THE RELATIONSHIPS BETWEEN CARDIORESPIRATORY FITNESS, WHITE MATTER INTEGRITY, AND COGNITIVE FUNCTION IN OLDER ADULTHOOD

by

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Bachelor of Arts, University of Connecticut, 2011

Submitted to the Graduate Faculty of the

Kenneth P. Dietrich School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2015

UNIVERSITY OF PITTSBURGH

DIETRICH SCHOOL OF ARTS AND SCIENCES

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December 11th, 2014

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White matter in the brain supports higher-order cognitive processes by facilitating signal transmission between diverse cortical regions. White matter integrity declines with advancing age, leading to impairments in memory and executive processes in older adulthood. Recent research suggests that higher-fit older adults may be less susceptible to white matter degeneration, although evidence for this relationship is limited. Here we examine whether cardiorespiratory fitness correlates with white matter integrity and whether this relationship further predicts cognitive performance in a large, older adult sample. Diffusion tensor imaging was used to determine microstructural white matter integrity in a group of 113 (mean age = 66.61) neurologically healthy adults. Measures of cardiorespiratory fitness (VO_2), working memory, and executive function were also collected. Using a whole-brain voxelwise analysis, we found that higher fitness levels predicted greater white matter integrity in multiple fiber pathways. We explored this relationship further using a region of interest approach, and found that higher fitness was associated with greater microstructural integrity in the anterior internal capsule and corona radiata, which contain fibers that project from subcortical to prefrontal structures. Further, statistical mediation analysis revealed that white matter integrity within the anterior internal capsule and corona radiata mediated the relationship between fitness and spatial

working memory performance. Results suggest that higher levels of aerobic fitness may protect against age-related declines in white matter integrity, which may, in turn, preserve memory performance in older adulthood.

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PREFACE

I would like to thank Dr. Kirk Erickson for providing me with funding from National Institutes of Health grant RO1 DK095172-03, which provided me with the opportunity to pursue this study.

1.0 INTRODUCTION

By the end of 2013, federal funds allocated to Alzheimer's Disease (AD) care will be upwards of $142 billion dollars (Alzheimer's Association, 2013). Currently, there are 5 million people in the United States with AD, but the prevalence is projected to triple within the next 40 years, making the study of dementia an important socioeconomic issue. Additionally, normal aging is accompanied by a progressive deterioration in cognitive processes. This systematic decline in cognitive function interferes with quality of life and increases the risk for Alzheimer's disease, personal injury, hospitalization, and death (Salthouse, 2004; Amieva et al., 2005; Zahodne et al., 2013; Johnson et al., 2007). Considering the lack of successful pharmaceutical therapies for AD and subclinical cognitive decline, it is important to examine whether favorable alterations in *modifiable* risk factors can attenuate, prevent, or treat age-related decline in cognitive function.

Along with progressive declines in cognitive function, aging is also associated with pronounced neural degeneration. But, the neural and cognitive changes associated with advancing age are not inevitable. Physical activity (PA) ameliorates age-related cognitive decline and increases the volume of grey matter regions that support higher-level cognitive processes, including the hippocampus and prefrontal cortex. In fact, PA and fitness-related changes in grey matter volume are one way in which PA may be linked to improvements in cognitive processes. But, grey matter changes do not fully account for the benefits of PA on cognitive function in older adulthood. Notably, very few studies have examined the relationship

between PA, white matter (WM) tissue integrity, and cognitive function. Given that higher-order cognitive processes rely on dynamic communication (signal transmission) between cortical regions, PA-related variation in WM structural integrity may partially mediate the relationship between PA and cognitive function in late life. The aim of the current study was to examine whether individual differences in aerobic fitness were associated with variations in WM integrity, and if so, whether this relationship was related to cognitive function in older adulthood.

Hypothesis 1: Higher levels of cardiorespiratory fitness will be associated with greater white matter integrity in a sample of healthy older adults.

Hypothesis 2: Higher cardiorespiratory fitness levels will be associated with better performance on tasks involving executive function and memory.

Hypothesis 3: Variation in white matter integrity will be one pathway by which cardiorespiratory fitness is associated with cognitive functioning. Using a statistical mediation model, we will test whether variation in WM integrity mediates the relationship between aerobic fitness and cognitive performance.

1.1 AGING AND COGNITIVE FUNCTION

Advancing age, even in the absence of disease, is frequently accompanied by systematic decline in memory and executive function, which broadly involves the coordination and control of processes involved in complex, goal-directed behavior (Buckner 2004; Salthouse 2005). The age at which cognitive decline first begins varies by domain and is a matter of contention in the literature, with some evidence that cognitive decline begins in early adulthood and other research suggesting stability of cognitive function until later adulthood (Schroeder & Salthouse 2004;

Salthouse 2009). Prospective research, such as the Seattle Longitudinal Study of Aging which followed 5,000 adults, some for as long as 35 years, suggests that significant age decrements in psychometric abilities are not observable until age 60 (Schaie 1994; Schaie & Hertzog 1986). Cross-sectional and other longitudinal research has, on the other hand, demonstrated that age-related cognitive decline may begin as early as the third decade (Salthouse, 2009). While the age at which cognitive decline first begins remains under debate, there is unequivocal evidence demonstrating that cognitive performance diminishes in older adulthood.

Between-subject designs comparing cognitive performance across different age groups emphasizes the pronounced declines frequently observed with advancing age. For example, Kray and Lindenberger (2002) examined differences in executive function in young adults (ages 18-25) and older adults (ages 60-75) using a task-switching paradigm. In this sample, older adults demonstrated reduced accuracy and greater response time relative to young adults, suggesting age-related impairment in executive control. Similarly, Gunstad and colleagues (2006) administered a battery of neuropsychological tests to a sample of 364 healthy adults between the ages of 21-84. After age stratification, they found a stepwise decrease in performance on executive function tasks with advancing age, with participants in the highest age tertile (50-84) demonstrating the lowest performance relative to all other groups (Gunstad et al., 2006).

Along with executive processes, advancing age also compromises memory function. In a study by Small and colleagues (1999), 212 cognitively healthy subjects ranging from 60 to 93 years old were followed prospectively and given annual neuropsychological evaluations. The neuropsychological examinations included assessments of memory, language, and visuospatial ability. After dividing the sample by age, specifically 60-69 and 70+, they found that age-related

decline was not represented across all cognitive domains. Rather, those that were at least 70 years old at baseline displayed a greater decline in memory performance over time relative to those aged 60-69 at baseline, while language and visuospatial abilities remained consistent across both age groups (Small et al., 1999). While memory decline in the seventh decade may be underrepresented in this study due to the limited age range, these results emphasize the domain specificity often observed with age-related cognitive decline. Similarly, progressive decline with advancing age has been observed in multiple memory domains, including working memory, long-term memory, and episodic memory (Nyberg et al., 1996; Buckner et al., 2004). Taken together, these results suggest that while cognitive decline may begin as early as young adulthood, cognitive abilities experience progressive decline with increasing age, with specific impairments in memory and executive function.

1.2 AGING AND WHITE MATTER

Advancing age is also associated with alterations in brain structure, including reductions in white matter integrity. Half of the human brain is composed of white matter, which consists primarily of glia and axons (Fields, 2008). Axons provide communicative connections in the central nervous system by regulating the distribution of action potentials, passing information in the form of electrical signals between areas of grey matter in the brain and the spinal cord. The axons are surrounded by myelin, a fatty tissue essential for alacrity of signal transmission, as well as maintenance of signal strength. Although some cognitive processes are localizable, many complex cognitive processes depend on communication between widely distributed neural systems (Madden et al., 2009a). Thus, degeneration of white matter can interfere with signal

transmission and lead to impairments in cognitive processes. Age-related white matter changes may be playing an underappreciated role in age-related cognitive decline since the focus of most prior research has been on changes in grey matter volume with age.

Fortunately, imaging methodologies have provided researchers with the ability to examine this relationship more closely. Magnetic resonance imaging (MRI) allows researchers to examine white matter structure *in vivo*. There are multiple ways to measure WM structure including assessment of white matter hyperintensities (WMH), which represent white matter lesions, as well as estimation of global and regional white matter volume. While both of these methods offer insight into WM development and pathology, they only provide information on macrostructural properties of WM, identifying complex fiber bundles as homogenous tissue, and neglecting important, microstructural properties. Within the last decade, Diffusion Tensor Imaging (DTI) has emerged as a novel approach to estimate white matter integrity. DTI is sensitive to subtle alterations or abnormalities in tissue properties and is thus able to detect variations in WM microstructural integrity (Madden et al., 2009a, Charlton et al., 2006). To do so, DTI measures the rate and directionality of water diffusion along WM fibers, with two primary outcome measures: 1) fractional anisotropy (FA), with higher levels suggestive of greater WM integrity and 2) mean diffusivity (MD), with higher levels suggestive of reduced WM integrity. Taken together, greater and more isotropic diffusion of water is indicative of compromised tissue integrity, specifically, demyelination, axonal fragmentation, and atrophy within WM fibers (Sullivan and Pfefferbaum, 2006). Additionally, DTI has emerged as a preferred estimate of WM integrity as it has been demonstrated to identify abnormalities in tissue structure that appear as normal areas of WM on conventional MR images (Pfefferbaum & Sullivan 2002; Vernooij et al., 2009).

