THE ROLE OF CARDIORESPIRATORY FITNESS IN WHITE MATTER AND COGNITION IN HEALTHY AGING

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We respectfully acknowledge that the University of Arizona is on the land and territories of Indigenous peoples. Today, Arizona is home to 22 federally recognized tribes, with Tucson being home to the O'odham and the Yaqui. Committed to diversity and inclusion, the University strives to build sustainable relationships with sovereign Native Nations and Indigenous communities through education offerings, partnerships, and community service.

DEDICATION

This dissertation is dedicated to my parents, Sharon and Glenn, as well as my sister, Sydney, whose support and encouragement allowed me to follow my aspirations to the desert and overcome any challenges that arose. I would also like to dedicate this dissertation to my grandfather, Dr. James Wallace Burt, whose pursuit of higher education later in life paved the way for me to contribute to the science of cognitive aging. I am honored to complete this dissertation in his memory and share the accomplishment of completing a dissertation with him.

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ABSTRACT

While certain cognitive domains remain relatively stable into old age in the absence of pathological processes, other domains are selectively vulnerable to healthy aging, including processing speed, executive functions, and memory. These age-related cognitive differences may be in part due to alterations in white matter responsible for propagating neural signals and efficiently connecting brain regions. Cardiorespiratory fitness (CRF) is an important indicator of vascular health that may mitigate age effects on cognition by protecting against age-related white matter degradation. In this study, I sought to evaluate the differential impact of CRF on tract-specific white matter integrity (WMI) and global free water (FW) as well as subsequent associations with cognitive aging in a cohort of healthy community-dwelling older adults.

First, I identified regional network covariance patterns of CRF-related WMI across four diffusion metrics, axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA). The resulting network patterns were characterized by enhanced integrity related to higher CRF in white matter bundles connecting anterior brain regions to one another, anterior to posterior regions, and subcortical regions. I additionally investigated associations of these patterns with important demographic and vascular health characteristics, including age, sex, macrostructural white matter hyperintensity (WMH) lesion load, and vascular risk level. Age and WMH volume were significantly negatively associated with expression of all four CRF-related WMI network patterns while male sex was significantly associated with higher expression of the CRF-AD and CRF-FA patterns. Vascular risk level also demonstrated a significant relationship with the CRF-RD, CRF-MD, and CRF-FA network patterns, such that increased vascular risk burden was associated with reduced expression of these patterns.

Second, I assessed the potential mediating effects of these CRF-related WMI network patterns in a pathway representing vascular impacts on cognitive aging. These results demonstrated

significant partial mediating effects of the CRF-RD and CRF-MD patterns on age-related processing speed, but not executive function or memory. Specifically, I found that older age was sequentially associated with greater WMH lesion load, which was further associated with reduced expression of the CRF-RD and CRF-MD patterns. Reduced expression of these patterns, in turn, predicted worse processing speed performance.

Third, I evaluated the impact of CRF on the relationship between cognition and a global metric of neural degradation, FW in white matter. Higher levels of FW were only predictive of worse processing speed and executive function at low (i.e., below average) levels of CRF, but not for memory. These results suggest that CRF may protect against brain-based cognitive differences in healthy aging at average and elevated levels of fitness. Further, differences in self-reported physical activity levels did not impact these effects.

Altogether, these findings add to our understanding of how vascular health may impact structural brain differences in white matter that, in part, underly the effects of cognitive aging. They additionally highlight the importance of investigating both regional and global impacts of CRF on brain markers of cognitive aging.

CHAPTER 1

INTRODUCTION

The proportion of adults over the age of 65 continues to rapidly increase both globally and in the United States (Cheng at al., 2020; Mather, Jacobsen, & Pollard, 2015). This growing aged population will place increased burden on institutions for healthcare and aid, as increasing age is commonly associated with higher incidences of disease, disability, and dementia (Jarbezski et al., 2021). It is thus imperative to better understand the mechanisms underlying and factors promoting healthy aging, such that older adults can remain cognitively healthy and functionally independent in the later decades of life (Lowry, Vallejo, & Studenski, 2012).

A complex interplay of genetic and lifestyle factors likely determines an individual's 'success' in aging, although the exact mechanisms and relative contributions remain unclear (Dixon & Lachman, 2019). It has been suggested that a unique combination of risk and protective factors, some modifiable, may determine an individual's likelihood of developing cognitive impairment in old age (McFall, McDermott, & Dixon, 2019). Further research is needed to determine the specific effects of health and lifestyle factors, such as cardiorespiratory fitness, on the cognitive and brain aging processes. Advancing our understanding of these mechanisms can provide specific targets for intervention to optimize the aging trajectory and prevent cognitive impairment, potentially reducing the negative impacts of aging over the lifespan.

Cognitive Aging

Healthy aging differentially affects multiple aspects of cognition. Crystallized, knowledge-based forms of cognition, such as vocabulary, as well as implicit memory processes tend to remain relatively well-preserved into old age (Craik & Salthouse, 2008; Salthouse, 2009). However, there is evidence that even healthy aging is associated with less efficient information processing, as evidenced by poorer performance on tasks of fluid cognition, including executive functions, processing speed, and long-term memory (Alexander et al., 2012a; Glisky, 2007; Park & Reuter-Lorenz, 2009; Salthouse, 1992). Quasi-longitudinal research has suggested great heterogeneity in the trajectories of decline for these functions, such that processing speed appears to be diminished linearly over the adult lifespan while memory and reasoning abilities show modest reductions throughout adulthood, which appears to accelerate only after the sixth decade (Salthouse, 2019). Processing speed may also contribute to performance in other areas of cognition, may precede declines in other domains, and thus, may be particularly important to examine in the healthy aging context (Salthouse, 1996; 2000). Executive functions encompass several cognitive processing features, including attentional monitoring, working memory, inhibition, and set shifting, which have been associated with poorer performances with increasing age (Miyake et al., 2000). Long-term memory, the ability to retain episodic information, has also been shown to be diminished in healthy aging, with poorer memory observed with increasing age (Albert, 1997; Glisky, Woolverton, McVeigh, & Grilli, 2022; Grady & Craik, 2007; Levine, Svoboda, Hay, Winocur, & Moscovitch; 2002). Given their vulnerability in old age, even in the absence of overt clinical symptoms related to neurological disease, identifying the brain-based biological underpinnings of these cognitive aging effects is vital to understanding the healthy aging process and the differences commonly observed among healthy older adults.

Brain Aging

Healthy aging has also been associated with differences in brain structure assessed through magnetic resonance imaging (MRI). Gray matter volume has shown preferential decreases in frontal and temporal lobes with increasing age (Alexander et al., 2012b; Bergfield et al., 2010; Brickman, Habeck, Zarahn, Flynn, & Stern, 2007; Fjell & Walhovd, 2010; Raz & Rodrigue, 2006). These reductions in gray matter volume typically show a linear association across adulthood and are correlated with cognition (Sele, Liem, Mérillat, & Jäncke, 2021; Lockhart & DeCarli, 2014).

Longitudinal studies of white matter volume have shown a nonlinear trajectory, with stable linear declines from ages 30 to 65 then accelerated decline until death (Fjell & Walhovd, 2010). Cross-sectional cohort studies have shown a similar trajectory of age-related white matter differences throughout the lifespan (Westlye et al., 2010).

These age-related differences in neuroanatomical structures may, in part, underlie worsening cognition observed in healthy aging. Patterns of region-specific correlations between measures of cognitive performance and brain structure have been observed. Specifically, executive functions (e.g., updating, inhibition) have been associated with both gray and white matter structure in overlapping but distinct sections of the frontal lobes (D'Esposito et al., 2000; Stuss & Levine, 2002; Stuss, 2011; Ziegler et al., 2010). Meanwhile, memory has been most often associated with structure of the temporal lobes, but also regions of the prefrontal cortex and parietal lobes (Kramer et al., 2005, Ziegler et al., 2010). While processing speed has not shown reliable associations with structure in a particular brain region, slowed processing speed has been related to deterioration of anterior white matter integrity, tracts of which project to many brain regions (Kennedy & Raz, 2009; Madden, Bennett, & Song, 2009a). Age-related differences and declines in specific cognitive capacities thus may arise from processes impacting the integrity of connections between regions as well as deterioration of the regional structures themselves.

White Matter in Cognitive Aging

White Matter Integrity

Diffusion tensor imaging (DTI) is a neuroimaging technique that provides quantitative values of microstructural integrity of cerebral white matter, the axons connecting brain regions that are wrapped in myelin sheaths insulating and enhancing the propagation of electrical signals both within and across neural cells (Basser, 1995). White matter integrity is thus thought to represent efficiency of neurotransmission by measuring how water diffuses along tracts (Basser & Pierpaoli, 1996). At each voxel along a tract, there is a directional preference of molecular diffusion. Fractional anisotropy (FA) measures the degree of non-directionality of the diffusion process at a voxel, with higher values representing greater directional (i.e., enhanced) integrity. While FA is sensitive to differences in white matter integrity, it does not provide information on the specific type of change occurring in the tracts (Alexander et al., 2011). Other metrics of integrity provided by DTI can provide more information about potential cellular alterations. Mean diffusivity (MD), for instance, reflects the molecular diffusion rate at a voxel independent of directionality and is sensitive to cellularity and edema (Sullivan & Pfefferbaum, 2006). Higher values of MD represent lower white matter integrity. While FA and MD are the most common 'summary' values reported in DTI research, other metrics can provide more specific information on potential cellular processes. Radial diffusivity (RD) measures diffusion perpendicular to a white matter tract and axial diffusivity (AD) measures diffusion parallel to the tract. There is evidence that RD and AD reflect myelin loss and axon integrity, respectively, and can provide information on the type of underlying differences that may be present (Soares et al., 2013; Song et al., 2002). These two DTI metrics, which are each components of MD, can be highly correlated but likely represent different neurobiological underpinnings of white matter integrity. It is thus important to consider each of these diffusion measures to help identify potential mechanisms of white matter integrity differences related to cognitive aging.

Previous research has most commonly reported reduced FA and higher MD in healthy aging, which have been hypothesized to reflect axonal loss and demyelination thereby reducing efficiency of neurotransmission (Barrick et al., 2010; Grieve et al., 2007; Sala et al., 2012; Salat, 2011; Sullivan, Rohlfing, & Pfefferbaum, 2010; Zhang et al., 2010). These age-related differences in white matter have been further associated with poorer performance on tasks of long-term memory, executive functions, and processing speed (Charlton, Barrick, Markus, & Morris, 2010; Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009; Haász et al., 2013; Lockhart et al., 2012; Lu et al., 2011).

Higher RD has also been associated with increasing age, while age effects on AD have been less consistent, showing both increases and decreases (Bennett et al., 2010; Burzynska et al., 2010). The specific pattern of differences across metrics may reflect different cellular mechanisms underlying microstructural white matter degeneration. For example, an observed pattern of lower FA accompanied by only higher RD values may reflect specific alterations to myelin sheaths, while a pattern characterized by lower FA and AD may reflect acute axonal injuries leading to low alignment parallel to the primary diffusion direction (Sen & Basser, 2005). These results emphasize the value of evaluating multiple integrity metrics and further interpreting them in relation to one another.

Differences in WMI throughout the aging process have often been conceptualized by an anterior-to-posterior shift in brain structure. Age-related reductions in integrity in frontal regions and the genu of the corpus callosum have been related to reduced processing speed and executive functions (Borghesani et al., 2013; Bucur et al., 2008; Callaghan et al., 2014; Madden et al., 2009b; Salami et al., 2012). These studies support the "last-in-first-out" hypothesis of brain aging, which posits the most recently myelinated sections of the brain in the frontal lobes may be the first to degrade with advanced age (Coelho et al., 2021). However, there has also been evidence of age-related differences in posterior white matter associated with processing speed that do not support this regional shift hypothesis (Barrick et al., 2010; Burgmans et al., 2010; Davis et al., 2009; Meier et al., 2012; Westlye et al., 2010). Indeed, Kennedy & Raz (2009) found that poorer processing speed and attentional abilities were related to reductions in anterior white matter integrity, poorer episodic memory was related to reduced central white matter integrity, and poorer inhibition and task-switching abilities were associated with degradation of posterior white matter integrity. Thus, regional analyses of white matter integrity may yield differential age effects and it is important to assess potential region-specific patterns of white matter alterations in aging.

Free Water

Extracellular free water (FW) in white matter is another metric acquired from diffusion MRI (dMRI) that is sensitive to age-related differences in brain structure and cognition (Maillard et al., 2019). Although this metric is less specific to precise pathophysiological processes, it may be more sensitive to cognitive and brain aging than conventional dMRI metrics of microstructural WMI (Chad et al., 2018; Maillard et al., 2019) and macrostructural WMH lesion load (Duering et al 2018; Dumont et al., 2019; Maillard et al., 2019). Elevated levels of FW have been associated with poorer performance in cognitive domains vulnerable to healthy aging, including processing speed (Duering et al., 2018), executive functions (Berger et al., 2022; Gugger et al., 2023; Gullett et al., 2020; Gustavson et al., 2023; Maillard et al., 2022a; Pappas et al, 2024), and memory (Gullett et al., 2020). In pathological aging cohorts including individuals with Alzheimer's disease (AD) and vascular disease, greater FW has also been associated with more severe clinical stages and worse executive functioning (Ji et al., 2017). FW, as a neuroimaging marker of aging, is thought to reflect several biological processes impacting the proportion of extracellular FW in white matter, including alterations in blood-brain barrier permeability (Pasternak et al., 2012; Maillard et al., 2017; Pappas et al., 2024), cerebral atrophy, neuroinflammation or injury (Gugger et al., 2023; Ji et al, 2017), and atherosclerosis secondary to poor vascular health (Maillard et al., 2017). It has yet to be investigated how CRF may impact the associations of FW with cognitive aging.

Vascular Health in Cognitive and Brain Aging

Vascular health conditions, including diabetes, hypertension, and hypercholesteremia, are common among older adults in the United States (McDonald, Hertz, Unger, & Lustik, 2009). Defining healthy cognitive aging in this context must include consideration for how these conditions impact the brain and subsequently cognition. Vascular health conditions have been associated with poorer brain and cognitive outcomes, especially in frontal brain regions and performance on frontally mediated, executive tasks (Levit, Hachinski, & Whitehead, 2020; Raz, Rodrigue, & Acker, 2003).

Modifiable lifestyle behaviors may interact with genetics and health conditions to alter the trajectory of cognitive decline both positively, such as with regular aerobic exercise (Colcombe et al., 2006), and negatively, such as with habitual cigarette smoking (Ho et al., 2012; Whitsel et al., 2022). While it is assumed these behavior-induced effects occur via alterations in cardiorespiratory system functioning, the mechanisms underlying these effects remain unclear.

Multiple cardiovascular risk factors often co-exist in individuals (Genest & Cohn, 1995). There is evidence that the effects of these risk factors can be additive, such that the risk of cognitive impairment increases with each additional risk factor (Luchsinger et al., 2005). Further, a greater number of cardiovascular risk factors has been associated with greater brain atrophy and poorer white matter health (Cox et al., 2019). Studies have developed aggregated risk scores to represent low (i.e., no or one vascular risk factor(s)) and high (i.e., two or more vascular risk factors) vascular risk burden. Bangen and colleagues (2014) found that advanced age was only associated with reduced cerebral blood flow in those with elevated cardiovascular risk level and that this relationship was associated with poorer cognitive performance. Testing effects of aggregated cardiovascular risk rather than individual conditions may provide a valuable health risk measure that more accurately represents those combined factors affecting cardiovascular and brain health in healthy cognitive aging.

Cardiorespiratory Fitness in Cognitive Aging

Measures of cardiorespiratory fitness (CRF) are often used to assess functional capacity of the cardiovascular system. A reliable metric of CRF and important indicator of vascular health is maximal oxygen consumption during exercise (VO_2max), which reflects the greatest amount of oxygen a person can take in from inspired air while exercising, expressed in milliliters of oxygen consumed per kilogram of body per minute (Huggett, Connelly, & Overend, 2005). It has been theorized that greater VO_2max represents more oxygen being transported and used in cellular metabolism, thereby increasing the amount of oxygen that can reach tissues throughout the body, promoting long-term angiogenesis and cerebral circulation (Cohn, 1987; Cohen et al., 1991; Fletcher et al., 2013; Stimpson, Davison, Javadi, 2018). During cardiopulmonary exercise testing, participants wear a nose clip and mouthpiece while gradually increasing speed and grade on a treadmill. Ventilation, oxygen, and carbon dioxide concentrations are measured simultaneously as exercise increases in intensity. Testing terminates once oxygen consumption plateaus with increased workload, respiratory exchange rate equalizes, or participants are near peak heart rate. Although highly reliable, exercise testing in this way is physically demanding, requires a great deal of time and resources, and may not be appropriate for all populations, such as those with physical disabilities or cardiovascular disease.

While numerous factors can affect an individual's CRF, including age and exercise habits, previous research has found that both gains in VO_2max due to increased physical activity (PA) as well as intrinsic VO_2max levels are up to 50% heritable (Bouchard et al., 2015; Williams et al., 2017). Engagement in PA may have varying effects on CRF across individuals in addition to distinct effects from VO_2max on cognitive and brain aging. Several studies have found independent associations of CRF and PA with cardiovascular risk (Ekblom-Bok et al., 2010; Myers et al., 2015) and all-cause mortality (Lee et al., 2011). Assessing both CRF and PA provides information on genetic and behavioral aspects of cardiovascular health, which may represent distinct effects on cognitive and physical health outcomes.

