan*

For women only: ❒ **Yes** ❒ **No** Are you post-menopausal?

Medical Screening Questions

Have you ever been diagnosed with and/or treated for the following medical conditions:

❒ **Yes** ❒ **No** Heart arrhythmia/ Heart murmur? – *If yes, reject*

❒ **Yes** ❒ **No** Asthma – *If yes, reject*

❒ **Yes** ❒ **No** Cardiac arrest/Heart attack? – *If yes, reject*

❒ **Yes** ❒ **No** Coronary artery disease? – *yes, reject*

❒ **Yes** ❒ **No** Diabetes? – *If yes, reject*

❒ **Yes** ❒ **No** Kidney problems? – *If yes, reject*

❒ **Yes** ❒ **No** Thyroid problems? – *If yes, reject*

❒ **Yes** ❒ **No** Liver problems? – *If yes, reject*

❒ **Yes** ❒ **No** Head injury including loss of consciousness >5min? – *If yes, reject*

❒ **Yes** ❒ **No** Stroke? – *If yes, reject*

❒ **Yes** ❒ **No** Epilepsy? – *If yes, reject*

❒ **Yes** ❒ **No** Alcohol or drug abuse? – *If yes, reject*

❒ **Yes** ❒ **No** Emotional problems, "such as anxiety, depression, ADHD…" – *reject if: ADHD, Bipolar, OCD, personality disorders; if anxiety or depression – ok if not current*

MRI Safety Screening Questions

Please indicate if you have any of the following:

❒ Yes ❒ No Electronic implant or device

❒ Yes ❒ No Magnetically-activated implant or device

❒ Yes ❒ No Cardiac pacemaker

❒ Yes ❒ No Any type of prosthesis

❒ Yes ❒ No Any metallic fragment or foreign body

❒ Yes ❒ No Surgical staples, clips, or metallic sutures

❒ Yes ❒ No Joint replacement (hip, knee, etc.)

❒ Yes ❒ No Bone/joint pin, screw, nail, wire, plate, etc.

❒ Yes ❒ No Dentures or partial plates

❒ Yes ❒ No Tattoo or permanent makeup
❒ Yes ❒ No Body piercing jewelry – *if removable, okay*
❒ Yes ❒ No Breathing disorder
❒ Yes ❒ No Motion disorder or tremors
❒ Yes ❒ No Claustrophobia
❒ Yes ❒ No Other _____

Current Medications:

_____
_____
_____

If eligible & currently sedentary: Would you be interested in being contacted in the future for a study that involves participation in a 12 week exercise program?
❒ Yes ❒ No

Subject approved: YES NO – *if no, please shred immediately*
Interviewer: _____

Appendix B: Medical History Questionnaire

Physical Fitness, Cardiovascular and Brain Health Study
MEDICAL HISTORY QUESTIONNAIRE

Please answer each question as honestly and accurately as possible, and bring this completed questionnaire with you to your appointment**. Your answers will be kept strictly confidential.**

Today's Date:_____/_____/_____
Age:_____ Date of Birth: ____/____/_____

Sex (please circle): Male Female

Relationship Status (please circle):

Married Domestic Partnership Single Divorced Separated Widowed

Race (please circle): Caucasian African American Latino Asian Other

Current Height:_____ Current Weight:_____

Women: Are you postmenopausal?_____

Address:_____

_____

Primary Telephone Number:_____ (please circle: home work cell)

Alternate Telephone Number:_____ (please circle: home work cell)

Emergency Contact Information
Name of closest relative or friend (for emergency contact): _____

Relationship to you: _____ Telephone: _____

Physician Information

Primary Care Physician Name: _____ Telephone:_____

Primary Care Physician Address:_____

_____

Cardiologist Name:_____ Telephone:_____

Cardiologist Address:_____

_____

IMPORTANT**: Do you experience adverse reactions to nitroglycerin?**

(please circle) Yes No Unsure

Has someone in your immediate family (that is, your biological mother, father, brother, sister, or any of your children) experienced any of the following problems: heart attack, bypass, angioplasty/stent placement, dementia, **hypertension BEFORE age 60**?