Using the above methodology, researchers have shown that aging is associated with a systematic increase in white matter degeneration. Unlike grey matter volume, which, on a global level, decreases linearly with age (Ge et al., 2002; Good et al., 2002), WM changes throughout the lifespan follow a quadratic, inverted-u shaped pattern (Bartzokis et al., 2001; 2003). This trajectory has been supported by Westlye and colleagues (2010), who assessed lifespan changes in WM using a sample of 430 subjects aged 8-85. Using DTI estimates, they found a protracted growth of WM into the fourth decade, at which time DTI indices plateaued, followed by a slow decline into late adulthood. Further, beginning at approximately 65 years, there was an accelerated decline in WM integrity, suggesting that older adults are particularly vulnerable to precipitous declines in WM integrity. Additionally, observational research suggests that the white matter damage observed with healthy aging may surpass grey matter damage in older adulthood (Salat et al., 1999; Jernigan et al., 2001; Guttmann et al., 1998). While the majority of work has placed an emphasis on grey matter changes in older adulthood, the above evidence underscores the pervasive degeneration of WM integrity with advancing age.

White matter degeneration observed with normal aging occurs in regions that support higher-level cognitive processes, with tract disruption following an anterior to posterior gradient (Head et al., 2004; Jernigan et al., 2001, Salat et al., 2005; O'Sullivan et al., 2001; Pfefferbaum et al., 2000; Pfefferbaum and Sullivan, 2003). Initial reports of this anterior-posterior trend showed that older adults displayed greater MD relative to younger adults, with maximal changes seen in the anterior WM (O'Sullivan et al., 2001). More recent studies have provided converging support for the susceptibility of anterior WM to aging (Pfefferbaum et al., 2000; Salat et al., 2005; Gunning-Dixon et al., 2009). For example, among 87 healthy subjects aged 20-73, Grieve and colleagues (2007) found an age-dependent variation in FA, such that older

adults displayed reduced FA in frontal, temporal, and parietal regions. The greatest differences were observed in the prefrontal regions, where FA declined at an approximate rate of 3% per decade (Grieve et al., 2007). Additionally, Pfefferbaum and colleagues (2005) assessed variations in white matter integrity among 10 younger (ages 22-37) and 10 older (ages 65-79) adults. Group differences were not observed in posterior regions of interest, suggesting that posterior fiber systems may be preserved with normal aging. In contrast, relative to the younger adults, the older adults demonstrated higher MD in a number of anterior regions including the superior and inferior longitudinal fasciculi, anterior cingulate bundle, and middle frontal gyrus. Importantly, functional imaging studies have identified the anterior cingulate and middle frontal gyrus as involved in supporting executive control, episodic memory, and problem solving (Pfefferbaum et al., 2005; Duncan and Owen, 2000). Even within particular fiber bundles there is an age-related preference toward degeneration in anterior WM segments. For instance, WM integrity was assessed within two longitudinal association tracts that transverse the frontal lobe, the inferior longitudinal fasciculus and the cingulum bundle. Differences in WM integrity between younger adults (mean age = 20.04) and older adults (mean age = 68.89) increased linearly from posterior to anterior regions within each tract, emphasizing the gradual increase in susceptibility of WM fibers to structural degeneration as fibers move along the posterior-anterior plane. (Davis et al., 2009). Taken together, these results suggest that normal aging is accompanied by WM alterations that are not diffusely represented across the brain. Instead, fiber tracts within discrete brain regions are particularly susceptible to age-related tissue degeneration, specifically those that integrate signals among grey matter regions that support higher-order cognitive processes.

1.3 ASSOCIATIONS BETWEEN WHITE MATTER AND COGNITIVE PROCESSES

The regionally specific white matter tract dysfunction observed with advancing age may provide a structural basis for age-related cognitive decline. While grey matter alterations have been implicated in age-related cognitive decline, research has demonstrated that changes in white matter circuits may also contribute to cognitive aging. A large-scale quantitative review demonstrated an inverse relationship between white matter hyperintensities (WMH) and performance on tasks involving executive function, processing speed, memory, and indices of global cognitive function (Gunning-Dixon and Raz, 2000), with subsequent work in agreement (Tullberg et al., 2004; De Groot et al., 2000). Recent work using DTI has also emphasized the contribution of degraded WM integrity to impairments in cognitive processes. For instance, in a study by Vernooij et al., (2009) 860 cognitively healthy adults aged 60 and over had DTI scans collected. All participants also underwent neuropsychological testing, which assessed various domains including memory, executive function, processing speed, motor speed, and global cognition. After adjusting for lesion volume and WM atrophy, higher MD was related to poorer performance on tasks of executive function, processing speed, and global cognition. Similarly, after accounting for variation in macrostructural WM, lower mean FA was related to poorer performance on tasks of processing speed. These results indicate that WM integrity as assessed by DTI may be uniquely associated with cognitive function above and beyond associations with white matter lesion volume or atrophy, suggesting that cognitive decline may also be compromised by minute microstructural variations in WM tissue.

While the aforementioned study examined global indices of WM integrity, additional work has found regionally specific relationships between WM integrity and cognitive function. For instance, in a sample of 20 older adults and 20 younger adults, Madden and colleagues

(2009b) found that older adults performed more poorly on an executive function task relative to younger adults. Further, they found that the relationship between age and cognitive performance was mediated by WM integrity (FA) in the genu and splenium of the corpus callosum (Madden et al., 2009b). Similarly, lower levels of FA in distinct regions have been linked to impaired performance on tasks involving working memory and executive processes (Charlton et al., 2006; Grieve et al., 2007; Gold et al., 2010). For example, in a sample of adults ranging in age from 20-73, Grieve and colleagues (2007) found that reduced FA within frontal, temporal, and parietal regions were associated with poorer cognitive performance on two executive function tasks. This relationship was particularly strong for FA within bilateral areas that extended from the prefrontal cortex to the parietal lobe (Grieve et al., 2007). Additionally, elevated MD in anterior WM circuits has been negatively correlated with performance on executive function tasks (O'Sullivan et al., 2001). These results emphasize the integral role of WM in cognitive function, and demonstrate the overlap between cognitive processes associated with normal aging and those associated with variations in WM integrity. The regional specificity suggests that tract degeneration within frontoparietal and frontotemporal regions is associated with impaired performance on tasks involving memory and executive processes.

1.4 EXERCISE, AGING, AND COGNITIVE FUNCTION

While cognitive and neural changes accompany normal aging, research has shown that these changes are not invariable, and instead may be altered by lifestyle factors. Specifically, maintenance of cognitive function with advancing age may be achieved by engaging in physical activity, and in particular aerobic exercise. Aerobic exercise is defined as a type of physical

activity that depends on increased oxygen consumption to stimulate aerobic metabolism, and thus meet increased energy demands during physical activity. Cardiorespiratory fitness (CRF) broadly refers to the ability of the body to supply oxygen to working muscles during sustained activity, and can be improved by aerobic exercise (Physical activity and health: A report of the Surgeon General, 1996). Maximal oxygen uptake (VO_2) is considered the "gold standard" measure of cardiorespiratory fitness, and acts as an objective proxy or outcome for habitual physical activity (Lee et al., 2010; Barnes et al., 2003).

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Further, intervention research, which allows for causal inference, has demonstrated favorable cognitive outcomes even after just 6 months of increased PA. Exercise intervention studies typically include a group that receives aerobic exercise training (i.e., brisk walking) along with one or more control groups. Importantly, these studies are often designed to ensure that each participant receives the same amount of social support/engagement, regardless of group

assignment. In a study by Kramer and colleagues (1999), 124 sedentary older adults were assigned to an aerobic exercise or non-aerobic (stretching and toning) control group. Participants also completed a battery of cognitive tasks assessing executive function before and after the intervention. Performance improvements were observed for the walking group, but not for the stretching and toning control group. For instance, in a task-switching paradigm, response time on switch trials, a condition that relies on executive control processes, improved post-intervention only for those assigned to the walking group. Further, in a comprehensive meta-analysis consisting of cognitive outcomes from exercise intervention trials, Colcombe & Kramer (2003) found that cognitive performance improved .5 standard deviations on average after exercise, regardless of the type of cognitive task. The most robust effects, though, were present for tasks involving executive processes. Thus, higher levels of PA or fitness can effectively stabilize or enhance cognitive function in both clinical and non-clinical older adult populations, which offers broad implications for a non-pharmacological method of combating cognitive decline.