Peak lifetime values of VO_2max occur between the ages of 15 and 30 years and progressively decrease with age (Fletcher et al., 2013). Even with age-related reductions, CRF has shown beneficial effects on cognition in healthy aging samples. Enhanced CRF has been associated with greater performance on tasks of both executive function and memory (Angevaren et al., 2008; Brown et al., 2010; Erickson et al., 2009; Kawagoe, Onoda, & Yamaguchi, 2017; Kramer et al., 1999; Weinstein et al., 2012; Wendell et al., 2014). Newson and Kemps (2006) found stronger effects of CRF on measures of attention and processing speed than on higher-order cognitive tasks, such as executive functions and memory. This suggests fitness may attenuate detrimental age effects on executive function and memory by improving the ‘lower order’ cognitive components required to complete those tasks efficiently. Overall, studies have found modest benefits of CRF on cognition, with the greatest effects on areas of cognition associated with efficiency, including attention and processing speed. Several reviews, however, have not found significant, beneficial effects of CRF on cognitive performance in aging (Etnier, Nowell, Landers, & Sibley, 2006; Young, Angevaren, Rusted, & Tabet, 2015). These inconsistencies in results may be due to differences in operationalizing fitness, measures of cognitive function, and/or demographic differences across the included studies.

Cardiorespiratory Fitness and White Matter

In healthy aging, greater VO_2max has been associated with greater global white matter volume as well as enhanced microstructural integrity in frontal and parietal lobes (Colcombe et al., 2006; Gordon et al., 2008; Voss et al., 2013). Greater CRF levels have been related to greater FA (Johnson et al., 2012; Zhu et al., 2015) even in the absence of significant effects of PA levels on integrity metrics (d’Arbeloff et al., 2021; Strömmer et al., 2020). These associations were found primarily in tracts connecting to anterior brain regions, including the genu of the corpus callosum, superior longitudinal fasciculus, inferior longitudinal fasciculus, and uncinate fasciculus, and were related to executive functions such as planning and coordination (Ding et al., 2018; Hayes et al., 2015; Johnson et al., 2012; Oberlin et al., 2016). There has been further evidence that these CRF-related differences in microstructural WMI mediate age-related effects on processing speed and spatial working memory performance (Oberlin et al., 2016; Strömmer et al., 2020). There have also been reported associations between elevated CRF and greater WMI in subcortical and posterior brain regions that attenuate age-related cognitive differences, even among very old adults with a range of chronic diseases, with differential associations with cognitive domains (Burgmans et al., 2010; Davis et al., 2009; Meier et al., 2012; Tian et al., 2014). It has been hypothesized that CRF may attenuate age-related cognitive differences in certain areas via increased delivery and upregulation of neurotrophins and other supporting factors in brain regions particularly vulnerable to demyelination in old age (Stimpson, Davison, Javadi, 2018), including increased cerebral perfusion, synaptogenesis, and/or angiogenesis (Maass et al., 2015; Stimpson, Davison, Javadi, 2018; Tsai et al., 2016). However, relatively few studies have examined whether and how CRF-related differences in tract-specific WMI influence cognition in healthy aging.

Overall Goals

Much of the extant literature on the influence of CRF on WMI in cognitive aging has used indirect measures of fitness (e.g., heart rate) to represent aerobic capacity, which may reflect similar yet different aspects of physiology and their impacts on brain health and cognition. Additionally, previous research has often evaluated microstructural WMI at the global level or regionally based on a limited number of tracts, making it difficult to draw conclusions about regionally distributed effects of CRF on WMI and subsequent associations with specific cognitive domains. Most studies have also not applied sensitive multivariate network covariance statistical techniques to examine the regional patterns of the association of CRF with WMI and cognition. Further, it has yet to be investigated how CRF influences FW in white matter, a global dMRI metric of degradation sensitive to age-related neural and cognitive differences.

The overall goal of my dissertation research, consisting of three first-authored manuscripts for publication, was to address each of these significant gaps in the literature by evaluating tract-specific effects of CRF on WMI, subsequent associations with cognition, and the influence of CRF on FW and cognition in the context of healthy aging.

In Chapter 2, I identified four network covariance patterns of CRF-related WMI across 42 white matter bundles for diffusivity metrics AD, RD, MD, and FA in a sample of 167 healthy older adults. Associations of important demographic factors, like age and sex, as well as vascular health factors, including vascular risk burden, APOE status, and macrostructural WMH lesion load were also assessed to investigate additional influences of these variables on the CRF-WMI patterns.

In Chapter 3, I evaluated the association of these network patterns with cognitive domains vulnerable to aging, including processing speed, executive function, and memory. A series of mediation models were used to investigate a potential pathway of vascular brain and cognitive aging that incorporate both macrostructural WMH lesion load and CRF-related WMI covariance patterns.

In Chapter 4, I investigated the influence of CRF on another brain marker of neural degradation in white matter, FW, and its association with cognition in 162 community-dwelling older adults. Specifically, I assessed the extent to which varying levels of CRF modified the association between FW and selected cognitive measures for domains susceptible to age effects, processing speed, executive function, and memory. Further, I examined the influence of PA levels on these relationships to evaluate the potentially distinct effects of CRF on brain and cognition.

CHAPTER 2

SMITH ET AL. (IN PREPARATION): CARDIORESPIRATORY FITNESS & WHITE MATTER INTEGRITY

Summary

Healthy aging in the absence of cognitive impairment or dementia has been associated with differences in brain structure (Alexander et al., 2006; Raz and Rodrigue, 2006; Farokhian et al., 2017), including diffusion metrics of WMI that have been most commonly assessed by AD, RD, MD, and FA. Increasing age is also associated with greater incidence of cardiovascular disease and many older adults have multiple cardiovascular health conditions (Cox et al., 2019; Luchsinger et al., 2005; Rodgers et al., 2019). These conditions can negatively impact brain structure and quality of life, as well as increase the risk for dementia (Kivipelto et al., 2001; Viswanathan et al., 2009). Maintaining good vascular health in old age may help to reduce the effects of brain aging. An important indicator of vascular health that is influenced by both genetic and lifestyle factors, CRF, can be directly assessed by obtaining VO_2max (maximal oxygen consumption) through graded cardiopulmonary exercise testing (Bouchard et al., 2015; Zadro et al., 2017; Sarzynski et al., 2022). Greater CRF has been associated with enhanced WMI (Strömmer et al., 2020; d'Arbeloff et al., 2021). Previous studies have typically relied on univariate analytic methods, which may limit the ability to fully characterize the regionally distributed associations of CRF with WMI. Multivariate statistical methods have been used to identify age-related differences in gray and white matter (Alexander and Moeller, 1994; Gazes et al., 2016; Geeraert et al., 2019; 2020), but have yet to be directly applied to evaluate the influence of CRF on regional measures of WMI.

I sought to apply multivariate Scaled Subprofile Model (SSM; Alexander and Moeller, 1994) analysis to directly investigate the relationship between CRF and regional tracts of WMI in the context of healthy aging while examining how 1) demographic factors (i.e., age and sex), 2) vascular

health and dementia risk characteristics (vascular risk burden and APOE ε4 carrier status), and 3) WMH lesion load were each associated with the identified regional SSM network patterns of CRF-related WMI. I hypothesized that greater integrity in white matter bundles connecting anterior and posterior brain regions, such as the genu of the corpus callosum and superior longitudinal fasciculus, would be related to elevated CRF. Further, I hypothesized that patterns of CRF-related WMI would be negatively impacted by poorer vascular health. Participants were comprised of 167 cognitively unimpaired adults ages 50 – 88 (mean $\pm$ *SD* = 69.0 $\pm$ 10.4 years) who underwent cardiopulmonary exercise testing and brain MRI scans on separate days. The current sample included 78 females (46.7%) and predominantly (93.4%) non-Hispanic White individuals. Participants completed a treadmill graded exercise test that began at a low intensity with a speed of 1 mph and an incline grade of 0%. Speed and grade were gradually increased based on the modified Naughton treadmill protocol during exercise (Berry et al., 1996; Strzelczyk et al., 2001). Pre-processing of diffusion MRI data involved eddy current correction (Smith et al., 2004) and EPI distortion correction using Brainsuite's INVERSION method (Bhushan et al., 2015). These images were further processed using Tracts Constrained by Underlying Anatomy (TRACULA; Yendiki et al., 2011; Maffei et al., 2021) for automated global probabilistic tractography of 42 white matter bundles using a diffusion model capable of modeling crossing fibers (Behrens et al., 2007) and extraction of standard diffusivity measures (i.e., AD, RD, MD, and FA). White matter hyperintensities were computed using T1 and T2-FLAIR MRI scans and the lesion segmentation toolbox (LST; Schmidt et al., 2012) with Statistical Parametric Mapping (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK).

Regional SSM network analysis (Moeller et al., 1987; Alexander and Moeller, 1994; Habeck et al., 2008; Alexander et al., 2012b) was performed using MATLAB (Math Works, Natick, Massachusetts, USA). A modified principal component analysis (PCA) was performed on diffusivity metrics, producing both a set of regional covariance pattern components and corresponding network subject scores reflecting the degree to which each participant expressed the identified network pattern. The Bayesian Information Criterion (BIC; Schwarz, 1978) was used as a model selection method to identify the best set of SSM components. A bootstrap re-sampling procedure (Efron and Tibshirani, 1994) with 10,000 iterations was applied in the SSM analysis (Gazes et al., 2016; Habeck et al., 2008; Alexander et al., 2012a) to provide reliability estimates for the regional pattern weights. Block-wise multiple linear regression analyses in Statistical Package for the Social Sciences (SPSS v22, Chicago, IL, USA) were used to test how demographics and vascular health and dementia risk factors were associated with expression of each CRF-related WMI pattern. TIV, age and sex, vascular risk level (0 or 1 vs. 2 or more clinical vascular risk factors) and APOE ε4 status (ε4 non-carrier vs. carrier) were sequentially then added in a three-block model to evaluate their associations with expression of each of the SSM patterns. Global WMH volume was then subsequently added as a final covariate in block 4 to evaluate the impact of macrostructural white matter lesion load on pattern expression.

While the CRF-WMI covariance patterns differed for some identified tracts, several were shared across the diffusion metric patterns, including the prefrontal body of the corpus callosum, fornix, and superior longitudinal fasciculus, suggesting these bundles may be especially sensitive to CRF-related differences across several MRI diffusion measures of WMI. CRF-RD and CRF-FA patterns were most strongly associated with both VO_2max and vascular risk level, suggesting that these DTI metrics may be most sensitive to differences in the integrity of white matter related to risk factors for cardiovascular health. Across all identified regional patterns of the diffusion WMI metrics, greater CRF was associated with enhanced integrity in white matter bundles primarily connecting anterior portions of the brain as well as in wider association areas. Multivariate patterns of MD and FA additionally identified white matter bundles connecting subcortical brain regions related to greater CRF.

Contributions

These findings support and extend previous research showing that CRF is an important indicator of cardiovascular health related to brain WMI by identifying tract-specific regional patterns of WMI that are preferentially associated with CRF in healthy older adults. They also provide support for multiple clinical vascular risk factors associated with added risk for brain aging in white matter. The results additionally highlight the value of multivariate network analysis methods to provide neuroimaging markers that can help to clarify the impacts of aging, vascular health, and CRF on the brain.

As first author for this work, I drafted the initial manuscript and was responsible for integrating revisions into the final document. With assistance from Dr. Gene Alexander, I developed the objectives and hypotheses for the study, carried out all statistical analyses, and interpreted the findings.

CHAPTER 3

SMITH ET AL. (IN PREPARATION): COGNITION & CARDIORESPIRATORY FITNESS-RELATED WHITE MATTER INTEGRITY

Summary

Greater CRF, an important indicator of cardiorespiratory system functioning has been correlated with enhanced WMI, primarily in frontal and parietal lobes that are vulnerable to age effects (Colcombe et al., 2006; Gordon et al., 2008; Voss et al., 2013). Elevated CRF has also been associated with lower risk for cognitive impairment and may attenuate cognitive aging effects on executive functions and memory (Angevaren et al., 2008; Erickson et al., 2009; Kramer et al., 1999; Kramer & Colcombe, 2018; Weinstein et al., 2012; Wendell et al., 2014). Moreover, there has been evidence that CRF may have stronger associations with measures of processing speed than with higher-order cognitive tasks, such as executive functions and memory (Newson & Kemps, 2006), suggesting that CRF may mitigate decremental age effects on these higher-order functions by enhancing the cognitive processing speed components required to efficiently complete those tasks. It remains unclear, however, the extent to which CRF effects on tract-specific WMI influence cognitive aging across these vulnerable cognitive domains as well as how these effects may be impacted by other indicators of white matter structure and vascular health, such as WMH lesion load.

The current work extended the findings of the previously described manuscript (Appendix A; Smith et al., in preparation) to assess cognitive associations of the identified CRF-WMI network covariance patterns in domains vulnerable to age effects. I also investigated whether microstructural CRF-related WMI and macrostructural WMH lesion load act as mediators to influence cognitive aging. The present cohort was comprised of the same 167 cognitively unimpaired older adults (mean age $\pm$ *SD* = 69.0 $\pm$ 10.4 years, 46.7% females, 93.4% non-Hispanic White). I hypothesized that lower expression of the CRF-WMI patterns would be associated with poorer cognitive performance on

measures of processing speed, executive function, and memory. I additionally expected that the CRF-WMI patterns would mediate the relationship between older age and poorer cognitive performance in these domains. Lastly, I predicted that greater age-related macrostructural white matter lesion load would be associated with diminished expression of the CRF-related WMI network patterns, which, in turn, would be predictive of worse cognitive performance.

One separate days, participants completed a graded treadmill exercise test based on the modified Naughton treadmill protocol with gradual increases in speed and incline over time (Berry et al., 1996; Strzelczyk et al., 2001) and MRI scans. Diffusion MRI data were pre-processed with Brainsuite's INVERSION method (Smith et al., 2004; Bhushan et al., 2015) then processed with TRACULA to extract standard diffusivity measures for 42 white matter bundles (Yendiki et al., 2011; Maffei et al., 2021). T1 and T2-FLAIR scans and the lesion segmentation toolbox (LST; Schmidt et al., 2012) with SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) were used to compute white matter hyperintensities. One measure was selected from each cognitive domain preferentially susceptible to aging (processing speed, executive function, and memory; Alexander et al., 2012b). Multivariate SSM analyses to identify the WMI covariance patterns related to CRF have been described in detail previously (Appendix A; Smith et al., in preparation).

I initially performed a set of multiple regression analyses to specifically evaluate the associations of each CRF-WMI network pattern with each cognitive measure and to further identify candidates for subsequent mediation analyses based on significant linear models. Block-wise linear regressions were performed with TIV, age, sex, years of education, vascular risk level, APOE ε4 carrier status, and WMH lesion load entered as initial covariates (block 1), each CRF-WMI pattern was entered next as the independent variable (block 2), and each cognitive measure was the dependent variable. The outputs of all multiple linear regression models across the cognitive domains were corrected for multiple comparisons using the false discovery rate (FDR) approach (Benjamini & Hochberg, 1995). Given that associations with higher-order cognitive functions may be driven by impacts of processing speed measures (Salthouse, 1992; 1996; Newson & Kemps, 2006), TMT A was included as an additional covariate for significant regression models of CRF-WMI network patterns predicting non-processing speed measures for inclusion in the FDR multiple comparison correction to evaluate candidacy for subsequent mediation analyses. Simple mediation analyses were conducted using the PROCESS macro (v3.5; Hayes, 2018) in SPSS 28.0 (IBM Corp., Armonk, NY) for those CRF-WMI patterns showing significant associations with cognitive measures in the multiple regression analyses using bootstrap resampling with 10,000 iterations to produce percentile 95% confidence intervals to examine if these patterns mediated the relationship between age and cognitive functions. Finally, serial mediation models were then used to evaluate if WMH volume influenced the CRF-WMI patterns' associations with age-related cognitive function.

From the multiple linear regression results, only CRF-RD and CRF-MD network patterns significantly predicted TMT A performance and were selected for follow-up mediation analyses with age and processing speed. Although TMT B performance was initially significantly predicted by the CRF-AD, CRF-RD, and CRF-MD patterns, these associations were no longer significant once TMT A was added as an additional covariate, suggesting the observed effects were explained by the processing speed component of this executive function task. In the simple mediation models for both CRF-RD and CRF-MD, older age predicted lower expression of the CRF-WMI patterns. In turn, lower expression of the network patterns then predicted slower completion time of TMT A, indicating poorer performance. After adding global WMH volume as a predictor to create serial mediation models, results indicated significant partial serial mediation effects on cognitive aging of both the CRF-RD and CRF-MD patterns. Specifically, increasing age predicted higher WMH volume, which was associated with lower expression of the network patterns, and less pattern expression predicted poorer performance on TMT A. These findings suggest that CRF may have the potential to selectively attenuate detrimental effects of aging and macrostructural lesion load on processing speed through enhancement of WMI in tracts connecting anterior brain regions to one another.

Contributions

The present work extends the findings of the previous manuscript (Appendix A; Smith et al., in preparation) by identifying cognitive associations with CRF-related WMI covariance network patterns characterized by enhanced integrity in areas connecting anterior brain regions to one another as well as anterior to posterior regions. These findings support the use of the network covariance patterns as potential neuroimaging markers as part of a white matter pathway linking age-related macrovascular lesion load with expression of CRF-related regional WMI to influence the cognitive aging effects on processing speed in healthy older adults. That significant effects were only observed in patterns representing CRF-related RD and MD additionally suggest that greater CRF may, in part, provide its protection from cognitive aging by mitigating age-related effects on myelin degradation.