YES or NO

If so, please indicate below all family members with problems. Also, be sure to **indicate the age at which their heart problem began**:

_____

_____

Primary/First Language: _____ Secondary Language: _____

Years of Education: _____ Highest Academic Degree: _____

Occupation: _____

(if retired, please indicate your occupation before retirement)

If you were not born in the USA, please indicate what year you moved to the USA

CURRENT MEDICATIONS

Please list current medications and dosages, use the back of the page if necessary:

Medication Type	**Name of Medication and Dose**	**Last time taken?**
Heart medicine		
Blood pressure medicine		
Cholesterol medicine		
Thromboembolic disease medicine		
Hypercoaguability medicine		
Steroids		
Hormones/HRT		
Birth Control		
Medicine for breathing/lungs		
Insulin		
Other medicine for diabetes		
Arthritis medicine		
Medicine for depression		
Medicine for anxiety		
Thyroid medicine		
Medicine for ulcers		
Allergy medicine		

Pain killers (prescription or over-the-counter)		
Dietary supplements (herbs, vitamins, etc)		
Other (please specify)		

Medical Problems

Have you ever had any of the following **general medical problems?**

Medical Problem	Please Circle	Year of Onset	If your answer is yes…
Angina (Chest pain)	YES or NO		Does this limit your ability to exercise?
Arrhythmia	YES or NO		
Arthritis	YES or NO		What type?
Asthma	YES or NO		How is it treated?
Atrial fibrillation	YES or NO		
Autoimmune Disease	YES or NO		What type?
Blood Clots	YES or NO		Where? (e.g. lungs, legs)
Blood pressure	YES or NO		Was it too high or too low?
Cardiac Arrest/Heart Attack/Heart Failure	YES or NO		
Coronary Artery Disease	YES or NO		
Angioplasty/Bypass Surgery/Heart Valve Surgery	YES or NO		
Cancer	YES or NO		What type (e.g., prostate, colon)?

Cushing's Syndrome	YES or NO		
Diabetes	YES or NO		Circle: Type I (child onset) or Type II (adult onset) Circle: Controlled by diet, oral medication, or insulin
Glaucoma	YES or NO		
Hepatitis	YES or NO		Circle: Hepatitis A, B, or C
High Cholesterol	YES or NO		
Kidney Problems	YES or NO		What type?
Liver Problems	YES or NO		What type?
Thyroid Problems	YES or NO		Hypothyroidism (underactive) or hyperthyroidism (overactive)
Viral Infection	YES or NO		What type? Date of last infection:

Have you ever had any of the following **neurological problems?**

Neurological Problem	**Please Circle**	**Year of Onset**	**If your answer is yes…**
1. Head Injury	YES or NO		Was there a loss of consciousness? For how long?
2. Brain hemorrhage	YES or NO		Please explain:
3. Stroke	YES or NO		Please explain:
4.Transient Ischemic Attack (TIA or Mini-Stroke)	YES or NO		Please explain:
5. Brain infection/meningitis	YES or NO		

6. Multiple Sclerosis	YES or NO		
7. Parkinson's disease	YES or NO		

Have you ever had any of the following **additional problems?**

Problem	**Please Circle**	**Year of Onset**	**If your answer is yes…**
1. Anxiety	YES or NO		Have you undergone treatment?
2. Depression	YES or NO		Have you undergone treatment?
3. Schizophrenia	YES or NO		Have you undergone treatment?
4. Other Psychiatric Illness	YES or NO		Please specify: Have you undergone treatment?
5. Alcohol/Drug Abuse	YES or NO		Have you undergone treatment?
7. Smoking	YES or NO		How many packs per day: If you quit, please indicate when:

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