1.4.1 Molecular Mechanisms

Mechanistically, PA induces changes in cell proliferation and brain vasculature that may partially explain exercise-induced effects on cognitive performance. In animal models, physical activity has been shown to stimulate neurogenesis, the growth of new neurons, in the hippocampus. In a seminal study by Kim and colleagues (2004), researchers examined the effect of treadmill exercise on cell proliferation in 4 week-old, 8 week-old, and 62 week-old rats. While the younger groups experienced the highest levels of new cell formation, the 62 week-old exercised rats also demonstrated significantly greater cell proliferation in the dentate gyrus

relative to sedentary controls, suggesting that exercise may combat age-related reductions in the rate of neurogenesis typically seen with advancing age.

Additionally, efficient brain vascularization is needed to supply neurons with the nutrients needed for maintenance and survival. Increased synaptic plasticity and neurogenesis resulting from increased PA require support from blood vessels. In fact, between 3 weeks to 1 month of regular PA has been shown to increase capillary growth in both young (Swain et al., 2003) and aged rats (Ding et al., 2006). Specifically, exercise may promote angiogenesis by up-regulating vascular endothelial growth factor (VEGF), which is principally involved in stimulating the growth of new blood vessels in the brain (Yao et al., .2004; Amaral et al., 2001). Additionally, exercise up-regulates brain-derived neurotrophic factor (BDNF) (Gomez-Pinilla et al., 2002; Vaynman et al., 2003;2004), which supports the birth and proliferation of newly developed neurons as well as the survival of existing neurons. Although, for the sake of brevity, only a cursory explanation of molecular mechanisms is provided here, research suggests that exercise favorably affects synaptic plasticity, neurogenesis, and capillary growth. The evidence surrounding neuromolecular changes associated with PA gives us cause to speculate about how exercise might influence brain morphology, particularly white matter integrity.

1.4.2 Exercise alters brain morphology

Given the capacity for sustained PA to induce synaptic plasticity and neurogenesis, research has suggested that related variations in brain morphology may account for the relationship between PA and cognitive function (Szabo et al., 2011; Weinstein et al., 2012). Intervention studies have found that aerobic exercise induces alterations in grey matter within frontal and temporal regions, specifically in the prefrontal cortex and the hippocampus (Colcombe et al., 2006;

Erickson et al., 2011; Erickson et al., 2010). Further, research indicates that improvements in cognitive performance may accompany domain-specific grey matter changes initiated by PA or fitness (Weinstein et al., 2012; Erickson et al., 2011). But, only a fraction of the relationship between fitness and cognitive function can be explained by variations in grey matter structure.

Although limited in number, studies using DTI have collectively demonstrated a positive association between PA and white matter integrity in older adulthood (Marks et al., 2007; Gons et al., 2013; Gow et al., 2012). For instance, Gons and colleagues (2013) examined the association between leisure-time PA, as assessed by self-report, and white matter integrity among 440 adults aged 50-85 with cerebral small vessel disease. After stratification of PA into quartiles, they found a step-wise increase in FA with increased levels of PA. Further, this relationship was present in almost all voxels of the WM skeleton, even after adjusting for age, sex, and cardiovascular risk factors. Additionally, in the largest sample to assess this relationship, Gow and colleagues (2012) gathered estimates of PA using subjective self-reports from 691 adults aged 70, and followed-up with MRI scans three years later. Within normal appearing white matter, higher levels of PA at baseline predicted greater white matter integrity three years later. While these large-scale studies suggest an inverse relationship between PA and white matter degeneration, they are limited by the use of subjective assessments of PA, which are prone to reporter bias and may not accurately reflect actual PA patterns.

Evidence using objective assessments of fitness has also demonstrated positive associations with white matter integrity (Johnson et al., 2012; Voss et al., 2012; Marks et al., 2011, Tseng et al., 2013). For instance, in a group of 26 older adults, Johnson and colleagues (2012) found that cardiorespiratory fitness correlated with callosal FA. Specifically, higher levels of cardiorespiratory fitness were positively associated with elevated FA in a majority of

the corpus callosum, a thick commissural fiber tract that regulates intrahemispheric communication. Similarly, using a sample of 15 older adults, Marks et al., (2011) found that higher $VO_{2\ peak}$ was associated with greater FA in the middle cingulum segment. Further, cardiorespiratory fitness explained 28.5% of FA's total variance in this region (Marks et al., 2011). Overall, results suggest that among older adults, PA and fitness are associated with higher levels of white matter integrity. But, at present, there is a very limited amount of research that has examined this relationship in non-clinical samples (8 studies to date). Additionally, there is a lack of consensus across studies regarding WM regions specifically associated with PA and fitness. Further, likely due to assessment cost and participant burden, current studies using aerobic fitness measures are comprised of small sample sizes. Thus, while studies have consistently demonstrated a positive linear relationship between fitness and white matter integrity, regional specificity remains unclear, with small sample sizes and methodological limitations further restricting interpretation. Finally, the cognitive processes associated with fitness-related variations in WM integrity have yet to be elucidated.

1.4.3 Linking exercise-induced changes in white matter to cognition

Taken together, previous research suggests that older adults are more susceptible to WM degeneration as well as declines in cognitive function. But, higher levels of aerobic fitness are associated with better cognitive performance as well as reduced white matter degeneration. While variation in grey matter structure may partially account for the association between fitness and cognitive function, there is a dearth of knowledge regarding the potentially influential role of WM in the relationship between fitness and cognitive processes. To date, only two studies have examined whether the microstructural changes induced by PA affect cognitive function (Voss et

al., 2013; Prakash et al., 2010), only one of which was conducted in the context of normal aging. Following a one-year aerobic exercise intervention, Voss et al. (2013) found that gains in aerobic fitness within the exercise group were associated with enhanced memory performance, as well as significant increases in prefrontal and temporal FA. Additionally, this result was not found in the stretching and toning control group. But, the increases in white matter integrity post-intervention were not associated with memory improvement (Voss et al., 2013). The authors suggested that null results may be a product of lack of statistical power for the cognitive measure employed (backward digit span). Additionally, in contrast to grey matter, it may be that a longer duration of aerobic exercise is needed to exert significant enough changes in WM to affect cognitive function. But, results from this study are promising as they demonstrate that one year of moderate-intensity exercise was sufficient to induce increases in frontotemporal white matter integrity. Further, positive associations between cardiorespiratory fitness, white matter integrity (FA) and cognitive performance (processing speed) have also been observed in populations with multiple sclerosis, a disorder characterized by demyelination (Prakash et al., 2010).

In sum, the relationship between fitness and WM integrity as assessed by DTI is promising, with research suggesting that higher levels of aerobic fitness may protect against age-related declines in white matter, although whether this relationship translates into cognitive change is currently unknown and requires further examination.

1.5 CURRENT STUDY

Aging is accompanied by degeneration in cerebral white matter, as well as declines in memory and executive functioning. Exercise interventions have demonstrated that physical activity is

protective, and even restorative against cognitive and brain morphological decline in aged populations. The relationship between aerobic fitness and cognitive performance may be partially mediated by white matter integrity, although most extant literature has focused on this relationship in structural grey matter. Therefore, we will test whether individual differences in fitness predict variations in white matter integrity, after accounting for demographic factors. We predict that there will be a direct association between aerobic fitness and white matter integrity, particularly in anterior regions. Second, we will test whether white matter integrity is positively associated with cognitive performance on executive function and memory tasks, after accounting for demographic factors. Finally, using statistical mediation, we will explore the extent to which variation in white matter integrity accounts for the relationship between cardiorespiratory fitness and cognitive function.

The present study has several advantages including a relatively large sample size and an objective measure of aerobic fitness. Additionally, the proposed study will expand on the present literature in two important ways: 1) assist in clarifying the present ambiguity regarding the regionally specific relationship between white matter and aerobic fitness by exploring direct associations between fitness and white matter integrity 2) test whether white matter integrity is a key statistical mediator in the relationship between fitness and cognitive function.