As first author for this work, I initially drafted manuscript and integrated revisions into the final document. With guidance and assistance from Dr. Gene Alexander, I developed the objectives and hypotheses for the study, carried out all statistical analyses, and formulated interpretations of the findings.

CHAPTER 4

SMITH ET AL. (IN PREPARATION): CARDIORESPIRATORY FITNESS INFLUENCE ON FREE WATER & COGNITION

Summary

MRI metrics of structural brain differences may partly drive differences in cognitive performance observed in healthy aging (Kennedy & Raz, 2009; Laukka et al., 2013). Extracellular FW within normal-appearing white matter obtained from diffusion MRI (dMRI) is sensitive to age-related differences in brain structure and cognition (Maillard et al., 2019). While less specific to underlying pathophysiological changes, FW is potentially more sensitive to age- and vascular-related neural degradation than conventional dMRI metrics of microstructural white matter integrity (Chad et al., 2018; Maillard et al., 2019) and macrostructural WMH lesion load (Duering et al 2018; Dumont et al., 2019; Maillard et al., 2019). Higher FW levels have been associated with poorer processing speed (Duering et al., 2018), executive function (Berger et al., 2022; Gugger et al., 2023; Gullett et al., 2020; Gustavson et al., 2023; Maillard et al., 2022a; Pappas et al, 2024), and memory (Gullett et al., 2020) in cognitively unimpaired cohorts. In clinical populations, elevated FW has also been associated with more severe dementia symptoms in patients with Alzheimer's disease, combined Alzheimer's disease with cerebrovascular disease, and vascular dementia. Furthermore, poorer executive functioning was associated with higher FW in all dementia subgroups (Ji et al., 2017). While the biological mechanisms that lead to increased FW are not fully understood, it is suspected to reflect several sources of age and vascular health factors that can lead to neural degeneration, including cerebral atrophy, neuroinflammation or injury (Gugger et al., 2023; Ji et al, 2017), atherosclerosis secondary to poor vascular health (Maillard et al., 2017), and alterations in blood-brain barrier permeability (Pasternak et al., 2012; Maillard et al., 2017; Pappas et al., 2024). However, CRF's influence, as a genetic and lifestyle marker of vascular health, on FW in cognitive

aging has yet to be investigated. Elevated CRF has been associated with better brain and cognitive aging outcomes (Angevaren et al., 2008; Erickson et al., 2011; Kramer et al., 1999; Kramer & Colcombe, 2018; Weinstein et al., 2012; Wendell et al., 2014), even when controlling for differences in physical activity (PA) (Burzynska et al., 2014; Erickson et al., 2014; Raichlen et al., 2020; Voss et al., 2016). Thus, elucidating how modifiable factors, like CRF, are associated with FW alterations in white matter may help identify new pathways related to age and vascular health, as well as targets for intervention.

In the present study, I sought to investigate the potential interactive effects of CRF with global FW in white matter on cognitive performance in a cohort of 162 healthy community-dwelling older adults (mean age $\pm$ SD = 68.9 $\pm$ 10.5 years, 42.0% females, 93.2% non-Hispanic White). I hypothesized that higher levels of FW would be associated with worse cognitive performance in age-sensitive domains, including processing speed, executive functioning, and memory in those with low levels of CRF, after controlling for differences in demographic and other common vascular and health risk factors; and that this association would be mitigated in participants with elevated CRF levels. I also assessed if potential interactive effects of CRF with FW were separable from differences in PA engagement by additionally adjusting for self-reported habitual activity levels.

CRF was assessed via graded cardiopulmonary exercise testing to obtain VO_2max based on the modified Naughton treadmill protocol (Berry et al., 1996; Strzelczyk et al., 2001). Pre-processing of raw dMRI involved motion and eddy current correction using the Functional Magnetic Resonance Imaging of the Brain Software Llibrary (FSL; Smith et al., 2004) and susceptibility induced EPI distortion was corrected using BrainSuite’s non-rigid registration-based INVERSION method (Bhushan et al., 2015). The diffusion signal in each voxel was then fitted to a two-compartment model: a tissue compartment modeled using a diffusion tensor and an extracellular FW compartment modeled as an isotropic diffusion tensor with a constant diffusion coefficient of water at 37°C (Pasternak et al., 2009). These FW fraction maps were estimated using a protocol developed by the

Mark Vascular Contributions to Cognitive Impairment and Dementia (VCID) consortium (https://markvcid.partners.org/; Maillard et al., 2022a; 2022b). Resulting FW values ranged from 0 to 1, representing the fractional contribution of FW in each voxel. Global WMH maps were generated using the T1 and T2-FLAIR MRI scans with the automated lesion segmentation toolbox (LST; Schmidt et al., 2012) for the SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). Participants completed the Physical Activity Questionnaire (PAQ; Voorrips, et al., 1991) to provide a total score reflecting their self-reported habitual engagement in physical activities. One cognitive measure was selected from each domain vulnerable to age effects to limit Type 1 error across tested models.

Block-wise multiple linear regression analyses were first used to test the hypothesized interactive effects of FW and CRF on the selected cognitive measures while controlling for demographic and vascular health characteristics. Significant interaction effects were then probed using simple slopes (Aiken et al., 1991) and Johnson-Neyman analyses (Hayes, 2018; Johnson & Neyman, 1936) using the PROCESS macro (v4.2; Hayes, 2018) for SPSS 28.0 (IBM Corp., Armonk, NY).

After controlling for demographic and vascular health factors, results indicated significant associations for higher levels of FW with poorer performance on measures of processing speed and executive function, but only in individuals with low levels of CRF, and not in those with moderate or higher levels. This attenuation effect was not seen on verbal memory performance, with no significant interaction observed. These results suggest mitigating effects of CRF in reducing the relation of this neuroimaging marker of white matter structural degradation with age-sensitive cognitive measures of processing speed and executive function, but not for verbal episodic memory. Moreover, the observed moderating effects of CRF on the relation of FW to cognitive performance was not affected after additionally adjusting for self-reported activity levels, suggesting that the benefits of greater levels of CRF for processing speed and executive function were distinct from engagement in habitual PA. These findings suggest that below average levels of CRF may not provide the attenuative benefits of moderate to high CRF levels for brain white matter degradation and age-related diminutions in processing speed and executive function.

Contributions

The present findings support the use of global FW elevation as a potentially important biomarker of cognitive aging and for evaluating impacts of CRF on these neural and cognitive processes. It also follows that CRF may serve as a useful intervention target to preserve cognition in aging with modifiable lifestyle factors to a certain average or higher threshold. The unique combination of genetic and lifestyle factors composing CRF may provide insights into individual variability related to benefits obtained from PA on brain structure and cognition.

As first author for this work, I drafted the original manuscript and was responsible for integrating revisions to produce the final document. With guidance and advice from Dr. Gene Alexander, I formulated the objectives and hypotheses for the study, carried out all statistical analyses, and interpreted the results.

CHAPTER 5

GENERAL DISCUSSION

The overall goal of this study was to investigate the influence of CRF on regional and global metrics of white matter structure and their associations with cognition in healthy aging. This research also aimed to evaluate the relative contributions of demographic, vascular health, and lifestyle characteristics on the hypothesized vascular pathways of brain and cognitive aging.

In Chapter 2, I identified four network covariance patterns of CRF-related WMI characterized by enhanced integrity in white matter bundles connecting anterior brain regions to one another, anterior to posterior brain areas, and subcortical regions. Reduced expression of these patterns was associated with advanced age, increased vascular risk burden, and increased macrostructural WMH lesion load. These results identified regional brain-based markers of CRF related to age and vascular health factors.

In Chapter 3, I found that expression of two of the previously identified patterns significantly contributed to a theorized model of a vascular health pathway of brain aging. Covariance network patterns of CRF-related RD and MD in white matter connecting anterior to anterior and anterior to posterior brain regions were associated with age-related WMH lesion load and in turn predicted worse processing speed performance. These findings suggest that CRF may have the potential to selectively attenuate detrimental effects of aging and macrostructural lesion load on processing speed through enhancement of myelin structure in tracts connecting anterior and posterior brain regions.

In Chapter 4, I showed moderating effects of CRF on FW associations with cognition, such that elevated FW predicted poorer processing speed and executive function performance only in individuals with low levels of CRF, regardless of PA engagement. That FW was not significantly associated with worse cognitive performance in these domains at moderate and high CRF levels suggest that CRF may attenuate the relationship of neural degradation in white matter and cognition at or above a threshold of average CRF. These findings provide insight into the interaction of modifiable factors with brain-based markers of cognitive differences in healthy aging and identify a potential target for future interventions to help mitigate cognitive dysfunction in both healthy and pathological aging samples.

Together, the findings of these three studies contribute to our understanding of how CRF is related to regional and global metrics of white matter structure and their subsequent relationships with cognition. By identifying CRF-related covariance patterns of tract-specific WMI and their role in a potential white matter pathway of cognitive aging, these results contribute to knowledge of microstructural white matter alterations sensitive to vascular health that may underly processing speed differences in healthy aging. Moreover, this research advances current knowledge in the field by identifying tract-specific regional patterns of WMI that are preferentially associated with CRF in healthy older adults. The outcomes additionally highlight the value of multivariate network analysis methods to provide neuroimaging markers that can help to clarify the impacts of aging, vascular health, and CRF on the brain. The findings of the present studies additionally contribute to the current body of literature by providing the first examination of the relationship between CRF and FW as it relates to cognitive aging. These results highlight the utility of FW as a neural marker sensitive to CRF and cognitive differences in healthy aging cohorts. This may prove useful as a future marker of successful protective effects on neural degradation of white matter in intervention studies targeting enhancement of CRF.

Future Directions

Neuroimaging Techniques

The present studies utilized novel neuroimaging methods to measure microstructural WMI across 42 tracts to enhance regional specificity of CRF effects. While these dMRI metrics have shown associations with specific biological mechanisms (e.g., myelin degradation, axonal integrity), they are limited in their resolution to examine pathophysiological underpinnings of white matter alterations. Additional neuroimaging measures may provide increased specificity to differences in microstructural white matter characteristics (e.g., axonal density) to enhance precision of WMI measurement (de Santis et al., 2014). Multi-shell acquisition of dMRI data, for example, can also provide enhanced resolution for intravoxel structures to ensure accurate delineation of white matter fibers (Jha et al., 2022). Additionally, while global measures of FW as a metric of overall neural white matter degradation are sensitive to age effects, FW values have been shown to be useful in enhancing regional specificity of white matter effects when assessing brain and cognitive aging (Ji et al., 2017; Ofori et al., 2019; Gullett et al., 2020). Other advanced neuroimaging techniques, such as Neurite Orientation Dispersion and Density Imaging, may also provide additional information about synaptic density, organization, and free water content, which may further elucidate neurobiological mechanisms driving cognitive dysfunction in aging (Raghavan et al., 2021).

Methodological Considerations

The current study relies on cross-sectional data for assessment of neural and cognitive differences at one time point, greatly limiting the ability to infer causality between observed relationships. Evaluating longitudinal changes in CRF, cognition, and white matter structure in older adults is warranted to better understand the dynamics of these relationships throughout both the healthy and pathological aging processes. It would additionally be important for future research to evaluate whether and how behavioral interventions can modify these relationships. At this time, I can also not rule out the possibility of unmeasured confounding factors that might contribute to the observed results. Intervention studies assessing change in CRF on cognition and in longitudinal studies with neuroimaging and cognitive measures over time would be important to extend these findings and to help determine causal mechanisms of the dynamics underlying changes in CRF, WMI, and cognition in healthy and pathological aging. Specifically, interventions targeting CRF would help clarify the extent to which CRF and its subsequent effects on brain and cognitive aging are modifiable and causally related.

Participant Characteristics

The present samples were obtained from a cohort of healthy community-dwelling older individuals, who were primarily non-Hispanic White, college educated adults with relatively low vascular risk burden. Given evidence of socioeconomic and racial/ethnic differences in brain outcomes in both healthy aging samples and cohorts with dementia (Ofori et al., 2024; Weiss et al., 2024), it will be important for future research to evaluate cohorts with a greater diversity across racial/ethnic groups, socioeconomic status, education levels, and health status to increase generalizability of findings. Expanding on this research by incorporating individuals with poor vascular health and cognitive impairment will provide additional insight into pathophysiological processes underlying neural and cognitive changes associated with CRF.

Conclusions

The current findings suggest: (1) elevated CRF was related to enhanced WMI in tracts connecting anterior to anterior, anterior to posterior, and subcortical to subcortical brain regions, which were further impacted by age and vascular risk burden; (2) these network patterns of CRF-related WMI may play a role in a white matter pathway of cognitive aging whereby CRF's relationship with aspects of WMI may attenuate detrimental effects of age and macrostructural lesion load on processing speed; and (3) CRF may attenuate a key marker of white matter neural degradation's relationship with worse processing and executive function in individuals with moderate and high levels, but not low levels of CRF. Altogether, these findings help to further characterize the relationship of CRF with regional and global metrics of white matter structural integrity and degradation as well as contribute to our understanding of CRF's potentially protective role in cognitive aging. This work may help spur new research efforts to better understand whether and how lifestyle interventions designed to enhance CRF can diminish the effects of brain and cognitive aging.

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APPENDIX A

Regional Network Covariance Patterns of White Matter Integrity Related to Cardiorespiratory Fitness in Healthy Aging

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Abstract

Cardiorespiratory fitness (CRF), measured by VO_2max, is an indicator of vascular functioning that can influence the integrity of brain microstructural white matter tracts in aging. How CRF is related to regional patterns of white matter bundles for magnetic resonance imaging (MRI) diffusion metrics (axial diffusivity, AD; radial diffusivity, RD; mean diffusivity, MD; fractional anisotropy, FA) has been less studied. We used a multivariate analysis method, the Scaled Subprofile Model (SSM), to identify network patterns of MRI tract-specific white matter integrity (WMI) for AD, RD, MD, and FA related to VO_2max and to evaluate their relation to demographic, vascular health, and dementia risk factors in 167 cognitively unimpaired older adults, ages 50 to 88. We identified four CRF-related regional patterns of WMI characterized by enhanced integrity in commissural pathways that connect areas within anterior brain regions (prefrontal body of the corpus callosum), connect subcortical regions to one another (fornix), and include selected association tracts (arcuate fasciculus, superior longitudinal fasciculus). Older age and greater white matter lesion load were each associated with reduced expression of all four CRF-WMI patterns, while high vascular risk level was associated with reduced expression of the RD, MD, and FA patterns. The regional patterns of RD and FA were most strongly associated with CRF. The results suggest that in healthy older adults, enhanced CRF is differentially associated with regional patterns of WMI, which are further impacted by age and macrostructural white matter lesion load. These findings support the use of the multivariate SSM in identifying regional patterns of white matter tracts that may provide markers of brain aging and cerebrovascular health.

Introduction

Advanced age is associated with greater incidence of cardiovascular disease (Rodgers et al., 2019). Maintaining vascular health in old age may help to reduce the effects of brain aging. Cardiovascular health conditions have been shown to negatively impact structural brain volumes and quality of life as well as increase the risk for dementia (Kivipelto et al., 2001; Viswanathan et al., 2009). Directly assessing cardiorespiratory fitness (CRF) by obtaining VO_2max (maximal oxygen consumption) through cardiopulmonary exercise testing can provide an important indicator of vascular health. Both genetic factors and modifiable lifestyle behaviors are significant contributors to the composition and trainability of CRF as well its role in healthy aging (Bouchard et al., 2015; Zadro et al., 2017; Sarzynski et al., 2022).

Aging in the absence of cognitive impairment or dementia (i.e., healthy aging) has been associated with differences in brain structure (Alexander et al., 2006; Raz and Rodrigue, 2006; Farokhian et al., 2017), including the integrity of white matter tracts. Magnetic resonance imaging (MRI) metrics of microstructural white matter integrity (WMI) derived from diffusion-weighted imaging have included measures of axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA) (Bennett et al., 2010; Burzynska et al., 2010). These have been suggested to reflect aspects of axonal loss and demyelination in the context of healthy aging (Lu et al., 2011; Salat, 2011; Soares et al., 2013).

In healthy older adults, greater VO_2max has been associated with enhanced microstructural integrity in tracts connecting regions in the frontal and parietal lobes (Colcombe et al., 2006; Gordon et al., 2008; Voss et al., 2013). Greater CRF levels have been related to enhanced FA and decreased RD (Johnson et al., 2012; Zhu et al., 2015), even in the absence of significant effects of physical activity levels on WMI metrics (Strömmer et al., 2020; d'Arbeloff et al., 2021). CRF and engagement in physical activity have also been shown to have distinct associations with aspects of structural and functional brain measures, including gray matter volume and thickness and functional connectivity

(Voss et al., 2016; Raichlen et al., 2020; Olivo et al., 2021), such that CRF has been associated with enhanced brain structure and function separate from physical activity effects. The associations have also involved white matter tracts connecting to anterior brain regions, including the genu of the corpus callosum, superior longitudinal fasciculus, inferior longitudinal fasciculus, and uncinate fasciculus (Johnson et al., 2012; Hayes et al., 2015; Oberlin et al., 2016; Ding et al., 2018). These studies have typically relied on univariate analytic methods that may limit the ability to fully characterize the regionally distributed associations of CRF with WMI in the context of healthy aging. Further research, using multivariate statistical methods that may be more sensitive in identifying patterns of tract-specific regional brain differences (Alexander and Moeller, 1994; Gazes et al., 2016; Geeraert et al., 2019; 2020), may help to better elucidate the relation of CRF to WMI in bundles preferentially vulnerable to aging. Such multivariate network analysis methods provide the opportunity to test regional WMI tract associations with CRF, without the need to control for multiple comparisons while having the opportunity to statistically adjust for related health and demographic characteristics. Given previous evidence of preferential alterations for selected white matter tracts with age, evaluating multivariate regional patterns of tract-specific metrics of integrity may help to better characterize the potential influence of CRF on regional WMI in the healthy aging population (Bennett and Madden, 2014; de Groot et al., 2015; Bender et al., 2016).