2.0 RESEARCH DESIGN AND METHODS

2.1 SUBEJCTS

One hundred and seventy-three participants between the ages of 60 and 81 (mean age 66.6 years; standard deviation = 5.6 years) were recruited to take part in a one-year, single-blind randomized walking intervention. Subjects were recruited through community advertisements and physician referrals. Participants underwent an initial phone screen, followed by a group orientation that provided study details and responses to participant inquiries. After the orientation, subjects participated in three baseline sessions. During these sessions, cognitive and cardiorespiratory fitness assessments were administered, and high-resolution magnetic resonance imaging (MRI) data was collected. As the objective of the present study is to examine associations between aerobic fitness, white matter integrity, and cognition rather than exercise-induced changes, only the baseline data will be used for analysis. Of the 173 participants, 16 were excluded due to incomplete information on relevant behavioral data (cognitive, VO_2, or demographic). An additional 12 subjects that did not complete the baseline MRI scan were excluded. Finally, 32 participants were excluded due to problems with their diffusion data including 1) failure to finish the entire scan (*n = 2*) 2) poor orientation during image acquisition (*n = 4*) and 3) excessive noise/field distortion (*n = 24*). Thus, the final sample consisted of 113 participants.

2.1.1 Exclusionary Criteria

As mentioned in Erickson et al. (2011), participants were required to score ≥ 51 on the modified Mini Mental Status Examination (high score of 57) to rule out clinically present cognitive impairment (Stern, 1987). Additionally, to rule out depression, individuals that scored > 3 on the Geriatric Depression Scale were excluded from the study (Sheikh, 1986). Participants were required to have normal color vision, a visual acuity of at least 20/40, and no history of neuropsychiatric conditions or neurological diseases or infarcts including Parkinson's disease, multiple sclerosis, Alzheimer's disease, or stroke. Further, participants were excluded if they demonstrated a history of vasculature problems, including diabetes and cardiovascular disease. No MRI contraindications could be present. In order to participate, subjects needed to obtain consent from their physician to engage in an exercise intervention, as well as a maximal graded exercise test (VO_2 max). Finally, participants were required to be 60+ years of age, and currently sedentary, defined as being physically active for 20 minutes or less each week within the six months prior to baseline examination, as assessed by the Physical Activity Scale for the Elderly (PASE) (Washburn et al., 1993).

2.2 INSTRUMENTS

2.2.1 Demographics

A brief questionnaire assessed basic demographic information including participants' age, gender, and education.

2.2.2 Cardiorespiratory fitness assessment

Maximal oxygen uptake (VO_2 max) was used in the present study as an objective measure of baseline cardiorespiratory fitness (CRF). As detailed by Voss et al., (2010), assessment of CRF was done using graded maximum exercise testing on a motor-driven treadmill with continuous monitoring of respiration, heart rate, and blood pressure by a cardiologist and nurse. During the assessment, subjects walked at a speed slightly faster than their normal walking pace with increasing graded increments of 2% every 2 minutes. Oxygen uptake was measured at 30 second intervals until a max VO_2 was attained or to the point of test termination due to exhaustion. VO_2 max was defined as the highest recorded VO_2 value when two of three criteria were satisfied:

1) a plateau in VO_2 peak between two or more workloads 2) a respiratory exchange ratio > 1.00 or 3) a heart rate equivalent to their age predicted maximum (i.e., 220-age).

VO_2 max scores are expressed in units of milliliters per kilogram per minute (ml/kg/min), after controlling for height and weight of the individual.

2.2.3 Diffusion Tensor Imaging

Diffusion weighted images were acquired using a 3 T Siemens head-only scanner. The echo time (TE) was 94 ms, with repetition time (TR) = 4,200 ms. Twenty-eight 4 mm slices were obtained along the anterior-posterior commissural plane. The protocol involved a T2-weighted acquisition followed by a 12-direction diffusion-weighted echo planar imaging scan (b-value = 1,000 s/mm^2), which was repeated six times.

2.2.4 Cognitive Assessments

2.2.4.1 Task-switching paradigm

The task-switching paradigm is used extensively in the literature to assess specific components of executive processes (Gold et al., 2010; Gratton et al., 2009; Kramer et al., 1999; Kray & Lindenberger 2000; Voss et al., 2012), including cognitive flexibility and inhibition (Verstynen et al., 2012). Associations between task-switching performance and WM integrity in older adults have been previously established (Kray and Lindenberger, 2002).

As described in Voss et al., (2010), participants utilized color-based cues to determine whether they were to judge whether a number was odd or even, or whether it was low or high (i.e., smaller or larger than 5). The numbers were presented individually for 1500 ms against a pink or blue background. If the background was blue, participants had to determine whether the number was high ("X" key) or low ("Z" key). If the background was pink, participants were to report whether the number was odd ("N" key) or even ("M" key). In both cases, participants were asked to answer as quickly as possible (Voss et al., 2010). Participants completed a practice block followed by a switching block, which included 120 trials with the task in each trial chosen randomly, from which performance results were recorded.

Reaction time and accuracy rates for single trials (trials during the non-switch condition which involved only one task at a time), repeat trials (trials during the dual-task condition in which the preceding trial involved the same task), and switch trials (trials during the dual-task condition in which the preceding trial involved a different task) were recorded. Cost estimates are often calculated using these measures to assess variation in performance with increasing task demand (Verstynen et al., 2012). Local and global costs tend to be more pronounced (increase)

with advancing age (Kray and Lindenberger, 2002; Madden et a., 2010) suggesting that older adults are more susceptible to deficits in executive control processes. Therefore, four estimates of cost performance were calculated, including local and global accuracy and local and global RT costs, which were used as outcome variables in subsequent analyses. Consistent with previous literature, differences in RT and accuracy rates between non-switch and switch trials within the same condition were calculated to reflect local accuracy and reaction time costs. Differences in RT and accuracy rates between the single-task condition and the dual-task condition were calculated to reflect global RT and accuracy costs.

2.2.4.2 Spatial Memory Task

This task assesses spatial memory, a cognitive domain that typically declines with advancing age. At the beginning of the task, participants were shown a fixation crosshair for 1 second, followed by the appearance of one, two or three dots placed in random locations on the screen for 500 ms. Then, a 3 second fixation crosshair appeared, during which time participants were asked to try to remember where the previous dot(s) were located. Following the 3-second delay, a red dot appeared on the screen. Subjects had to indicate whether the red dot displayed was in the same location (match) or a different location (non-match) than one of the previously presented black dot(s) by pressing a designated key on a computer keyboard (x = nonmatch; m = match). There were 40 trials per set size (1, 2, and 3 black dots), with 20 match and 20 non-match trials in each, totaling to 120 trials. Prior to task administration, several practice trials were conducted to familiarize the participant with the task, during which time they were directed to respond as quickly and accurately as possible. Accuracy rates and reaction time were

recorded separately for the 1, 2, and 3 dot conditions, which were then averaged to create mean RT and accuracy scores.

2.2.4.3 Flanker Task

Participants completed a modified flanker paradigm, which assesses attentional control and perceptual speed (Erickson et al., 2005). During this task, participants were asked to identify the orientation of a central arrow cue that was embedded in an array of five arrows that pointed in either the left or right direction. In half of the trials, the surrounding arrows pointed in the same direction as the central cue (e.g., <<<<<), in the other half the flanking arrows pointed in the opposite direction of the central cue (e.g., <<<><). Reaction times on congruent and incongruent trials were recorded, and flanker cost was calculated by subtracting the reaction times of congruent trials from those of incongruent trials.

2.3 IMAGE PROCESSING

DTI data was analyzed in order to determine the extent to which cardiorespiratory fitness is associated with white matter integrity, as well as the degree to which variation in white matter integrity accounts for the relationship between cardiorespiratory fitness and cognitive function. DTI estimates the rate and directionality of water diffusion in the interstitial fluid (between individual white matter fibers) and within the intracellular space. The diffusion of water in brain tissue is highly affected by the local microstructural integrity of the tissue. Water diffusion will be anisotropic, or directionally homogenous, when restricted by barriers such as axons,

neurofilaments, and myelin (Hagmann et al., 2006). In contrast, when integrity is compromised by demyelination or axonal injury, diffusion within white matter becomes less constrained and therefore more isotropic. Quantification of diffusion is completed by computing a tensor, a mathematical model of the directionality and magnitude of water diffusion in 3D space. The shape of the tensor is determined by: 1) three eigenvectors, which represent directions of diffusion along 3 primary axes and 2) three eigenvalues, which represent the magnitude or rate of diffusion along the 3 axes. The largest eigenvalue corresponds to the principle eigenvector and represents water diffusion that is parallel to the axon. The other two eigenvalues represent the rate of diffusivity in the two orthogonal planes (Wozniak and Lim, 2006). Fractional anisotropy (FA) is the most commonly used estimate of WM integrity, and represents overall anisotropy within a voxel. FA is computed using a weighted ratio of the standard deviation of the 3 extracted eigenvalues of the mean, therefore taking into consideration the rate of diffusion parallel and perpendicular to the axon, or principle direction of diffusion. FA values fall between a range of 0 and 1, indicating the degree of integrity. For instance, an FA value of 0 represents a spherical diffusion (i.e. no axons), as would be seen in the ventricles, while an FA value of 1 indicates that water diffusion only occurs along the primary axis and is fully restricted in all other directions (Sullivan and Pfefferbaum, 2006).

In the present study, diffusion data was processed using tools in the FMRIB Software Library (FSL) (Image Analysis Group, FMRIB, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl/; (Smith et al., 2004). Using FMRIB's Diffusion Toolbox (v2.0; http://fmrib.ox.ac.uk/fsl/fdt/index.html), each participant's data was eddy current corrected, to adjust for field distortions, by affine registration to the reference, or *Bo* image. This was followed by the removal of non-brain tissue using the Brain Extraction Tool (BET) (Smith and

Nichols, 2009). Next, DTIfit was used to calculate the diffusion tensor at each voxel. Specifically, this step computes the voxelwise eigenvalues and eigenvectors of the diffusion tensor from each participant's image, calculating various diffusion parameters, including FA. The FA data was then fed into the FSL tract-based spatial statistics toolbox (TBSS; v1.2, http://www.fmrib.ox.ac.uk/fsl/tbss/index.html; Smith et al., 2006) pipeline. TBSS is used frequently in DTI processing, and its algorithms for alignment of FA images across multiple subjects into a standard space have been tested and validated (Smith et al., 2006). First, FA images were eroded to remove likely outliers from the diffusion tensor-fitting step. Then, FA images were normalized to 1 X 1 X 1 mm MNI152 standard space via alignment to a common registration target. As considerable atrophy occurs in older adulthood, it is standard to compute a study specific template when using older adult populations, as the standard FSL FA template (FMRIB58_FA) reflects an average of young to middle aged adults. Therefore a study-specific template was created and was used as the target for registration. To create the study specific template, we first affine registered all native-space FA images to the FA template in MNI space, then averaged across subjects to generate the study-specific template. Registration to the study-specific template is done by combining two transformations: 1) a non-linear transformation of each subject's FA image to the study specific template and 2) an affine registration of the template to MNI152 space. Following the MNI transformation for all subjects, a mean FA image was computed and an average skeleton was generated that represented major tracts common across all participants. The skeleton was thresholded at an FA value of 0.2 (Smith et al., 2007), to ensure that major WM tracts were included and to exclude regions that may contain multiple types of tissue. Then, in order to account for any residual misalignments not corrected for during registration, each participant's normalized FA image was projected onto the mean FA

skeleton. This resulted in a common tract skeleton onto which each subjects FA image was aligned. An example of the mean FA skeleton produced from the present study can be found in Figure 1.

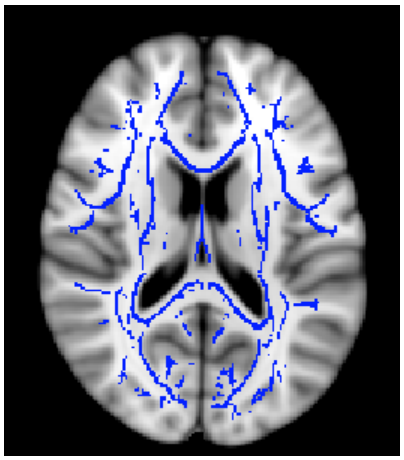


Figure 1. White matter skeleton

2.4 STATISTICAL ANALYSIS

To determine voxels significantly associated with fitness, a whole-brain voxel-wise analysis was performed using the randomize tool in FSL. The randomize tool examines the association between VO_2 and FA at each voxel by testing the t value at each voxel against the null distribution generated using 5000 permutations. Rather than specifying clusters using *a priori* thresholds, the Threshold-Free Cluster Enhancement (TFCE) technique was employed at a threshold of $p < .05$. After whole-brain TFCE cluster analysis, we identified and labeled the specific pathways where FA values correlated with VO_2 by overlaying the Johns Hopkins

University (JHU) white matter atlas to our statistically-derived images. Then, we used the average of the significant voxels within these anatomically identified regions in SPSS to test (1) whether the association between VO_2 and FA continued to survive after the inclusion of confounding demographic factors and 2) whether FA values in specific white matter tracts mediated the association between fitness and cognitive performance.

Once entered into SPSS, FA values were averaged across hemispheres for several reasons. First, collapsing across hemispheres reduced the number of comparisons by half. Additionally, the left and right hemispheres for each region of interest (ROI) were highly correlated (all $p < .0001$), demonstrating considerable covariance across hemispheres. Finally, we did not have any hypotheses about laterality effects of fitness or mediation. Thus, using multivariate linear regression, we examined whether the association between VO_2 and FA within each bilateral ROI remained significant after including relevant covariates in the model, specifically age, gender, and education. Only regions that were significantly associated with VO_2 after adjusting for demographic factors were used as mediator variables in subsequent mediation analysis.

A mediating variable is a variable that accounts for all or some of the relationship between an independent variable (IV) and a dependent variable (DV). Mediation does not require a direct effect of the IV on the DV. Rather, mediation requires an indirect effect, such that the effect of the IV through the mediator on the DV is significantly different from zero. In the present study, we examined whether variation in white matter integrity partially accounts for the association between cardiorespiratory fitness and cognitive performance. This relationship is illustrated further, below (Figure 2).

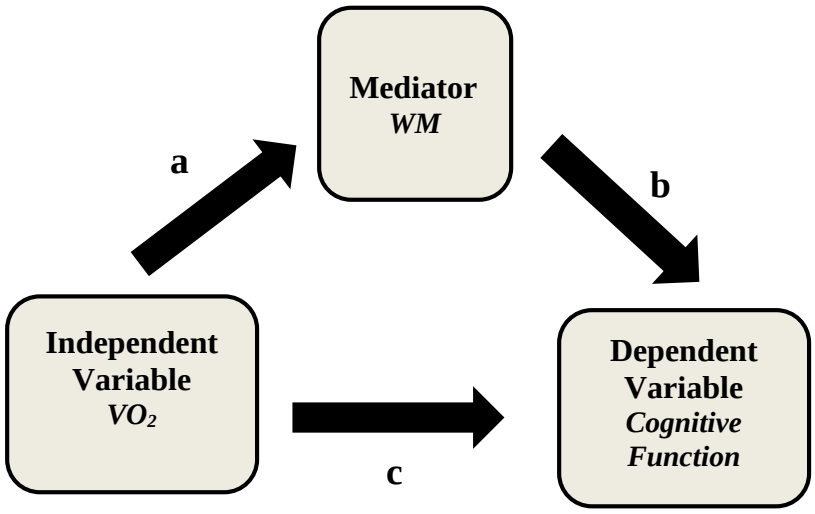


Figure 2. Mediation model

To determine if there was a significant indirect association between cardiorespiratory fitness and cognitive performance that is mediated by white matter integrity, we used the PROCESS macro created by Andrew Hayes (Preacher & Hayes, 2008). The sampling distribution of the indirect effect (a*b) is often skewed. To account for this non-normality, the PROCESS macro uses bootstrapped sampling with replacement. Therefore, the indirect effect of VO_2 on cognitive performance was estimated using 10,000 bootstrapped samples that were drawn with replacement from observations within the study sample. Along with the indirect effect, this technique also provides an estimate of 1) the direct pathway, specifically the association between VO_2 and cognitive performance after accounting for covariates *and* the mediator and 2) the total effect pathway, which represents the relation between VO_2 and cognitive performance, adjusting only for covariates (sum of indirect and direct pathways). Indirect, direct, and total path effects are represented by 95% bias corrected and accelerated

confidence intervals (CI), with results considered significant if the confidence intervals do not contain 0. The regression coefficients are displayed in unstandardized form, as the bootstrapped CI's correspond to the unstandardized effects rather than the standardized effects. All regression models controlled for potentially confounding variables that were correlated with the mediator or dependent variable, including age, gender, and years of education. Outcome variables for mediation analyses included cost scores for RT and accuracy on task-switch trials, RT cost on the Flanker task, and average accuracy rates and RT on the spatial working memory task.

3.0 RESULTS

Characteristics of the sample including demographics, average FA within each ROI, and task performance are described in Tables 1-3. Demographic characteristics including age, gender, education, as well as average fitness levels of the 113 participants included in the study were similar to those who were not included in the analyses (all p >.05). Additionally, those included in the study did not differ from excluded subjects on measures of cognitive performance.