Clinical vascular health risk factors (e.g., hypertension, hyperlipidemia, smoking, and diabetes) increase the risk of cerebrovascular disease (CVD) and can adversely impact WMI (Fuhrman et al., 2019). Poor vascular health has also been shown to increase risk for developing Alzheimer’s disease (AD) (Meng et al., 2014; O’Brien and Markus, 2014). The common genetic risk factor for late-onset Alzheimer’s disease (AD), the apolipoprotein E (APOE) ε4 allele, has specifically been associated with increased risk of coronary heart disease and CVD, as well as detrimental effects on white matter (Kaprio et al., 1991; Fullerton et al., 2000; Raichlen and Alexander, 2014; Wang et al., 2015). Multiple cardiovascular risk factors often co-exist in older adults (Genest and Cohn, 1995). There is evidence that the contributions of these risk factors can be additive, such that the risk of cognitive impairment increases with each additional risk factor (Luchsinger et al., 2005). Moreover, having a greater number of cardiovascular risk factors has been associated with greater brain atrophy and poorer white matter health (Cox et al., 2019). Among older adults, the presence of multiple vascular risk factors has been associated with greater reductions in cerebral blood flow compared to those with one or no vascular risk factors and has been further associated with poorer cognitive performance (Bangen et al., 2014). White matter hyperintensities (WMH) on MRI, that reflect chronic small vessel disease, increase with both age and with vascular health risk factors and may be an indicator of disrupted WMI (Gunning-Dixon and Raz, 2000; Habes et al., 2016).

The present study used a multivariate network covariance analysis method, the Scaled Subprofile Model (SSM; Moeller et al., 1987; Alexander and Moeller, 1994) to identify regional patterns of WMI related to CRF, as measured by VO_2max, to evaluate which white matter bundles and which integrity metrics (i.e., FA, MD, RD, AD) may be most sensitive to differences in CRF in healthy aging. The multivariate SSM has been used in many structural neuroimaging studies to identify patterns of regional gray matter differences associated with age and multiple health factors (Alexander et al., 2006; 2008; 2012; 2020; Bergfield et al., 2010; Kern et al., 2017; Song et al., 2023; Van Etten et al., 2024). Differences in WMI using the SSM have been evaluated in relation to aging and cognitive function (Gazes et al., 2016), but this analytic method has yet to be directly applied to CRF with regional measures of WMI. We sought to apply multivariate SSM to directly investigate the relationship between CRF and regional tracts of WMI in the context of healthy aging. This study also sought to examine how 1) demographics (i.e., age and sex), 2) vascular health and dementia risk, including clinical vascular risk factors and APOE e4 carrier status, and 3) WMH lesion load were each associated with the identified regional SSM network patterns of CRF-related WMI. We hypothesized that greater integrity in white matter bundles connecting anterior and posterior brain regions, such as the genu of the corpus callosum and superior longitudinal fasciculus, would be related to elevated CRF. Further, we hypothesized that patterns of CRF-related WMI would be negatively impacted by poorer vascular health.

Method

Participants

Healthy adults (N =167; Age = 69.0 $\pm$ 10.4 years) ages 50 – 88, underwent cardiopulmonary exercise testing and brain MRI scans. The sample was cognitively unimpaired, as indicated by the Mini Mental Status Exam (MMSE = 28.98 $\pm$ 1.26; Folstein et al., 1975), and included 78 females (46.7%) and predominantly (93.4%) non-Hispanic white participants. To exclude significant neurological, medical, and psychiatric disorders, participants underwent an extensive medical screen, and a physical and neurological examination performed by a neurologist (GAH) who specializes in aging and dementia. Participants were excluded if they had a MMSE score less than 26 or a Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960) score greater than 9. The present participants comprise a subsample of a larger cohort of 210 older adults, including only those who were able to complete a treadmill test of CRF and diffusion-weighted MRI scans. Fourteen participants were excluded based on inability to complete VO_2max testing, 16 additional participants were excluded due to not undergoing neuroimaging, and 13 participants were excluded based on poor data quality for the MRI scans (described in Section 2.5.1). Sixty participants (35.9%) were determined to have high cardiovascular risk based on endorsement of two or more available risk factors: history of cardiac arrest, hypertension, high cholesterol, diabetes, and historical or current smoker status (Bangen et al., 2014; Boutzoukas et al., 2022; see Table 1). All procedures were approved by the Institutional Review Board at the University of Arizona and all participants provided informed written consent.

Exercise testing

Participants completed a treadmill graded exercise test at the Pulmonary Function Laboratory at the University of Arizona Medical Center (Tucson, AZ). Each treadmill session began at a low intensity with a speed of 1 mph and an incline grade of 0%. Speed and grade were gradually increased based on the modified Naughton treadmill protocol during exercise (Berry et al., 1996; Strzelczyk et al., 2001). Expiratory gases were measured using a metabolic cart and standard techniques of open-circuit spirometry and oxygen uptake was obtained using indirect calorimetry. Maximal oxygen consumption (VO_2max [ml/kg/min]) to assess CRF was considered achieved when two of the following three criteria were satisfied: (1) plateau in VO_2 with an increase in workload; (2) respiratory exchange ratio of 1:1 (VCO_2:VO_2) or higher; and (3) heart rate within ten beats of the age-predicted maximum. Termination criteria were consistent with the guidelines of the American College of Sports Medicine (Pescatello et al., 2014).

Genetic testing

APOE genotyping was performed at the Translational Genomics Research Institute (TGen, Phoenix, AZ, USA) from venous blood samples and was determined using restriction-fragment-length polymorphisms (RFLP), which has been described in previous studies (Van Etten et al., 2021; Song et al., 2023). Briefly, high molecular weight DNA were extracted, assayed, and then amplified using AmpliTaq Gold Fast PCR Master Mix (Applied Biosystems: Thermo Fisher Scientific, Waltham, MA). Samples were assessed for characteristic banding patterns of the six common APOE genotypes according to previously published methods (Addya et al., 1997). In the present cohort, there were 49 APOE ε4 carriers (29.3%) and 118 non-carriers (70.7%).

2.4 Image acquisition

T1-weighted Spoiled Gradient Echo (SPGR) MRI scans (slice thickness = 1.0mm, TR = 5.3ms, TE = 2.0ms, TI = 500ms, flip angle = 15°, matrix = 256x256, FOV = 25.6cm) and T2 Fluid-Attenuated Inversion Recovery (FLAIR) scans (slice thickness = 2.6mm, TR = 11000ms, TE = 120ms, TI = 2250ms, flip angle = 90°, matrix = 256x256, FOV = 25.0cm) were acquired on a 3T GE Signa scanner (HD Signa Excite, General Electric, Milwaukee, WI). Diffusion-weighted images (DWI) in 51 directions with 8 B0 images (b = 1000 s/mm2, slice thickness = 2.6mm, TR = 12500ms, TE = 70ms, flip angle = 90°, matrix = 128 x 128, FOV = 25cm, and 58 slices) were also acquired during the same MRI session.

1.5 Image processing

Diffusion tensor imaging

Pre-processing of diffusion MRI data involved eddy current correction (Smith et al., 2004) and EPI distortion correction using Brainsuite's INVERSION method (Bhushan et al., 2015). These images were processed using TRACULA (Yendiki et al., 2011; Maffei et al., 2021) for automated global probabilistic tractography of 42 white matter bundles using a diffusion model capable of modeling crossing fibers (Behrens et al., 2007) and extraction of standard diffusivity measures (AD, RD, MD, and FA). Briefly, this involved using the specific structural brain regions of interest created by the FreeSurfer processing stream (Fischl et al., 2002) in conjunction with a manually labeled training dataset of WM pathway priors to estimate the posterior probability distribution for the 42 WM tracts for each participant. Reconstructed tracts were visually inspected for quality of reconstruction and were re-processed, as needed. Participants were excluded if: 1) during visual inspection, any tract contained just one streamline or many incorrect streamlines, 2) more than one of the four diffusion metrics were extreme values (beyond 3 IQR) for a tract, or 3) any of the four diffusion metrics were extreme values (beyond 3 IQR) on more than one tract. Based on these parameters, 13 participants were excluded for poor DWI data quality. Estimates of total intracranial volume (TIV) were obtained in native brain space from each of the T1 images using Statistical Parametric Mapping (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK) by calculating the sum of the total gray matter, white matter, and cerebrospinal fluid segments (Alexander et al., 2012).

WMH volume

White matter hyperintensities (WMH) were computed using T1 and T2-FLAIR scans and the lesion segmentation toolbox (LST; Schmidt et al., 2012) with SPM12. WMH probability maps were generated with the multispectral lesion growth algorithm by LST in a subset of the sample at a range of values for the optimization parameter kappa and spatially compared with reference WMH maps to determine the optimal threshold (0.35) for the present cohort of cognitively unimpaired older adults (Franchetti et al., 2020; Van Etten et al., 2021). Voxel volumes in the WMH maps were summed to compute the total WMH volume in milliliters (ml) and the global values were log transformed for subsequent analyses (Franchetti et al., 2020; Van Etten et al., 2021).

Statistical analyses

Network covariance patterns

Regional SSM network analysis (Moeller et al., 1987; Alexander and Moeller, 1994; Habeck et al., 2008; Alexander et al., 2012) was performed using MATLAB (Math Works, Natick, Massachusetts, USA). First, diffusivity metrics for each white matter bundle underwent natural log transformation and mean values across regions and participants were subtracted at each tract. Next, a principal component analysis (PCA) was performed, producing a set of regional covariance pattern components and corresponding network subject scores, which reflected the degree to which each participant expressed the identified regional pattern. The Bayesian Information Criterion (BIC; Schwarz, 1978) was used as a model selection method to identify the best set of SSM components. This method was chosen as it accounts for sample size differences in selecting the best model, providing a conservative selection of components for identifying the covariance pattern that may provide for greater generalizability and reproducibility of the network pattern across samples (Dziak et al., 2020). A bootstrap re-sampling procedure (Efron and Tibshirani, 1994) with 10,000 iterations was applied in the SSM analysis (Gazes et al., 2016; Habeck et al., 2008; Alexander et al., 2012) to provide reliability estimates for the regional white matter integrity values for the observed pattern weights related to tract-specific diffusion metrics and VO_2max. The influence of TIV was subsequently assessed for each WMI SSM pattern using multiple regression to test the association of CRF-related WMI pattern expression with VO_2max after adjusting for TIV. Network analyses were also followed by univariate regression for the individual tracts in relation to VO_2max for the four WMI metrics to assess how each tract contributed to the CRF-related network patterns, with false discovery rate (FDR) correction for multiple comparisons (Benjamini and Hochberg, 1995).

Regressions with demographic and health factors

Block-wise multiple linear regression analyses in SPSS (v22, Chicago, IL, USA) were used to test how demographic and vascular health and dementia risk factors were associated with expression of each CRF-related WMI pattern. While adjusting for TIV in block one, age and sex were entered into block two to evaluate their association with CRF-related WMI pattern expression. Vascular risk level (0 or 1 vs. 2 or more clinical vascular risk factors) and APOE e4 status (e4 non-carrier vs. carrier) were then added in block three to evaluate their associations with expression of the SSM patterns. Global WMH volume was then subsequently added as the final covariate in block 4 to evaluate the impact of macrostructural white matter lesion load on the models.

Follow-up sensitivity analyses were conducted by adding individual vascular risk factors (i.e., history of cardiac arrest, hypertension, high cholesterol, diabetes, and historical or current smoker status) to the models in place of vascular risk level to 1) assess potentially differential contributions of individual risk factors to expression of the CRF-WMI patterns and 2) evaluate the potential unique additive effect of vascular risk factors on network patterns. Follow-up analyses were also performed with use of hypertensive medication status as a separate covariate to assess potential effects.

3 Results

3.1 Network covariance patterns

The SSM analyses with BIC model selection criteria identified the linear combination of components that best predicted CRF (i.e., VO_2max [ml/kg/min]) for each diffusion metric. The CRF-related AD pattern (lower AD indicates better tract integrity) included the first six components and accounted for 11.2% of the variance in CRF (β = .342, p = 6.00E-7) with higher expression of the network pattern associated with greater CRF. Bootstrap resampling of the linearly combined regional pattern was characterized by reductions of AD in the bilateral arcuate fasciculus, bilateral frontal aslant, and bilateral superior longitudinal fasciculus (SLF) 2 and 3 tracts, with relative increases in the left anterior thalamic radiation, right external capsule, left optic radiation, and bilateral uncinate fasciculus tracts (Figure 1). After adjustment for TIV, pattern expression remained significantly associated with CRF (β = .313, adjusted R^2 change = .094, p = 1.20E-5). Follow-up univariate analyses revealed that AD in bilateral arcuate fasciculus (β = -.334, p-FDR = 2.90E-5 for left; β = -.346, p-FDR = 1.60E-5 for right), bilateral frontal aslant (β = -.345, p-FDR = 1.60E-5 for left; β = -.337, p-FDR = 2.60E-5 for right), bilateral SLF 2 (β = -.328, p-FDR = 3.70E-5 for left; β = -.297, p-FDR = 1.76E-4 for right), bilateral SLF 3 (β = -.315, p-FDR = 7.30E-5 for left; β = -.350, p-FDR = 1.20E-5) were negatively associated with VO_2max, indicating greater WMI in these tracts with enhanced CRF. Tracts identified as relative increases in the SSM pattern all showed significant decreases univariately: left anterior thalamic radiation (β = -.252, p-FDR = .001), right external capsule (β = -.207, p-FDR = .008), left optic radiation (β = -.165, p-FDR = .033), and bilateral uncincate fasciculus (β = -.193, p-FDR = .013 for left; β = -.237, p-FDR = .003 for right), suggesting that these tracts reflect relatively less enhanced integrity with increasing CRF.

The CRF-related RD pattern included the first four components and accounted for 30.2% of the variance in VO_2max (β = .553, p = 8.88E-15) with higher expression of the network pattern related to greater CRF. This pattern was characterized by reductions in RD (lower RD indicates better tract integrity) in the prefrontal body of the corpus callosum with relative increases in the bilateral arcuate fasciculus, bilateral acoustic radiation, bilateral corticospinal tract (CST), left SLF 1, and bilateral SLF 3 bundles (Figure 2). After adjusting for TIV, pattern expression was significantly associated with CRF (β = .511, adjusted R^2 change = .253, p = 9.21E-14). Univariately, the prefrontal body of the corpus callosum was negatively associated with CRF (β = -.463, p-FDR = 2.96E-10). Tracts showing relative increases in the SSM pattern were also negatively associated with CRF, including the bilateral arcuate fasciculus (β = -.292, p-FDR = 2.00E-4 for left; β = -.307, p-FDR = 1.00E-4 for right), bilateral acoustic radiation (β = -.198, p-FDR = .011 for left; β = -.219, p-FDR = .005 for right), left SLF 1 (β = -.315, p-FDR = 7.30E-5), and bilateral SLF 3 (β = -.296, p-FDR = 1.79E-4 for left; β = -.384, p-FDR = 2.00E-6 for right). Bilateral CST was not significantly related to CRF (β = -.036, p-FDR = .641 for left; β = -.056, p-FDR = .473 for right). These results suggest that the relative reductions in the prefrontal body of the corpus callosum observed in the SSM RD pattern reflects greater integrity in relation to increasing CRF, whereas the tracts identified as relative increases in the SSM pattern reflect relatively less enhanced WMI with greater CRF.

The CRF-related MD pattern included the first six components and accounted for 18.2% of the variance in VO_2max (β = .432, p = 5.45E-9) with higher expression of the network pattern related to greater CRF. The pattern was characterized by reductions in MD (lower values indicate better tract integrity) in the prefrontal body of the corpus callosum, the genu of the corpus callosum, bilateral frontal aslant, and left fornix bundles with relative increases in the bilateral acoustic radiation, bilateral CST, bilateral optic radiation, and middle cerebellar peduncle (Figure 3). After TIV adjustment, pattern expression was significantly associated with CRF (β = .412, adjusted R^2 change = .166, p = 4.05E-9). Follow-up univariate analyses revealed that MD in the prefrontal body of the corpus callosum (β = -.446, p-FDR = 1.51E-9), genu of the corpus callosum (β = -.368, p-FDR = 5.00E-6), bilateral frontal aslant (β = -.371, p-FDR = 5.00E-6 for left; β = -.369, p-FDR = 5.00E-6 for right), and left fornix (β = -.292, p-FDR = 2.00E-4) were significantly negatively associated with VO_2max. MD in tracts identified as relative increases in the SSM pattern showed significant univariate decreases with CRF, including the bilateral acoustic radiation (β = -.231, p-FDR = .004 for left; β = -.251, p-FDR = .001 for right), bilateral optic radiation (β = -.275, p-FDR = 4.67E-4 for left; β = -.313, p-FDR = 7.80E-5 for right), and middle cerebellar peduncle (β = -.250, p-FDR = .001). MD values in bilateral CST were not significantly associated with VO_2max (β = -.122, p-FDR = .118 for left; β = -.119, p-FDR = .126 for right). White matter bundles identified as relative decreases in the SSM MD pattern reflect regions with greater integrity with increasing CRF, while those identified as relative increases reflect tracts with relatively less enhanced integrity with greater CRF.

The CRF-related FA pattern included the first eight components and accounted for 25.7% of the variance in CRF (β = .512, p = 1.60E-12) with higher expression of the network pattern related to greater fitness. The pattern was characterized by relative reductions in FA (higher FA indicates better tract integrity) in the right arcuate fasciculus, right acoustic radiation, bilateral CST, and right middle longitudinal fasciculus (MLF) with increases in the prefrontal body of the corpus callosum and right fornix bundles (Figure 4). After adjustment for TIV, pattern expression remained significantly associated with CRF (β = .478, adjusted R^2 change = .223, p = 3.94E-12). Follow-up univariate analyses revealed that FA in the prefrontal body of the corpus callosum (β = .346, p-FDR = 1.51E-9) and right fornix (β = .351, p-FDR = 1.20E-5) were significantly positively associated with VO_2max. No FA values in tracts identified in the pattern as relative decreases were significantly associated with VO_2max univariately, including right arcuate fasciculus (β = .041, p-FDR = .599), right acoustic radiation (β = -.012, p-FDR = .876), bilateral CST (β = -.110, p-FDR = .155 for left; β = -.046, p-FDR = .554 for right), and right MLF (β = .042, p-FDR = .593). These results suggest that the observed relative increases in the SSM FA pattern in the prefrontal body of the corpus callosum and right fornix reflect greater WMI with increasing CRF, whereas those areas identified as relative FA decreases in the pattern reflect relatively less enhanced tract integrity with increasing CRF.

Overall, the network covariance patterns consistently identified several white matter bundles that demonstrated greater tract integrity with greater VO_2max values across multiple WMI diffusion metrics, including the prefrontal body of the corpus callosum, genu of the corpus callosum, bilateral fornix, bilateral arcuate fasciculus, and bilateral frontal aslant. The SSM patterns also identified covarying white matter bundles that are relatively less associated with greater CRF, indicating relatively less enhanced integrity with increasing levels of VO_2max, including the bilateral CST, bilateral acoustic radiation, and bilateral uncinate fasciculus.

3.2 Demographics and vascular health risk factors

Expression of each CRF-related WMI network pattern was then tested with multiple linear regression for associations with TIV (block 1), age and sex (block 2), vascular risk level and APOE ε4 status (block 3), and global WMH volume (block 4). Full results for each pattern are shown in Tables 2 (AD pattern results), 3 (RD pattern results), 4 (MD pattern results), and 5 (FA pattern results). Only CRF-related RD pattern expression was significantly associated with TIV in the first models (AD: $\beta = .093$, $p = .231$; RD: $\beta = .163$, $p = .035$; MD: $\beta = .066$, $p = .396$; FA: $\beta = .117$, $p = .131$). After adding age and sex to the models, age was significantly inversely related for all SSM patterns (AD: $\beta = -.390$, $p = 5.02\text{E-}7$; RD: $\beta = -.547$, $p = 1.00\text{E-}13$; MD: $\beta = -.470$, $p = 8.38\text{E-}10$; FA: $\beta = -.628$, $p = 2.93\text{E-}18$). Neither TIV (AD: $\beta = -.084$, $p = .379$; RD: $\beta = .052$, $p = .546$; MD: $\beta = -.078$, $p = .397$; FA: $\beta = -.073$, $p = .369$) nor sex (AD: $\beta = .182$, $p = .055$; RD: $\beta = .035$, $p = .679$; MD: $\beta = .108$, $p = .235$; FA: $\beta = .142$, $p = .080$) were significant predictors for any patterns in model 2.

After subsequently adding vascular risk and APOE ε4 status to the models, we found that vascular risk level was inversely associated with expression of the RD and FA patterns (AD: $\beta = -.058$, $p = .434$; RD: $\beta = -.132$, $p = .048$; MD: $\beta = -.140$, $p = .051$; FA: $\beta = -.161$, $p = .010$) while APOE ε4 status did not significantly contribute predictive value for any patterns (AD: $\beta = -.019$, $p = .797$; RD: $\beta = .018$, $p = .786$; MD: $\beta = -,012$, $p = .863$; FA: $\beta = -.007$, $p = .915$). In these models, age remained significant across all patterns (AD: $\beta = -.385$, $p = 8.78\text{E-}7$; RD: $\beta = -.533$, $p = 3.39\text{E-}13$; MD: $\beta = -.456$, $p = 2.27\text{E-}9$; FA: $\beta = -.612$, $p = 8.82\text{E-}18$), sex emerged as a significant predictor for AD and FA patterns with greater pattern expression associated with male sex (AD: $\beta = .193$, $p = .045$; RD: $\beta = .060$, $p = .484$; MD: $\beta = .135$, $p = .143$; FA: $\beta = .172$, $p = .033$), and TIV was not significant for all regional diffusion patterns (AD: $\beta = -.095$, $p = .326$; RD: $\beta = .035$, $p = .686$; MD: $\beta = -.100$, $p = .279$; FA: $\beta = -.098$, $p = .229$).

Finally, adding global WMH volume to the models to assess the association of macrostructural WMI with expression of the CRF-related WMI patterns revealed that WMH volume was significantly negatively associated with SSM participant scores across all diffusion patterns (AD: β = -.260, p = .005; RD: β = -.285, p = 5.99E-4; MD: β = -.191, p = .034; FA: β = -.165, p = .036). In these final models, TIV remained non-significant across all patterns (AD: β = -.031, p = .752; RD: β = .105, p = .221; MD: β = -.053, p = .572; FA: β = -.057, p = .489), age remained a significant predictor across all patterns (AD: β = -.221, p = .020; RD: β = -.353, p = 3.30E-5; MD: β = -.336, p = 2.91E-4; FA: β = -.508, p = 1.81E-9), sex remained a significant predictor in AD and FA pattern models (AD: β = .199, p = .035; RD: β = .066, p = .423; MD: β = .139, p = .126; FA: β = .176, p = .028), vascular risk level emerged as a significant predictor for expression of the MD pattern and remained a significant predictor for the RD and FA patterns (AD: β = -064, p = .377; RD: β = -.139, p = .032; MD: β = -.144, p = .041; FA: β = -.165, p = .008), and APOE ε4 status remained non-significant across all patterns (AD: β = -.007, p = .919; RD: β = .030, p = .632; MD: β = -.004, p = .958; FA: β = .001, p = .990). In follow-up sensitivity analyses, no individual vascular risk factors (p's > .05) or use of hypertensive medication (p > .05) was significantly associated with CRF-WMI pattern expression.

4 Discussion

The present study used a multivariate network covariance approach to identify regional patterns of tract-specific WMI associated with CRF in a healthy aging cohort. Across the identified regional patterns of diffusion WMI metrics, including for AD, RD, MD, and FA, greater VO_2max was associated with enhanced integrity in white matter bundles primarily connecting anterior portions of the brain as well as in wider association areas. Multivariate patterns of MD and FA additionally identified white matter bundles connecting subcortical brain regions related to greater CRF. These results extend previous studies, which have identified both frontal (Gordon et al., 2008; Voss et al., 2013) and subcortical associations of CRF with WMI in tracts vulnerable to cognitive aging (Davis et al., 2009; Burgmans et al., 2010; Meier et al., 2012; Tian et al., 2014).

Specifically, relative reductions of AD were observed in the bilateral arcuate fasciculus (connecting temporal gyri and frontal regions), bilateral frontal aslant (connecting posterior inferior frontal gyrus, pars opercularis, and medial aspects of superior frontal gyrus), bilateral (SLF) 2 (connecting caudal middle frontal gyrus and inferior parietal lobe), and bilateral SLF 3 tracts (connecting posterior inferior frontal gyrus and anterior supramarginal gyrus) indicating better integrity with increased CRF. The pattern also identified relative increases, which reflect comparatively less decreases relative to greater CRF based on univariate follow-up analyses, in the left anterior thalamic radiation (connecting thalamus and prefrontal cortex), right external capsule (connecting putamen and claustrum), left optic radiation (connecting thalamus and occipital cortex), and bilateral uncinate fasciculus tracts (connecting anterior temporal pole and anterior middle temporal gyrus with medial and orbital prefrontal cortex). While previous studies have not always found CRF associations with AD (Johnson et al., 2012; Voss et al., 2013), the results are consistent with other studies that have demonstrated a relationship between AD and CRF in SLF 1 (Ding et al., 2018). AD, a measurement of diffusivity that extends parallel to WM tracts, is generally thought to reflect axonal integrity (Concha et al., 2006). Disruptions to AD may additionally be influenced by macrostructural effects, such as the formation of WMH, due to disrupted diffusion of water molecules (Bennett et al., 2010; Salat 2011). This was shown in the follow-up regression analyses in the present study where expression of the CRF-AD pattern was significantly negatively associated with global WMH volume while accounting for age, sex, and cardiovascular and dementia risk factors (i.e., vascular risk level, APOE status). These findings additionally indicate that multivariate statistical methods, like the SSM, may be able to detect subtle regional differences in tract-specific WMI related to CRF that univariate methods may not.

The CRF-RD pattern revealed relative reductions in the prefrontal body of the corpus callosum (terminating in the Freesurfer subdivision of the rostral middle frontal region) with relative increases, which again appeared as comparatively less decreases, in the bilateral arcuate fasciculus. (connecting temporal gyri and frontal regions), bilateral acoustic radiation (connecting posterior thalamus and transverse temporal gyrus of Heschl in superior temporal gyrus), bilateral CST (connecting spinal tract to pre- and postcentral gyri as well as supplementary motor area), left SLF 1 (connecting superior frontal gyrus and WM posterior to the posterior central gyrus and dorsal to the cingulate sulcus), and bilateral SLF 3 bundles (connecting posterior inferior frontal gyrus and anterior supramarginal gyrus). These results are consistent with previous work that found lower RD associated with greater CRF levels in the corpus callosum and pre-motor areas (Johnson et al., 2012, Tarumi et al., 2022). RD is commonly thought to reflect myelin integrity through measurement of diffusion perpendicular to WM tracts (Song et al., 2005). Thus, the CRF-RD pattern may indicate WM bundles with enhanced or preserved myelination related to greater CRF in addition to bundles with preferentially less reductions in diffusivity. Additionally, expression of the pattern was significantly associated with age, vascular risk level, and global WMH volume, such that greater pattern expression was related to younger age, lower vascular risk, and lower overall WMH lesion load. Compared to the CRF-AD pattern, the CRF-RD pattern was more strongly associated with cardiovascular health in this healthy older adult cohort.

The CRF-MD pattern was characterized by reductions in the prefrontal body of the corpus callosum (terminating in the Freesurfer subdivision of the rostral middle frontal region), the genu of the corpus callosum (mid-anterior corpus callosum frontal projections), bilateral frontal aslant (connecting posterior inferior frontal gyrus, pars opercularis, and medial aspects of superior frontal gyrus), and left fornix (connecting the hippocampal formation [CA1, CA3, and fimbria] with anterior thalamic nuclei, mammillary bodies, medial septal nucleus, and basal forebrain) with relative increases in the bilateral acoustic radiation (connecting posterior thalamus and transverse temporal gyrus of Heschl in superior temporal gyrus), bilateral CST, (connecting spinal tract to pre- and postcentral gyri as well as supplementary motor area), bilateral optic radiation (connecting thalamus and occipital cortex), and middle cerebellar peduncle (fibers from cerebral hemispheres relay data through the contralateral middle cerebellar peduncles, to reach the opposite cerebellar hemisphere terminating in the frontal, parietal, occipital, and temporal lobes). MD represents a combination of AD and RD, reflecting the average rate (Madden et al., 2009; Bennett and Madden, 2014), magnitude (Sala et al., 2012), and motility of water diffusion, independent of the directionality (Sullivan and Pfefferbaum, 2006). The CRF-MD pattern displayed significant overlap with the AD and RD patterns but also uniquely detected CRF associations in the fornix connecting subcortical regions. Similarly to the CRF-RD pattern, expression of the CRF-MD was significantly associated with younger age, lower cardiovascular risk levels, and lower global WMH volume.

Finally, unlike AD, RD, and MD, higher values of FA indicate better directional tract integrity. The CRF-FA pattern was characterized by increases in the prefrontal body of the corpus callosum (terminating in the Freesurfer subdivision of the rostral middle frontal region) and right fornix (connecting hippocampal formation [CA1, CA3, and fimbria] with anterior thalamic nuclei, mammillary bodies, medial septal nucleus, and basal forebrain). The pattern also identified relative reductions, i.e., tracts with comparatively less increases in FA, in the right arcuate fasciculus (connecting temporal gyri and frontal regions), right acoustic radiation, (connecting posterior thalamus and transverse temporal gyrus of Heschl in superior temporal gyrus), bilateral CST, (connecting spinal tract to pre- and postcentral gyri as well as supplementary motor area) and right MLF (connecting superior and middle anterior temporal gyri and temporal pole with superior and inferior parietal cortex). Although FA is sensitive to directional white matter microstructural differences, this metric lacks specificity for the type of white matter alterations that may be present (Alexander et al., 2011). These findings are consistent with previous studies showing that CRF levels have been related to enhanced FA in tracts connecting anterior brain regions (Johnson et al., 2012; Zhu et al., 2015). The pattern also suggests a potential hemispheric difference in FA for associations with CRF in the right hemisphere. Greater expression of this pattern was significantly associated with younger age, male sex, lower vascular health risk, and reduced macrostructural white matter lesion load.

While the patterns differed for some identified tracts, several were shared across diffusion metric patterns, including the prefrontal body of the corpus callosum, fornix, and superior longitudinal fasciculus, suggesting these bundles may be especially sensitive to CRF-related differences across several MRI diffusion measures of WMI. Results additionally suggest that RD and FA may be most sensitive to differences in the integrity of white matter related to risk factors for cardiovascular health, as these patterns were most strongly associated with both VO_2max and vascular risk level in this cohort of healthy older adults. This is consistent with previous studies that have found significant associations with CRF in both RD and FA in the absence of AD and MD relationships (Johnson et al., 2012). Together, these findings support network regional covariance patterns of brain white matter tracts sensitive to CRF differences in healthy aging. Moreover, these patterns may represent useful biomarkers of cardiovascular health in older age.

Previous literature has been mixed on the extent to which CRF impacts white matter in healthy aging, as well as which bundles are sensitive to differences in cardiovascular health. Multivariate statistical approaches have been useful for detecting local, tract-specific associations of aging and CRF while reducing the need for multiple comparisons and accounting for shared variance between closely related white matter tracts (de Santis et al., 2014a; Geeraert et al., 2019; 2020). The present results indicate that greater CRF was mainly associated with preferential preservation of WMI in the prefrontal body and genu of the corpus callosum, arcuate fasciculus, superior longitudinal fasciculus, frontal aslant tract, and fornix, suggesting that these bundles are sensitive to CRF differences in a cognitively unimpaired, generally healthy aging sample. Follow-up regression analyses revealed that all CRF-related WMI patterns were significantly associated with age, with increasing age related to reduced expression of the patterns, and each reflecting greater WMI with increasing CRF. Additionally, reduced expression of the RD, MD, and FA patterns was related to vascular risk level, such that those with at least two clinical vascular risk factors displayed less preservation of the CRF-related WMI above and beyond effects of age, sex, and TIV. Notably, greater expression of the AD and FA patterns in males was observed after adjusting for other covariates. Future research is warranted for evaluation of potential sex differences in WMI related to CRF. Although previous work has shown detrimental effects of APOE ε4 on WMI (Heise et al., 2024), none of the present CRF-WMI patterns were significantly associated with APOE ε4 status in our generally healthy cognitively unimpaired cohort. These results provide support for multiple clinical vascular risk factors associated with added risk for brain aging. It has been hypothesized that CRF may attenuate age-related cognitive decline via increased delivery and upregulation of neurotrophins and other supporting factors in brain regions particularly vulnerable to demyelination in old age (Stimpson et al., 2018). Other potential mechanisms include increased cerebral perfusion, synaptogenesis, and angiogenesis (Maass et al., 2015; Tsai et al., 2016; Stimpson et al., 2018). CVD and associated risk factors may interfere with these processes. Thus, high CRF may help attenuate age-related effects on myelin and subsequently associated cognitive functions. Follow-up research studies are needed to determine the impact of CRF on white matter and associated aspects of cognition. Longitudinal data would be especially important to better understand the relationship between CRF and WMI over time and the extent to which this relationship can be modified by different lifestyle factors (e.g., diet, exercise).

Global WMH volume was a significant predictor of all four SSM CRF-related patterns while we controlled for age, sex, vascular health, and APOE e4 carrier status. These findings suggest that chronic small vessel disease may further disrupt WMI sensitive to CRF in older age, distinct from the accumulation of common cardiovascular health risk factors. Although the exact mechanisms remain unclear, it has been suggested that WMH can disrupt local and distal WMI by alterations in water mobility in the interstitial space (Wardlaw et al., 2013), increased blood-brain barrier permeability (Farrall and Wardlaw, 2009), and/or Wallerian degeneration (Pierpaoli et al., 2001).