Table 1. Demographics

Demographics	**Mean (SD)** ***(n = 113)***
Age	66.61 (5.653)
Education (years)	15.48 (2.919)
Gender (% female)	63.70%
VO_{2peak}	21.401 (4.890)

SD = standard deviation

Table 2. Average FA values for significant voxels in white matter regions of interest

White Matter ROI's (FA)	**Mean (SD)** ***(n= 113)***
Genu of CC	.6107 (.049)
Body of CC	.5087 (.056)
Splenium of CC	.6317 (.031)
Anterior Internal Capsule	.5955 (.040)
Anterior Corona Radiata	.4584 (.040)
Superior Corona Radiata	.4941 (.037)
Fronto-occipital Fasiciculus	.5197 (.053)
Posterior Corona Radiata	.4982 (.033)
Fornix	.3739 (.070)
SLF	.4466 (.033)

SD = standard deviation; CC = corpus callosum; SFL = superior longitudinal fasciculus; FA = fractional anisotropy; ROI = region of interest

Table 3. Means and standard deviations for task performance on individual tasks

Cognitive Task	Mean (SD) *(n = 113)*
Flanker Reaction Time (ms)	
Flanker Congruent RT	591.314 (94.73)
Flanker Incongruent RT	673.954 (123.60)
Flanker Cost	82.883 (52.051)
Task Switch Reaction Time (ms)	
Single trial RT	784.226 (104.77)
Repeat trial RT	985.855 (142.80)
Switch trial RT	1372.637 (217.56)
Mixed RT	1181.538 (163.54)
Local RT Cost	380.772 (179.56)
Global RT Cost	396.334 (137.57)
Task Switch Accuracy (%)	
Single Accuracy	92.617 (8.83)
Mixed Accuracy	80.230 (20.77)
Repeat Accuracy	83.082 (19.64)
Switch Accuracy	77.325 (22.76)
Local Accuracy Cost	-5.757 (8.39)
Global Accuracy Cost	-12.038 (19.295)
Spatial WM Task Reaction Time (ms)	
1-Dot RT	820.66 (188.13)
2-Dot RT	933.16 (183.455)
3-Dot RT	1016.434 (192.90)
Average RT	923.419 (180.227)
Spatial WM Task Accuracy (%)	
1-Dot Accuracy	86.347 (14.31)
2-Dot Accuracy	81.694 (12.88)
3-Dot Accuracy	77.389 (15.16)
Average Accuracy	81.81 (12.86)

Note: Italicized variables (above) were used as outcome variables in analyses.
RT = reaction time; WM = working memory

3.1 CORRELATIONS BETWEEN FITNESS AND COGNITIVE PERFORMANCE

Males ($r = .437$; $p < .001$) and those with greater years of education ($r = .291$; $p = .002$) had higher fitness levels compared to women and those with fewer years of education. Additionally, older age was associated with lower levels of fitness ($r = -.460$; $p < .001$).

After accounting for age, gender, and years of education, partial correlation analysis revealed that higher levels of VO_2 were correlated with shorter reaction times on the spatial working memory task ($r = -.221$; $p = .021$). Additionally, there was a trending association between VO_2 and local accuracy cost, such that higher fitness levels were correlated with a lower switching cost ($r = .180$; $p = .059$) during the task-switch task. There was not a relationship between VO_2 and Flanker RT cost ($r = -.001$ $p = .988$), global RT cost ($r = -.169$; $p = .078$), local RT cost ($r = -.053$; $p = .581$), global accuracy cost ($r = .106$; $p = .268$) or average spatial working memory accuracy ($r = .138$; $p = .151$). See Table 4 for more details.

Table 4. Correlations between cardiorespiratory fitness and cognitive performance

Cognitive Tasks	r	p-value
Flanker Cost	-0.001	0.988
TS Global RT Cost	-0.169	0.078
TS Local RT Cost	-0.053	0.581
TS Global Acc. Cost	0.106	0.269
TS Local Acc. Cost	0.18	0.059
Spatial WM RT	-0.221	0.02
Spatial WM Acc.	0.138	0.151

Results from partial correlations, adjusting for age, gender, and years of education.

3.2 FITNESS PREDICTS WHITE MATTER INTEGRITY

Prior to adjusting for covariates, whole-brain voxel-wise analysis revealed an association between fitness and multiple regions of the WM skeleton, particularly among voxels located in anterior fiber tracts. These associations remained significant after familywise-error correction at $p < .05$. An FA map of the voxels within the WM skeleton significantly associated with VO_2 can be found in Figure 3A. Regions in which significant associations between VO_2 and FA were located include the genu, body, and splenium of the corpus callosum, the fornix, and the left and right anterior corona radiata, superior corona radiata, posterior corona radiata, anterior internal capsule, superior longitudinal fasciculus, and superior fronto-occipital fasciculus (Figure 3B).

FA values from each region of interest were extracted and subjected to multivariable linear regression analyses to assess the association between cardiorespiratory fitness and FA after correcting for age, gender and education. Out of the ten ROI's, three survived adjustment for demographic characteristics, with results demonstrating a positive relationship between VO_2 and FA within each ROI. Specifically, higher VO_2 predicted greater FA in the splenium of the corpus callosum ($\beta = .252$; S.E. = .001; $p = .036$), the anterior internal capsule ($\beta = .278$; S.E. = .001; $p = .016$), and the superior fronto-occipital fasciculus ($\beta = .238$; S.E. = .001; $p = .044$). Additionally, the anterior corona radiata showed a trending association ($\beta = .223$; S.E. = .001; $p = .051$). R^2 estimates extracted from regression models suggest that fitness explained an additional 3-5% of the variation in FA within these regions, after including age, gender, and years of education in the model (Table 5).

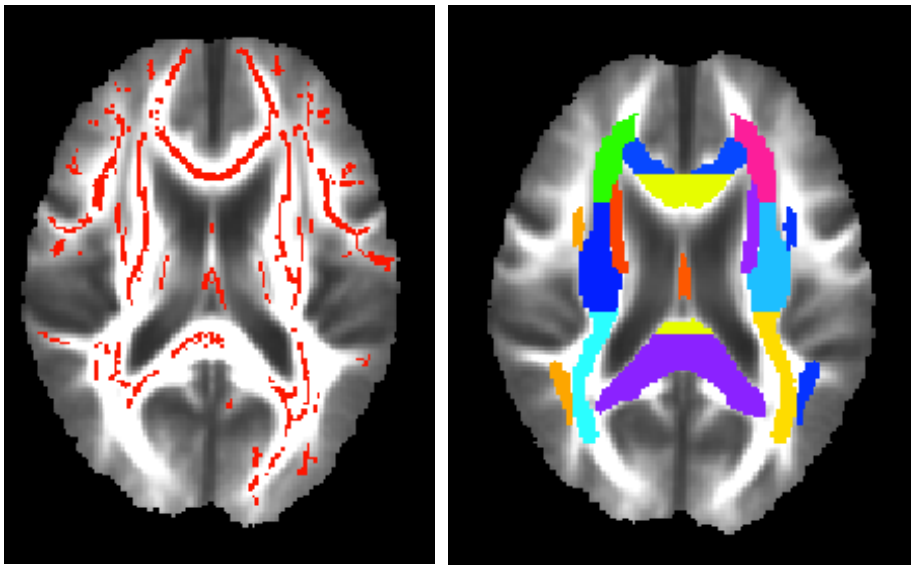
In contrast, fitness did not contribute significantly to explaining the variance in mean FA in the genu, body, fornix, superior longitudinal fasciculus, posterior corona radiata, or superior corona radiata after adjustment for demographic factors. Only regions significantly associated

with fitness were included in subsequent mediation models. The anterior corona radiata was also included in further analyses due to the trend at p<.06.

Table 5. Results of multivariate linear regression analyses, predicting FA in each ROI with fitness

	β	St. Error	p-value	R^2
Genu	0.173	0.001	0.115	0.017
Body	0.147	0.001	0.205	0.012
Splenium	0.252	0.001	0.036*	0.036
Anterior Internal Capsule	0.278	0.001	0.016*	0.044
Anterior Corona Radiata	0.223	0.001	0.051	0.028
Superior Corona Radiata	0.155	0.001	0.198	0.014
Posterior Corona Radiata	0.207	0.001	0.084	0.024
Superior Fronto-occipital fasciculus	0.238	0.001	0.044*	0.032
Fornix	0.057	0.002	0.608	0.002
Superior Longitudinal Fasciculus	0.114	0.001	0.332	0.007

Above analyses adjusted for age, gender, and years of education. R^2 = R^2 change following addition of VO_2 to the model.
* indicates significance at p < .05.



A. **B.**

A) Results from voxel-wise whole-brain analysis. Highlighted in red are voxels within the FA skeleton that were significantly associated with VO_2, prior to adjusting for age, gender, and years of education. B) Anatomically defined regions of interest using the JHU white matter atlas.