The present study has several limitations. First, the sample was largely homogenous with primarily non-Hispanic white, highly educated participants with relatively low cardiovascular health burden compared to the general population of older adults. Given evidence of racial/ethnic and socioeconomic disparities in WMI outcomes (Weiss et al., 2024), further research with more diverse samples in dimensions of race/ethnicity, education, socioeconomic status, and cardiovascular health would be beneficial to further evaluate the generalizability of our findings. Additionally, the present results rely on CRF and brain metrics from one time point, limiting our ability to assess causal inference. Future research would benefit from evaluating longitudinal changes in CRF and WMI in older adults to better understand the dynamics of these relationships throughout both the healthy and pathological aging process. It would be important for future studies to evaluate whether and how lifestyle behavioral interventions can help to modify these dynamics, as well as the extent to which they are associated with cognitive and clinical outcomes. Finally, further research should utilize additional neuroimaging measures with increased specificity to alterations in microstructural white matter characteristics (e.g., axonal density) to enhance precision of WMI measurement and to further clarify specific mechanisms in the associations between CRF and WMI in aging (de Santis et al., 2014b).

5 Conclusion

The present study used a multivariate covariance approach to identify regional network patterns of tract-specific WMI for brain diffusion measures of AD, RD, MD, and FA related to CRF in cognitively unimpaired older adults. The resulting patterns were characterized by enhanced WMI in relation to greater CRF across all four diffusion metrics, involving white matter bundles mainly connecting anterior brain regions as well as wider association tracts. Greater expression of these patterns was also strongly and consistently associated with younger age and less macrostructural white matter lesion load; and higher expression of several network patterns (i.e., RD, MD, FA) were related to lower levels of vascular risk, suggesting differential associations of WMI diffusion metrics with cardiovascular health. Together, these results highlight unique contributions from the presence of multiple vascular risk factors and age on brain WMI in healthy older adults. Finally, our analyses support the use of multivariate network analyses, like SSM, with MRI diffusion metrics of localized white matter tracts as a potential marker of lifestyle influences on brain aging that can help to assess relationships between CRF, WMI, age, and cardiovascular health in the context of healthy aging.

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

SS: Conceptualization, Formal analysis, Visualization, Writing – original draft. PB: Formal analysis, Visualization, Writing – review and editing. DR: Data curation, Writing – review and editing. MG: Data curation, Writing – review and editing. JAH: Data curation, Writing – review and editing. GH: Data curation, Writing – review and editing. MH: Data curation, Writing – review and editing. TT: Data curation, Writing – review and editing. GA: Data curation, Formal analysis, Funding acquisition, Project administration, Writing – review and editing.

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10 Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Appendix A: Figure 1

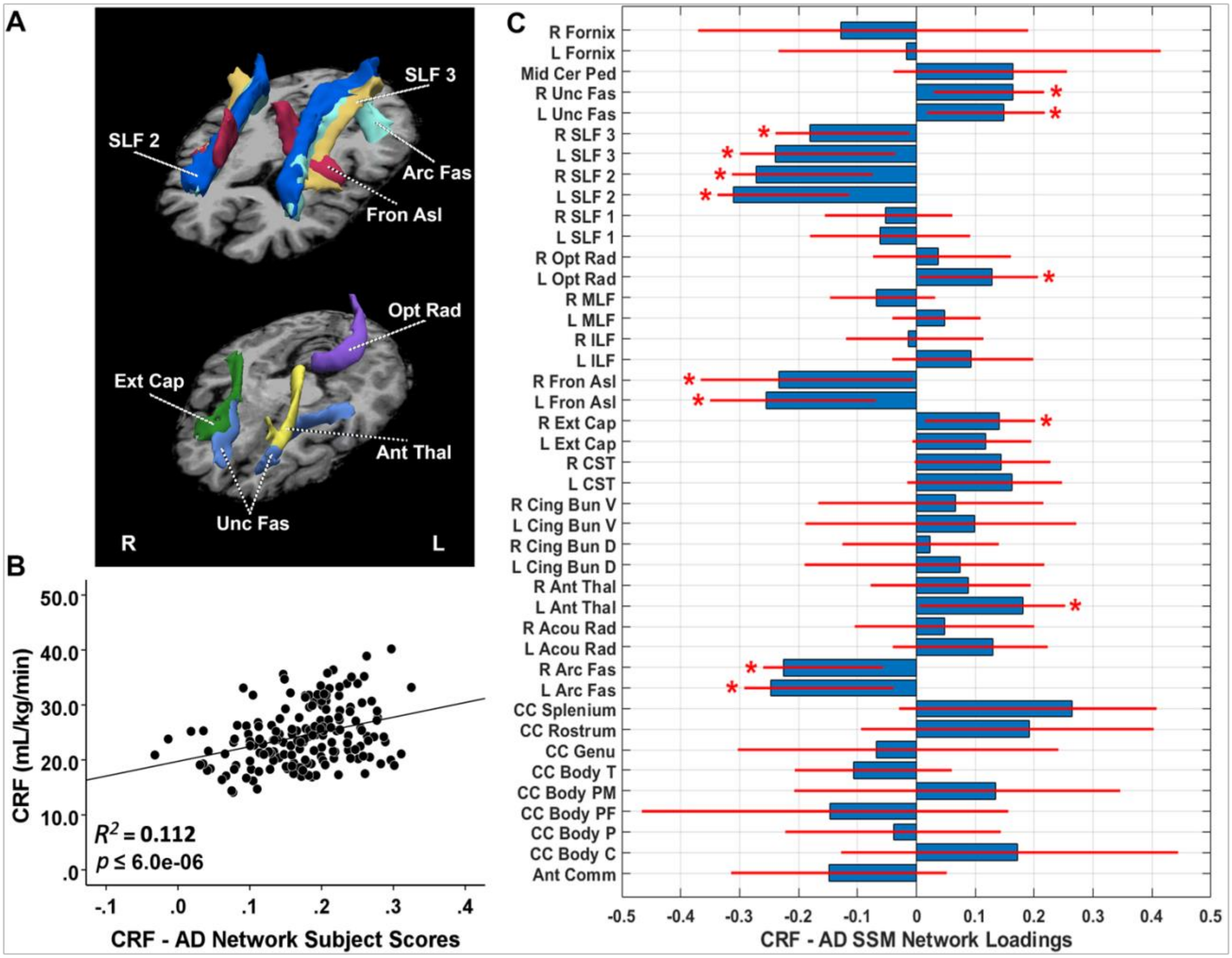

Figure 1. **(A)** White matter tracts identified as significant loadings in the cardiorespiratory fitness (CRF)-related axial diffusivity (AD) network pattern. **(B)** CRF-AD network subject scores and CRF. The subject scores of the CRF-AD network pattern derived from the first six SSM components. The scatterplots show that greater CRF was associated with higher expression of the network pattern. Adjusted R^2 and p values are displayed. **(C)** CRF-related tract-specific AD loadings for the SSM network pattern of AD. Blue bars indicate point estimates for the loadings and red lines indicate the 95% confidence intervals. Asterisks reflect significant ROIs contributing to the covariance pattern. SSM, Scaled Subprofile Model; R, right; L, left; Mid Cer Ped, middle cerebellar peduncle; Unc Fas, uncinate fasciculus; SLF, superior longitudinal

fasciculus; Opt Rad, optic radiation; MLF, middle longitudinal fasciculus; ILF, inferior longitudinal fasciculus; Fron Asl, frontal aslant; Ext Cap, external capsule; CST, corticospinal tract; Cing Bun V, cingulum bundle ventral; Cing Bun D, cingulum bundle dorsal; Ant Thal, anterior thalamic radiation; Acou Rad, acoustic radiation; Arc Fas, arcuate fasciculus; CC, corpus callosum; Body T, temporal body; Body PM, premotor body; Body PF, prefrontal body; Body P, body parietal; Body C, body central; Ant Comm, anterior commissure.

Appendix A: Figure 2

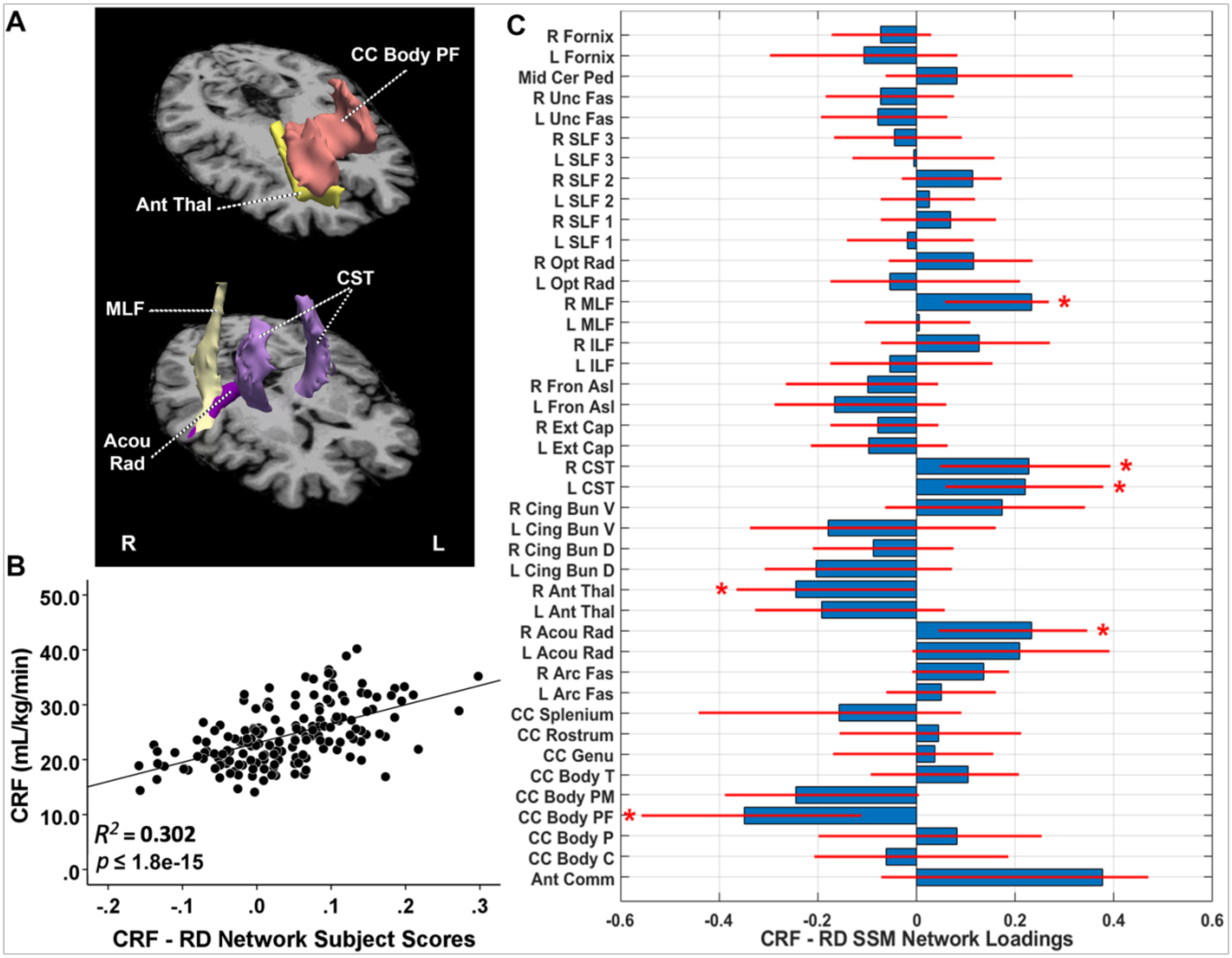

Figure 2. **(A)** White matter tracts identified as significant loadings in the cardiorespiratory fitness (CRF)-related radial diffusivity (RD) network pattern. **(B)** CRF-RD network subject scores and CRF. The subject scores of the CRF-RD network pattern were derived from the first four SSM components. The scatterplots show that greater CRF was associated with higher expression of the network pattern. Adjusted R^2 and p values are displayed. **(C)** CRF-related tract-specific RD loadings for the SSM network pattern of RD. Blue bars indicate point estimates for the loadings and red lines indicate the 95% confidence intervals. Asterisks reflect significant ROIs contributing to the covariance pattern. SSM, Scaled Subprofile Model; R, right; L, left; Mid Cer Ped, middle cerebellar peduncle; Unc Fas, uncinate fasciculus; SLF, superior longitudinal

fasciculus; Opt Rad, optic radiation; MLF, middle longitudinal fasciculus; ILF, inferior longitudinal fasciculus; Fron Asl, frontal aslant; Ext Cap, external capsule; CST, corticospinal tract; Cing Bun V, cingulum bundle ventral; Cing Bun D, cingulum bundle dorsal; Ant Thal, anterior thalamic radiation; Acou Rad, acoustic radiation; Arc Fas, arcuate fasciculus; CC, corpus callosum; Body T, temporal body; Body PM, premotor body; Body PF, prefrontal body; Body P, body parietal; Body C, body central; Ant Comm, anterior commissure.

Appendix A: Figure 3

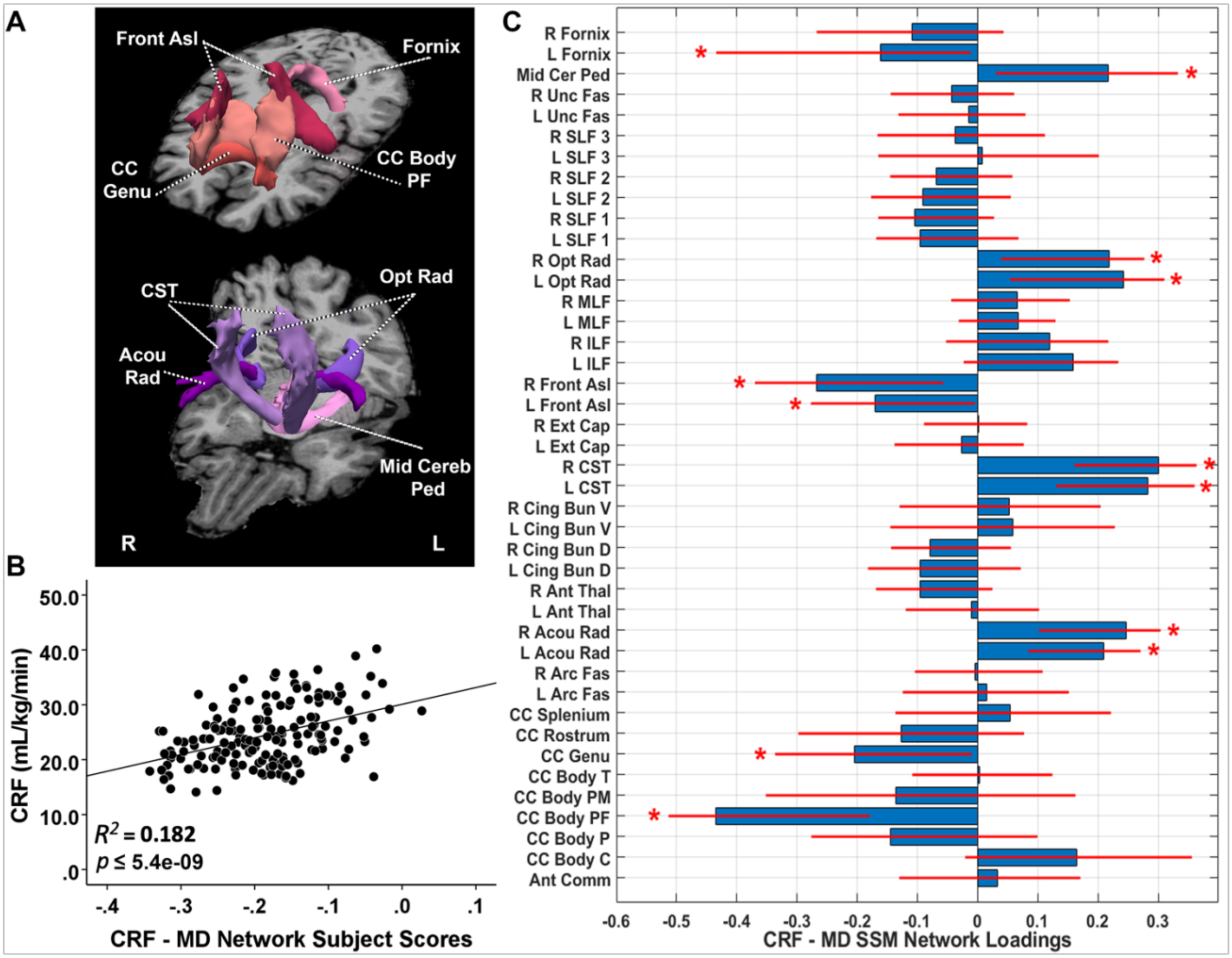

Figure 3. **(A)** White matter tracts identified as significant loadings in the cardiorespiratory fitness (CRF)-related mean diffusivity (MD) network pattern. **(B)** CRF-MD network subject scores and CRF. The subject scores of the CRF-MD network pattern were derived from the first six SSM components. The scatterplots show that greater CRF was associated with higher expression of the network pattern. Adjusted R^2 and p values are displayed. **(C)** CRF-related tract-specific MD loadings for the SSM network pattern of RD. Blue bars indicate point estimates for the loadings and red lines indicate the 95% confidence intervals. Asterisks reflect significant ROIs contributing to the covariance pattern. SSM, Scaled Subprofile Model; R, right; L, left; Mid Cer Ped, middle cerebellar peduncle; Unc Fas, uncinate fasciculus; SLF, superior longitudinal

fasciculus; Opt Rad, optic radiation; MLF, middle longitudinal fasciculus; ILF, inferior longitudinal fasciculus; Fron Asl, frontal aslant; Ext Cap, external capsule; CST, corticospinal tract; Cing Bun V, cingulum bundle ventral; Cing Bun D, cingulum bundle dorsal; Ant Thal, anterior thalamic radiation; Acou Rad, acoustic radiation; Arc Fas, arcuate fasciculus; CC, corpus callosum; Body T, temporal body; Body PM, premotor body; Body PF, prefrontal body; Body P, body parietal; Body C, body central; Ant Comm, anterior commissure.