Figure 3. FA skeleton and regions of interest

3.3 WHITE MATTER MEDIATES THE ASSOCIATION BETWEEN FITNESS AND COGNITIVE PERFORMANCE

Mediation analyses were conducted to test the hypothesis that brain white matter is one pathway by which cardiorespiratory fitness is associated with cognitive function. After controlling for variance associated with age, gender, and years of education, mediation analysis showed significant indirect associations between VO_2 and spatial working memory accuracy through the anterior internal capsule (B = .2084; CI (SE) .0301; .5472 (.1279)), the anterior corona radiata (B = .138; CI (SE) 0075; .3975 (.095)), and the superior fronto-occipital fasciculus (B =.152; CI (SE) .013; .426 (.101)) (Table 6). The positive direction of the coefficients show that higher levels of cardiorespiratory fitness were associated with greater FA in the anterior internal

capsule, anterior corona radiata, and the superior fronto-occipital fasciculus, which, in turn, predicted better performance (fewer errors) on the spatial working memory task. As the spatial working memory accuracy variable represented an average of the accuracy rates across the three task conditions, we also ran separate *post hoc* mediation models for each of the spatial working memory conditions, namely, the 1-dot, 2-dot, and 3-dot conditions (Figure 4). All three regions showed significant indirect effects for the 1-dot and 3-dot conditions, while only the superior fronto-occipital fasciculus mediated the association between fitness and 2-dot accuracy rates (Figure 4). In contrast, the splenium of the corpus callosum did not mediate the association between VO_2 and average spatial working memory performance.

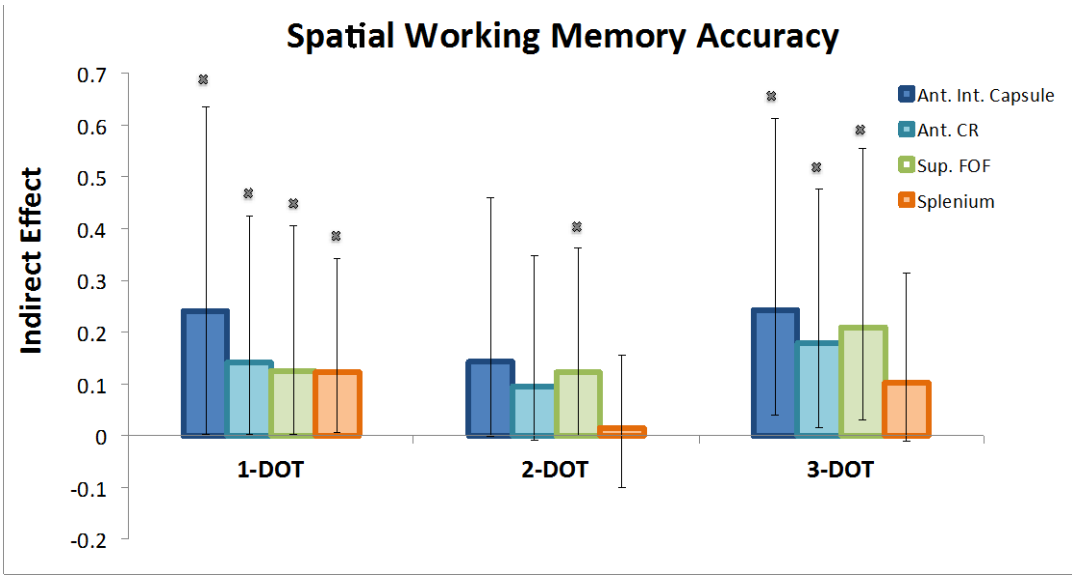
WM integrity in the splenium significantly mediated the association between VO_2 and Flanker RT cost (B = -.4741; CI (SE) -1.4454; -.0033 (.3426)). In particular, the association between VO_2 and reaction time on Flanker trials occurred through an indirect pathway in which higher VO_2 was associated with greater white matter integrity, which in turn predicted lower RT costs between congruent and incongruent trials. No other regions significantly mediated the relationship between VO_2 and Flanker RT cost (Figure 5).

FA values within each ROI did not independently mediate the relationship between VO_2 and performance on the remaining cognitive outcomes, including global and local task-switch accuracy and RT costs, and average spatial working memory reaction time (Table 6).

Table 6. Mediation: indirect effects, standard errors, and 95% confidence intervals for each cognitive variable

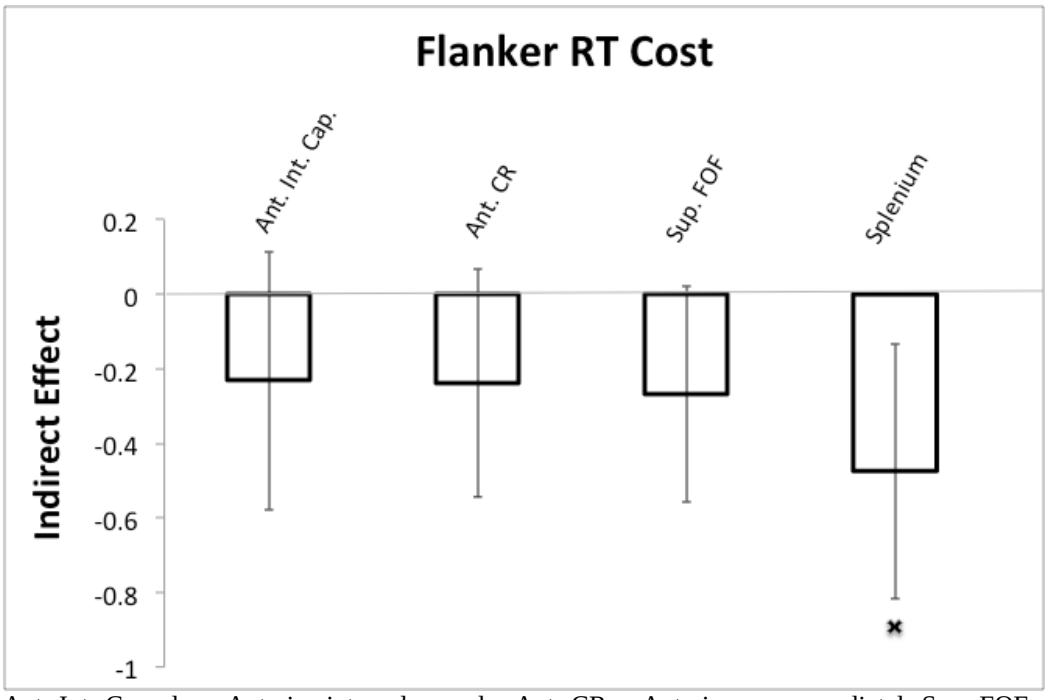
	Anterior Internal Capsule	**Anterior Corona Radiata**	**Splenium**	**Fronto-Occipital Fasciculus**
Flanker Cost B (SE)	-.230 (.348)	-.238 (.306)	**-.474 (.343) ***	-.269 (.289)
95% CI	(-1.055; .353)	(-1.106; .185)	**(-1.445; -.003)**	(-1.063; .142)
TS Global RT Cost B (SE)	-.388 (.948)	-.574 (.891)	-.055 (.919)	.470 (.761)
95% CI	(-2.995; 1.025)	(-3.247; .596)	(-1.909; 1.888)	(-.659; 2.542)
TS Local RT Cost B (SE)	-1.415 (1.371)	-1.585 (1.367)	-.798 (1.171)	-.876 (1.122)
95% CI	(-5.468; .411)	(-5.601; .134)	(-3.87; 1.038)	(-4.041; .680)
TS Global Acc. Cost B (SE)	-.219 (.153)	-.195 (.142)	-.119 (.137)	-.154 (.110)
95% CI	(-.625; .007)	(-.602; .001)	(-.462; .101)	(-.456; .002)
TS Local Acc. Cost B (SE)	.054 (.063)	.065 (.062)	.046 (.051)	.036 (.05)
95% CI	(-.026; .231)	(-.011; .248)	(-.027; .184)	(-.036; .171)
SPWM RT B (SE)	-.412 (1.068)	-.788 (.983)	-.429 (.865)	-.449 (.948)
95% CI	(-3.237; 1.223)	(-3.737; .404)	(-2.51; 1.059)	(-2.891; 1.014)
SPWM Acc. B (SE)	**.208 (.128) ***	**.138 (.093) ***	.08 (.066)	**.152 (.101) ***
95% CI	**(.030; .547)**	**(.008; .398)**	(-.014; .255)	**(.013; .426)**

CI=confidence interval; RT=reaction time; Acc=accuracy; SPWM=spatial working memory; TS=task-switch.
* indicates significant indirect effects (CI does not contain zero).



Accuracy stratified into accuracy rates on the 1-dot, 2-dot, and 3-dot spatial working memory trials. Error bars indicate 95% confidence intervals. **x** indicates significant indirect effects (CI does not contain zero).

Figure 4. Mediation indirect effects for Spatial Working Memory accuracy



Ant. Int. Capsule = Anterior internal capsule; Ant. CR = Anterior corona radiatal; Sup. FOF = Superior Fronto-occipital fasciculus. Error bars indicate 95% confidence intervals. **x** indicates significant indirect effects (CI does not contain zero).