Appendix A: Figure 4

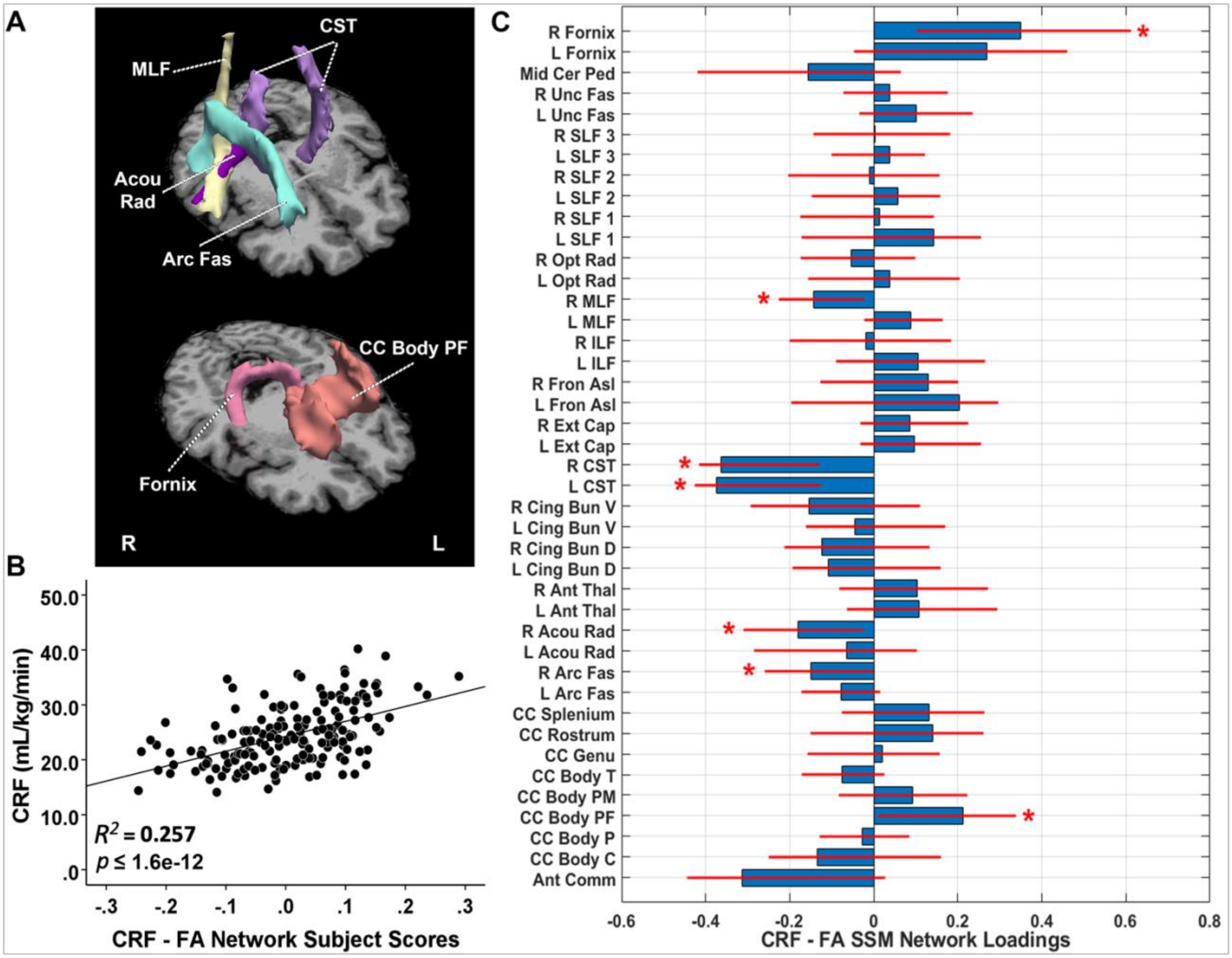

Figure 4. **(A)** White matter tracts identified as significant loadings in the cardiorespiratory fitness (CRF)-related fractional anisotropy (FA) network pattern. **(B)** CRF-FA network subject scores and CRF. The subject scores of the CRF-FA network pattern were derived from the first eight SSM components. The scatterplots show that greater CRF was associated with higher expression of the network pattern. Adjusted R^2 and p values are displayed. **(C)** CRF-related tract-specific FA loadings for the SSM network pattern of RD. Blue bars indicate point estimates for the loadings and red lines indicate the 95% confidence intervals. Asterisks reflect significant ROIs contributing to the covariance pattern. SSM, Scaled Subprofile Model; R, right; L, left; Mid Cer Ped, middle cerebellar peduncle; Unc Fas, uncinate fasciculus; SLF, superior longitudinal

fasciculus; Opt Rad, optic radiation; MLF, middle longitudinal fasciculus; ILF, inferior longitudinal fasciculus; Fron Asl, frontal aslant; Ext Cap, external capsule; CST, corticospinal tract; Cing Bun V, cingulum bundle ventral; Cing Bun D, cingulum bundle dorsal; Ant Thal, anterior thalamic radiation; Acou Rad, acoustic radiation; Arc Fas, arcuate fasciculus; CC, corpus callosum; Body T, temporal body; Body PM, premotor body; Body PF, prefrontal body; Body P, body parietal; Body C, body central; Ant Comm, anterior commissure.

Appendix A: Table 1: Participant Characteristics

N	167
Age [years; M (SD)]	68.99 (10.41)
Education [years; M (SD)]	15.76 (2.58)
Sex (F/M)	78/89
MMSE [M (SD)]	28.98 (1.26)
VO_2max [ml/kg/min; M (SD)]	24.43 (5.40)
Cardiac arrest (Y/N)	5/161
Hypertension (Y/N)	55/112
Hyperlipidemia (Y/N)	73/94
Diabetes (Y/N)	8/159
Smoking history (Y/N)	66/101
Vascular risk level (H/L)	60/107
APOE ε4 Status (Y/N)	49/118

Table 1. Means (standard deviations) for the sample. F/M, Female/Male; Y/N, Yes/No; MMSE, Mini Mental Status Exam; APOE, apolipoprotein E ε4; H/L, High/Low, High = two or more vascular risk factors, Low = less than two vascular risk factors.

Appendix A: Table 2. Summary of multiple regression analyses for variables predicting the CRF-AD network pattern

Variable	Model 1				Model 2				Model 3				Model 4			
	β	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*
TIV	.093	.066	.055	.231	-.084	-.059	.067	.379	-.095	-.067	.068	.326	-.031	-.022	.069	.752
Age					**-.390**	**-.003**	**.000**	**5.02E-7**	**-.385**	**-.003**	**.000**	**8.78E-7**	**-.221**	**-.001**	**.001**	**.020**
Sex					.182	.025	.013	.055	**.193**	**.027**	**.013**	**.045**	**.199**	**.027**	**.013**	**.035**
Vascular risk level									-.058	-.008	.011	.434	-.064	-.009	.010	.377
APOE									-.019	-.003	.011	.797	-.007	-.001	.011	.919
WMH													**-.260**	**-.041**	**.015**	**.005**
Adjusted R^2	.003				.139				.133				.169			
F for R^2	1.44			.231	**9.97**			**5.00E-6**	**6.08**			**3.50E-5**	**6.61**			**3.00E-6**

β represents the standardized coefficient and B the unstandardized coefficient. SE indicates t and standard error of natural log-transformed B. Sex, vascular risk level, and APOE ε4 status were dummy coded 1 for males, high vascular risk level, and APOE ε4 carriers, respectively. Bolded variables are significant (*p* < .05).

Key: APOE, apolipoprotein E; TIV, total intracranial volume; WMH, white matter hyperintensity volume.

Appendix A: Table 3. Summary of multiple regression analyses for variables predicting the CRF-RD network pattern

Variable	Model 1				Model 2				Model 3				Model 4			
	β	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*
TIV	**.163**	**.142**	**.067**	**.035**	.052	.045	.075	.546	.035	.031	.075	.686	.105	.092	.075	.221
Age					**-.547**	**-.004**	**.001**	**1.00E-13**	**-.533**	**-.004**	**.001**	**3.39E-13**	**-.353**	**-.003**	**.001**	**3.30E-5**
Sex					.035	.006	.014	.679	.060	.010	.015	.484	.066	.011	.014	.423
Vascular risk level									**-.132**	**-.023**	**.012**	**.048**	**-.139**	**-.025**	**.011**	**.032**
APOE									.018	.003	.012	.786	.030	.006	.012	.632
WMH													**-.285**	**-.055**	**.016**	**5.99E-4**
Adjusted R^2	.021				.300				.308				.353			
F for R^2	**4.51**			**.035**	**24.7**			**3.28E-13**	**15.8**			**1.25E-12**	**16.1**			**1.87E-14**

β represents the standardized coefficient and B the unstandardized coefficient. SE indicates t and standard error of natural log-transformed B. Sex, vascular risk level, and APOE ε4 status were dummy coded 1 for males, high vascular risk level, and APOE ε4 carriers, respectively. Bolded variables are significant ($p < .05$).

Key: APOE, apolipoprotein E; TIV, total intracranial volume; WMH, white matter hyperintensity volume.

Appendix A: Table 4. Summary of multiple regression analyses for variables predicting the CRF-MD network pattern

Variable	Model 1				Model 2				Model 3				Model 4			
	β	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*
TIV	.066	.052	.061	.396	-.078	-.061	.072	.397	-.100	-.079	.072	.279	-.053	-.042	.074	.572
Age					**-.470**	**-.003**	**.001**	**8.38E-10**	**-.456**	**-.003**	**.001**	**2.27E-9**	**-.336**	**-.002**	**.001**	**2.91E-4**
Sex					.108	.017	.014	.235	.135	.021	.014	.143	.139	.021	.014	.126
Vascular risk level									-.140	-.022	.011	.051	**-.144**	**-.023**	**.011**	**.041**
APOE									-.012	-.002	.012	.863	-.004	-.001	.012	.958
WMH													**-.191**	**-.033**	**.016**	**.034**
Adjusted R^2	-.002				.196				.206				.223			
F for R^2	.723			.396	**14.5**			**2.09E-8**	**9.60**			**4.95E-8**	**8.94**			**2.06E-8**

β represents the standardized coefficient and B the unstandardized coefficient. SE indicates t and standard error of natural log-transformed B. Sex, vascular risk level, and APOE ε4 status were dummy coded 1 for males, high vascular risk level, and APOE ε4 carriers, respectively. Bolded variables are significant (*p* < .05).

Key: APOE, apolipoprotein E; TIV, total intracranial volume; WMH, white matter hyperintensity volume.

Appendix A: Table 5. Summary of multiple regression analyses for variables predicting the CRF-FA network pattern

Variable	Model 1				Model 2				Model 3				Model 4			
	β	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*	*β*	B	SE	*p*
TIV	.117	.122	.080	.131	-.073	-.076	.085	.369	-.098	-.102	.084	.229	-.057	-.059	.086	.489
Age					**-.628**	**-.006**	**.001**	**2.93E-18**	**-.612**	**-.006**	**.001**	**8.82E-18**	**-.508**	**-.005**	**.001**	**1.81E-9**
Sex					.142	.029	.016	.080	**.172**	**.035**	**.016**	**.033**	**.176**	**.036**	**.016**	**.028**
Vascular risk level									**-.161**	**-.034**	**.013**	**.010**	**-.165**	**-.035**	**.013**	**.008**
APOE									-.007	-.001	.014	.915	.001	.000	.014	.990
WMH													**-.165**	**-.038**	**.018**	**.036**
Adjusted R^2	.008				.371				.389				.402			
F for R^2	2.31			.131	**33.6**			**5.88E-17**	**22.1**			**7.81E17**	**19.6**			**4.66E-17**

β represents the standardized coefficient and B the unstandardized coefficient. SE indicates t and standard error of natural log-transformed B. Sex, vascular risk level, and APOE ε4 status were dummy coded 1 for males, high vascular risk level, and APOE ε4 carriers, respectively. Bolded variables are significant ($p < .05$).

Key: APOE, apolipoprotein E; TIV, total intracranial volume; WMH, white matter hyperintensity volume.

APPENDIX B

Cardiorespiratory Fitness-Related Regional White Matter Integrity Associated with Processing Speed in Healthy Aging

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Abstract

Cardiorespiratory fitness (CRF), measured by VO_2max, is an important indicator of cardiovascular health that may influence age-related differences in cognitive functioning through its potential impact on brain white matter integrity (WMI). In a sample of 167 healthy older adults 50 to 88 years of age, we investigated how cognitive aging was associated with expression of previously identified CRF-related multivariate network patterns of WMI derived from magnetic resonance imaging (MRI) measures of axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA). Multiple regression analyses indicated that the CRF-related WMI patterns for RD and MD, but not AD or FA, were significantly associated with a measure of visuomotor processing speed, after we controlled for demographic and vascular risk factors. Follow up mediation analyses revealed that slowed processing speed related to aging was mediated by lower expression of both the CRF-RD and CRF-MD patterns, which were in turn mediated by age-related increases in MRI white matter lesion load. Lower expression of the regional CRF-RD and CRF-MD network patterns characterized by diminished integrity in areas connecting anterior brain regions to one another as well as anterior to posterior regions, may provide neuroimaging markers of cognitive aging related to processing speed, as part of a white matter pathway linking age-associated microvascular lesion load with CRF-related integrity of myelin in healthy older adults.

1. Introduction

Healthy aging has been associated with decrements in multiple cognitive domains, with processing speed, executive functions, and aspects of memory preferentially affected (Salthouse, 1992; Glisky, 2007; Alexander et al., 2012b). Differences in brain tissue assessed by magnetic resonance imaging (MRI) are associated with cognitive aging, including diffusion-weighted imaging measures of microstructural white matter integrity (WMI) commonly evaluated with axial diffusivity (AD), radial diffusivity (RD), mean diffusivity (MD), and fractional anisotropy (FA). Specifically, reduced global WMI has been associated with, and may mediate, age-related reductions in cognitive performance, particularly within processing speed and executive functions (Borghesani et al., 2013; Cremers et al., 2016; Madden et al., 2009; Raz et al., 2006; Salami et al., 2012; Samanez-Larkin et al., 2012). Age-related differences in tract-specific WMI have also been found primarily in association and commissural fibers in anterior brain regions, with relative preservation of sensorimotor fibers (de Groot et al., 2015; Fjell & Walhovd, 2010; Madden et al., 2009; Bennett et al., 2010; Burzynska et al., 2010; Salat, et al., 2009) and have been associated with poorer performance on tasks of processing speed, executive functions, and long-term memory (Charlton, Barrick, Markus, & Morris, 2010; Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009; Haász et al., 2013; Lockhart et al., 2012). It has been suggested that reductions in WMI underly observed cognitive differences via axonal loss and demyelination, which can interfere with the propagation of neural signals (Lu et al., 2011; Salat, 2011; Soares et al., 2013). Further, there has been evidence that regional WMI differences involving certain tracts may be differentially associated with cognitive domains, such that poorer processing speed and executive abilities have been related to reductions in anterior WMI, poorer episodic memory has been associated with reduced central WMI, and poorer inhibition and task-switching abilities have been related to degradation of posterior WMI (Kennedy & Raz, 2009). These findings highlight the potential importance of further evaluating how alterations in regional patterns of different WMI metrics may contribute to cognitive aging.

Poor cardiovascular health has been associated with detrimental effects on cognitive aging (Bangen et al., 2014; Kulshreshtha et al., 2019). Vascular health conditions, including diabetes and hypertension are common among older adults (McDonald, Hertz, Unger, & Lustik, 2009; Rodgers et al., 2019) and have been associated with poorer brain and cognitive outcomes, especially involving frontal brain regions and performance on frontal-mediated, executive tasks (Levit, Hachinski, & Whitehead, 2020; Raz, Rodrigue, & Acker, 2003). Additionally, poor vascular health may increase risk for developing Alzheimer's disease (O'Brien & Markus, 2014). The common genetic risk factor for late-onset Alzheimer's disease, the ε4 allele of apolipoprotein E (APOE), has also been specifically associated with increased risk of cardiovascular disease and detrimental effects on white matter even in older adults without dementia (Kaprio et al., 1991; Raichlen and Alexander, 2014; Wang et al., 2015). Cognitive differences in healthy aging samples have also been associated with macrostructural whiter matter lesion load, as evidenced by greater global MRI white matter hyperintensity (WMH) lesion volume, which is thought to reflect chronic small vessel disease (Gunning-Dixon & Raz, 2000; Habes et al., 2016). WMH lesions are observed often in healthy aging and to a greater extent in the presence of increasing cardiovascular risk factors (Firbank et al., 2007).