Figure 5. Mediation indirect effects for Flanker RT cost

4.0 DISCUSSION

In a large, cognitively healthy older adult sample, we found that fitness predicted white matter integrity in multiple white matter tracts, including the anterior limb of the internal capsule, the anterior corona radiata, the superior fronto-occipital fasciculus, and the splenium of the corpus callosum. Further, for the first time, we demonstrate that fitness-related variation in white matter integrity within these regions mediates the relationship between fitness and cognitive performance. These findings could not be explained by age, gender, or years of education.

We observed a positive linear relationship between VO_2, a measure of cardiorespiratory fitness, and white matter integrity within the anterior limb of the internal capsule and the anterior corona radiata. This is consistent with recent evidence demonstrating a positive cross-sectional association between fitness and FA in the anterior corona radiata among a sample of middle-aged adults with multiple sclerosis (Prakash et al., 2010). The corona radiata is represented by a large, fan-like array of fibers responsible for cortico-thalamo-cortical projections. The internal capsule is continuous with the corona radiata and corresponds to the ventral segment of fibers within the corona radiata located between the basal ganglia and the thalamus. While the caudal portions of the internal capsule primarily include auditory, visual, and somatosensory projection fibers, the anterior limb carries ascending thalamic projections concerned with cognitive, limbic, and basal ganglia functions to the prefrontal cortex (Catani and de Schotten, 2012). Given this anatomical connectivity, the involvement of these fiber tracts in cognitive processes, as observed

in the present study, is not surprising. In fact, reduced integrity in the anterior internal capsule has been observed both in Alzheimer's disease (Liu et al., 2011) and mild cognitive impairment (Liu et al., 2011; Cho et al., 2008). This pathway has also been linked to cognitive performance in non-clinical populations. Specifically, in a cognitively healthy sample of 52 adults, lower microstructural integrity within the anterior internal capsule predicted poorer performance on a variety of working memory tasks (Kennedy & Raz, 2009). Similarly, in our sample, the anterior internal capsule and corona radiata mediated the association between fitness and spatial working memory performance. Thus, fitness may preserve WM integrity in corticostriatal and corticothalamic circuits responsible for facilitating communication between subcortical and prefrontal regions involved in working memory processes.

The superior fronto-occipital fasciculus was also found to be a significant mediator of the relationship between fitness and cognitive performance in the present study. This fiber bundle contains projections from frontal association cortices to the parietal cortex, and has been previously implicated in nonverbal processing speed (Kennedy and Raz, 2009). Overall, the functional role of this pathway is not well understood (Catani and de Schotten, 2012), although our results suggest that it may also be involved in visuospatial processing or working memory.

Finally, white matter integrity within the splenium of the corpus callosum mediated the association between cardiorespiratory fitness and Flanker RT cost. In a small sample of healthy older adults, Johnson and colleagues (2012) also observed a relationship between fitness, measured using VO_{2peak}, and FA within a large portion of the corpus callosum, although the strongest effects were observed in the body of the corpus callosum (Johnson et al., 2012). The splenium includes the caudal segment of the corpus callosum, and contains interhemispheric fibers from the occipital and superior temporal cortical areas, which broadly include auditory and

visual processing regions. Given the anatomical connectivity of the splenium, it would be expected that its functional role is limited to lower-level sensory processes. On the contrary, white matter integrity in the splenium has been predictive of performance on tasks of memory and executive function (Kennedy and Raz, 2009; Madden et al., 2009b). In fact, reduced FA in the splenium has previously been associated with greater reaction time during a visual detection task that required different responses depending on the stimuli presented (Madden et al., 2004). Thus, results from the present study support previous work demonstrating the involvement of the splenium in higher-order cognitive functions, and suggest that fitness is linked to greater white matter integrity in fibers within the splenium that may mediate attentional control processes.

Although it is unknown through what molecular pathways exercise affects white matter integrity, there is some suggestion that this relationship may occur via cardiovascular pathways. Exercise reduces peripheral cardiometabolic risk factors including hypertension, adiposity, hypercholesterolemia, and insulin insensitivity (Carroll & Dudfield, 2004; Dunn et al., 1999; Healy et al., 2008), which converge to cause cognitive impairment and neural degeneration (Elias et al., 2003, Kivipelto et al., 2005; Whitmer et al., 2005). Additionally, exercise may reduce levels of chronic low-grade systemic inflammation, a common feature of many of the aforementioned conditions which, when present, stimulates a host of neural and cognitive consequences (Rosano et al., 2012). Notably, higher levels of C-reactive protein, (CRP), an acute-phase protein considered a biomarker of inflammation, predicts reduced white matter integrity specifically in the corona radiata and the corpus callosum (Wersching et al., 2010). Given the positive associations observed in the present study between fitness and WM integrity within these regions, it may be that physical activity favorably affects white matter morphology by reducing levels of systemic inflammation, thereby sparing fiber bundles that are particularly

susceptible to inflammation-based degeneration. Further elucidation on the possible mechanisms through which exercise might affect the brain has come from rodent models. In particular, studies have shown that exercise increases the production of nerve growth factors including brain-derived neurotrophic factor and insulin like growth factor-1, which facilitate neural repair and promote synaptic plasticity and neurogenesis (Cotman, Berchtold, & Christie, 2007; Trejo, Carro, & Torres-Alemán, 2001: Vaynman et al., 2003; Yang et al., 2014; Pang et al., 2004). In addition, microscopic, angiogenic benefits have been observed in rodent models of exercise, which may also help to preserve white matter integrity (Yao et al., 2004; Amaral et al., 2001). Importantly, the mechanisms discussed above are speculative, as the biological pathways through which physical activity exerts effects on white matter morphology have not yet been investigated in humans.

The proposed study is not without limitations. First, the quantitative estimates of WM integrity are susceptible to variations in fiber-crossing and fiber density, and thus may not accurately represent WM integrity in all regions, although this is a limitation present in all research that uses diffusion weighted imaging. Additionally, the diffusion data was collected using only 12 gradient directions, which limits the precision of the tensor estimation. Also, white matter hyperintensities were not controlled for in this sample. It is possible that unmeasured third variables may contribute to the relationship between cardiorespiratory fitness and cognitive performance or may moderate the fitness-white matter association, including health factors such as blood pressure and adiposity. But, examination of these moderating factors may be more appropriate after the link between PA, white matter, and cognitive performance has been firmly established. Further, causal inferences cannot be made regarding results obtained from the above analyses as the cross-sectional nature of the data does not permit

assessment of true causal pathways. Additionally, because the population was required to be sedentary at baseline, we were only able to examine the relationship between WM integrity and aerobic fitness within a relatively limited range of fitness. This may be why we did not observe a direct relationship between fitness and task-switch performance, which is in contrast to previous findings (Verstynen et al., 2012). But, the significant mediation results observed in the present study suggest that these findings may be even stronger in a population with greater inter-individual variability in fitness levels. Finally, there is a lack of diversity within our sample, with a large portion of participants Caucasian, well-educated, and derived from a similar location in Illinois, limiting the generalizability of the results.

Despite these limitations, the current study has several strengths that distinguish this research from previous work. The few existing studies that have assessed the association between fitness or PA and white matter integrity have been limited either by small sample sizes ($n < 30$) (Johnson et al., 2012; Marks et al., 2007; 2010; Tseng et al., 2013) or subjective, questionnaire-based assessments of PA (Gow et al., 2012; Gons et al., 2013), which are subject to recall bias. In contrast, the present investigation utilized a larger sample size, decreasing the likelihood of type 2 error, as well as a well-validated, objective measure of fitness. Additionally, our analytical assessment of white matter differs from existing work. Past research has examined the association between PA or fitness and white matter integrity within either one or two *a priori* regions of interest (Tian et al., 2014; Marks et al., 2007; 2011), or using a voxel-wise, whole-brain approach without subsequent cluster or region analysis (Gow et al., 2012; Gons et al., 2012). The former approach limits understanding to a few discrete brain regions, while the latter does not provide information on regional specificity. In the present study we utilized both voxel-based whole-brain and region of interest analysis to globally examine this

relationship and, in addition, further specify particular neural circuits associated with fitness. Finally, this is the first study that has examined the cognitive correlates of the fitness-white matter relationship.

In sum, we have demonstrated, for the first time, that white matter integrity serves as a mediating pathway for the relationship between fitness and cognitive performance. In particular, higher fitness levels predict greater white matter integrity in multiple fiber bundles, which, in turn, differentially relate to improved performance on tasks of attentional control, visuospatial processing, and working memory. Future directions include using exercise interventions to assess causality, as well as elucidating the mechanisms by which fitness and PA affect white matter integrity.

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