Cardiorespiratory fitness (CRF), assessed through cardiopulmonary exercise testing, is an important indicator of the quality of cardiorespiratory system function. Greater CRF has been correlated with greater WMI, primarily in frontal and parietal lobes that are vulnerable to age effects (Colcombe et al., 2006; Gordon et al., 2008; Voss et al., 2013). Additionally, CRF has been linked to cognitive aging such that enhanced CRF is associated with lower risk for cognitive impairment and may attenuate cognitive aging effects on executive functions and memory (Angevaren et al., 2008; Erickson et al., 2009; Kramer et al., 1999; Kramer & Colcombe, 2018; Weinstein et al., 2012; Wendell et al., 2014). Moreover, there has been evidence suggesting that CRF may have stronger associations with measures of processing speed than with higher-order cognitive tasks, such as executive functions and memory (Newson & Kemp, 2006). These findings suggest that CRF may attenuate detrimental age effects on executive function and memory by enhancing the 'lower order' cognitive processing speed components required to efficiently complete those tasks. Beneficial effects of elevated CRF may be most protective against age-related alterations in white matter and cognition in the later decades of life (Burzynska et al., 2014; Kramer & Colcombe, 2018; Tian et al. 2014; Sexton et al., 2016). Several reviews of the literature, however, have not found significant beneficial effects of CRF on cognitive performance in aging (Etnier, Nowell, Landers, & Sibley, 2006; Young, Angevaren, Rusted, & Tabet, 2015). These inconsistencies in results across studies may be due to differences in operationalizing fitness, measures of cognitive function, and/or differences in demographic and health characteristics.

Multivariate covariance methods enable comprehensive simultaneous examination of all white matter tracts and may be more sensitive in identifying patterns of tract-specific brain differences related to CRF and their impact on cognitive aging (Gazes et al., 2016; Geeraert et al., 2019; 2020). In a prior study (Smith et al., in preparation), we used multivariate network covariance analysis with the Scaled Subprofile Model (SSM; Alexander & Moeller, 1994) to identify network patterns of CRF-related WMI, reflecting tract-specific covariance in white matter bundles in a cohort of healthy older adults. This method has previously been used to investigate network covariance patterns of structural gray and white matter related to healthy aging, as well as associated differences in cognitive function and other health-related risk factors

(Bergfield et al., 2010; Gazes et al., 2016; Song et al., 2023; Van Etten et al., 2024), but had yet to be applied to CRF in relation to microstructural WMI. Our prior work revealed a set of regional network SSM covariance patterns that were characterized by enhanced WMI in relation to greater CRF across four diffusion metrics (i.e., AD, RD, MD, and FA), involving white matter bundles mainly connecting anterior brain regions (prefrontal body and genu of corpus callosum) as well as wider association tracts connecting anterior to posterior brain areas (superior longitudinal fasciculus) (Smith et al., in preparation). Additionally, the SSM patterns of CRF-related MD and FA identified enhanced WMI in bundles connecting subcortical brain regions (fornix) associated with higher levels of CRF. Greater expression of the four CRF-WMI regional network patterns were each strongly, inversely associated with age and several network patterns (i.e., RD, MD, FA) were additionally inversely related to levels of vascular risk and macrostructural white matter lesion load after adjusting for age. These findings suggested that greater expression of these CRF-related WMI diffusion metrics were associated with enhanced cardiovascular health. Together, these analyses supported the use of multivariate SSM network analysis with MRI diffusion metrics of localized white matter tracts as a potential marker of vascular-related brain aging that can help to assess relationships between CRF, WMI, age, and cardiovascular health in healthy older adults. However, this prior study did not yet examine how these previously identified CRF-WMI network patterns are related to cognition in this healthy aging cohort. Investigating the cognitive associations with the identified CRF-WMI patterns may help to elucidate the underlying structural brain white matter differences related to CRF that may attenuate the effects of cognitive aging.

In the present study, we evaluated 1) how, in the same sample of community-dwelling healthy older adults, differences in the expression of the previously identified CRF-AD, CRF-RD, CRF-MD, and CRF-FA network patterns (Smith et al., in preparation) were associated with cognitive functions preferentially affected by aging and 2) whether microstructural CRF-related WMI and macrostructural WMH lesion load act as mediators to influence cognitive aging. Using multiple regression analyses, we first analyzed the associations of each of the four identified CRF-WMI network covariance patterns with selected cognitive functions preferentially vulnerable to aging (i.e., processing speed, executive functions, memory), while we controlled for total intracranial volume (TIV), age, sex, education, level of vascular risk, APOE ε4 carrier status, and WMH volume. We then used mediation analyses to investigate the potential role of the microstructural CRF-related WMI patterns and macrostructural WMH lesion load as mediators of the association of age with cognition. In this case, we sought to evaluate a multimodal neuroimaging white matter pathway through which age-related macrostructural white matter lesion load impacts the effects of CRF on regional patterns of WMI to influence the association of age with cognition. We hypothesized that 1) lower expression of the CRF-WMI patterns would be associated with poorer cognitive performance (i.e., processing speed, executive function, and memory) in this healthy aging cohort and would mediate the relationship between older age and poorer cognitive performance on measures vulnerable to aging, and 2) greater age-related macrostructural white matter lesion load would, in turn, be associated with diminished expression of the CRF-related WMI network patterns, as part of a white matter pathway that would influence the relation between older age to poorer cognition in healthy aging.

2. Materials and methods

2.1 Participants

Healthy adults (N =167; Age = 69.0 $\pm$ 10.4 years) ages 50 – 88, underwent neuropsychological testing, brain MRI scans, and cardiopulmonary exercise testing. The sample was cognitively unimpaired (indicated by the Mini Mental Status Exam (MMSE) = 28.98 $\pm$ 1.26; Folstein, Folstein, & McHugh, 1975), with predominantly (93.4%) non-Hispanic white participants including 78 self-reported females (46.7%). Participants were determined to have a low vascular risk level (35.9%) if they endorsed the presence of zero or one clinical vascular risk factor, including history of cardiac arrest, hypertension, high cholesterol, diabetes, and historical or current smoker status, and were determined to have high vascular risk if they endorsed having two or more of these vascular risk factors (Bangen et al., 2014; Boutzoukas et al., 2022). Participant characteristics have been previously reported in detail (Smith et al., in preparation) and, for those included in the present analyses, are shown in Table 1. All participants provided written informed consent, all procedures were approved by the Institutional Review Board (IRB) at the University of Arizona, and all privacy rights of participants were observed.

2.2 Exercise Testing

Full details of the exercise testing have been described previously (Smith et al., in preparation; Song et al., 2023). Briefly, participants completed a graded treadmill exercise test with gradual increases in speed and incline over time based on the modified Naughton treadmill protocol during exercise (Berry et al., 1996; Strzelczyk et al., 2001) with standard techniques of open-circuit spirometry to measure respiratory gas exchanges (Song et al., 2023). To assess CRF, maximal oxygen consumption (VO_2max [ml/kg/min]) was considered achieved when two of the following three criteria were met: (1) heart rate within ten beats of the age-predicted maximum; (2) plateau in VO_2 with an increase in workload; and (3) respiratory exchange ratio of 1:1 (VCO_2:VO_2) or higher, consistent with the American College of Sports Medicine guidelines (Pescatello et al., 2014).

2.3 Cognitive Testing

Cognitive testing was performed on a separate day from exercise testing. Analyses focused on tests of cognitive domains preferentially susceptible to aging: processing speed, executive functions, and memory (Alexander et al., 2012b). Three tests were selected from each domain. Processing speed was assessed with the Trail Making Test A (TMT A), a measure of visual scanning and visuomotor speed that requires participants to connect in numerical order a sequence of 25 numbered circles (Reitan, 1956). Executive functions were evaluated by the Trail Making Test B (TMT B; Reitan, 1956), a task of cognitive flexibility and set shifting where participants are required to connect 25 circles labeled with numbers or letters by alternating between ascending numerical and alphabetical sequences. Verbal memory was measured by consistent long-term retrieval (CLTR), the number of words consistently recalled on at least three succeeding trials without the benefit of reminding, on the Buschke Selective Reminding Test (BSRT; Buschke, 1973). For this version of the word-list learning test, 12 unrelated items were presented across a maximum of 12 trials, where only items that were not recalled on the previous trial were subsequently presented.

2.4 Genetic testing

APOE genotyping was performed at the Translational Genomics Research Institute (TGen, Phoenix, AZ, USA) and was determined using restriction-fragment-length polymorphisms (RFLP) from venous blood samples, which has been described in previous studies (Van Etten et al., 2021; Song et al., 2023). Briefly, high molecular weight DNA were extracted, assayed, and then amplified using AmpliTaq Gold Fast PCR Master Mix (Applied Biosystems: Thermo Fisher Scientific, Waltham, MA). Samples were then evaluated by identifying banding patterns of the six common APOE genotypes according to previously published methods (Addya et al., 1997). In the present cohort, there were 49 APOE ε4 carriers (29.3%) and 118 non-carriers (70.7%).

2.5 Image Acquisition and Processing

Neuroimaging acquisition procedures have been described in detail previously (Smith et al., in preparation). In summary, T1-weighted Spoiled Gradient Echo (SPGR) MRI scans (slice thickness = 1.0mm, TR = 5.3ms, TE = 2.0ms, TI = 500ms, flip angle = 15°, matrix = 256x256, FOV = 25.6cm), T2 Fluid-Attenuated Inversion Recovery (FLAIR) scans (slice thickness = 2.6mm, TR = 11000ms, TE = 120ms, TI = 2250ms, flip angle = 90°, matrix = 256x256, FOV = 25.0cm), and diffusion-weighted images (DWI) in 51 directions, including 8 B0 images, (b = 1000 s/mm2, slice thickness = 2.6mm, TR = 12500ms, TE = 70ms, flip angle = 90°, matrix = 128 x 128, FOV = 25cm, and 58 slices) were acquired on a 3T GE Signa scanner (HD Signa Excite, General Electric, Milwaukee, WI) during the same MRI session. DWI data were pre-processed with Brainsuite's INVERSION method (Smith et al., 2004; Bhushan et al., 2015) then processed with TRACULA to extract standard diffusivity measures for 42 white matter bundles (Yendiki et al., 2011; Maffei et al., 2021). TIV was calculated using Statistical Parametric Mapping (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK) by summing the total segments of gray matter, white matter, and cerebrospinal fluid in native brain space (Alexander et al., 2012a). White matter hyperintensities (WMH) were computed using T1 and T2-FLAIR scans and the lesion segmentation toolbox (LST; Schmidt et al., 2012) with SPM12. Voxel volumes in the WMH maps were summed to compute the total WMH volume in milliliters (ml) and the global values were log transformed for subsequent analyses (Franchetti et al., 2020; Van Etten et al., 2021).

2.6 Statistical Analyses

2.6.1 Network Covariance Patterns

Multivariate SSM analyses to identify the WMI covariance patterns related to CRF have been described in detail previously (Smith et al., in preparation). Regional SSM network analysis (Moeller et al., 1987; Alexander and Moeller, 1994; Alexander et al., 2012; Habeck et al., 2008) was performed using MATLAB (Math Works, Natick, Massachusetts, USA) to produce a set of regional covariance patterns of WMI for AD, RD, MD, and FA associated with CRF. Briefly, a principal component analysis (PCA) was performed on the natural log-transformed participant by WMI region data, after subtraction of the means across participants and regions, to produce regional covariance pattern components and corresponding network subject scores, which reflected the degree to which each participant expressed the identified regional pattern. The best set of SSM components predicting CRF were then selected using the Bayesian Information Criterion (BIC; Schwarz, 1978). A bootstrap re-sampling procedure (Efron and Tibshirani, 1994) with 10,000 iterations was applied in the SSM analysis (Habeck et al., 2008; Alexander et al., 2012; Gazes et al., 2016) to provide reliability estimates for the regional WMI values for the observed pattern weights related to each of the four tract-specific diffusion metrics (i.e., AD, RD, MD, FA) and CRF. The CRF-related AD pattern was characterized by reductions (lower AD indicates better integrity) in the bilateral arcuate fasciculus, bilateral frontal aslant, and bilateral superior longitudinal fasciculus (SLF) 2 and 3 tracts, with relative increases (less CRF-related reductions) in the left anterior thalamic radiation, right external capsule, left optic radiation, and bilateral uncinate fasciculus tracts. The CRF-RD network pattern demonstrated reductions in RD (lower RD reflects enhanced integrity) in the prefrontal body of the corpus callosum with relative increases in the bilateral arcuate fasciculus, bilateral acoustic radiation, bilateral corticospinal tract (CST), left SLF 1, and bilateral SLF 3 bundles. SSM analyses revealed that the CRF-MD pattern identified MD reductions (lesser MD reflects better integrity) the prefrontal body of the corpus callosum, the genu of the corpus callosum, bilateral frontal aslant, and left fornix bundles with relative increases in the bilateral acoustic radiation, bilateral CST, bilateral optic radiation, and middle cerebellar peduncle. Finally, the CRF-FA network pattern was characterized by increases (greater FA reflects enhanced integrity) in the prefrontal body of the corpus callosum and right fornix bundles, with relative decreases (i.e., less CRF-related elevation) in the right arcuate fasciculus, right acoustic radiation, bilateral CST, and right middle longitudinal fasciculus.

2.6.2 Linear Regressions

To limit Type I error across multiple models, we focused on one selected cognitive measure in each domain expected to be especially vulnerable to aging, i.e., processing speed, executive function, and memory (Alexander et al., 2012b). Additionally, since PROCESS macro mediation models (Hayes, 2018) do not allow for multiple comparison correction, we initially performed a set of multiple regression analyses to specifically evaluate the associations between each CRF-WMI network pattern and each cognitive measure. Significant CRF-WMI pattern associations with cognitive measures were then selected as candidates for subsequent mediation analyses. Specifically, block-wise linear regressions were performed with TIV, age, sex, years of education, vascular risk level, APOE ε4 carrier status, and WMH lesion load entered as initial covariates (block 1), each CRF-WMI pattern was entered next as the independent variable (block 2), and each cognitive measure was the dependent variable. The output of these 12 multiple linear regression models (four CRF-WMI patterns tested for three cognitive outcomes) were corrected for multiple comparisons using the false discovery rate (FDR) approach (Benjamini & Hochberg, 1995). CRF-WMI patterns that significantly predicted cognitive outcomes, with all covariates included and after FDR correction, were then selected for testing in mediation models of age-related cognition. Given that associations with higher-order cognitive functions may be driven by impacts of processing speed measures (Salthouse, 1992; 1996; Newson & Kemp, 2006), TMT A was included as an additional covariate for significant regression models of CRF-WMI network patterns predicting non-processing speed measures for inclusion in the FDR multiple comparison correction described above to evaluate candidacy for subsequent mediation analyses.

2.6.3 Simple Mediation Models

Mediation analyses were conducted using the PROCESS macro (v3.5; Hayes, 2018) in SPSS 28.0 (IBM Corp., Armonk, NY) for those CRF-WMI patterns showing significant associations with cognitive measures in the multiple regression analyses. The mediation models were performed using bootstrap resampling with 10,000 iterations to produce percentile 95% confidence intervals, to examine if the previously identified CRF-WMI patterns mediated the relationship between age and cognitive functions. Covariates for these simple mediation analyses included TIV, sex, education, vascular risk level, APOE ε4 carrier status, and global WMH volume. Significant mediation effects were determined by the percentile bootstrap CIs that did not contain zero. Completely standardized indirect effects were reported as effect size measures (Preacher and Kelley, 2011).

2.6.4 Serial Mediation Models

Serial mediation models were then used to evaluate if WMH volume influenced the CRF-WMI patterns' associations with age-related cognitive function. In these models, we tested age as a predictor and separately included only those cognitive outcomes as dependent variables that showed significant simple mediations with the patterns as the sole mediator, with WMH volume and the CRF-WMI pattern tested as serial mediators. We also included TIV, sex, years of education, vascular risk level, and APOE ε4 carrier status as covariates to control their potential influence on WMH lesion load, CRF-WMI pattern expression, and cognitive performance.

3. Results

3.1 Linear Regressions

After we controlled for TIV, age, sex, years of education, vascular risk level, APOE e4 carrier status, and global WMH volume, initial linear regressions revealed significant inverse relationships between TMT A performance and expression of the CRF-RD ($\beta = -0.244$, $p = 0.006$, $FDRp = 0.024$, R^2 change = 0.033) and CRF-MD ($\beta = -0.288$, $p = 0.0003$, $FDRp = 0.004$, R^2 change = 0.060). Expression of the CRF-AD ($\beta = -0.124$, $p = 0.117$, $FDRp = 0.176$, R^2 change = 0.008) and CRF-FA ($\beta = -0.203$, $p = 0.029$, $FDRp = 0.058$, R^2 change = 0.020) patterns did not significantly predict TMT A. Significant negative relationships between TMT B and expression of the CRF-AD ($\beta = -0.197$, $p = 0.013$, $FDRp = 0.036$, R^2 change = 0.027), CRF-RD ($\beta = -0.220$, $p = 0.015$, $FDRp = 0.036$, R^2 change = 0.026) and CRF-MD ($\beta = -0.239$, $p = 0.003$, $FDRp = 0.018$, R^2 change = 0.039) patterns were also indicated. TMT B performance was not significantly predicted by the CRF-FA ($\beta = -0.182$, $p = 0.054$, $FDRp = 0.093$, R^2 change = 0.014) network pattern. There were no significant relationships between expression of any CRF-WMI pattern and BSRT CLTR after FDR correction ($FDRp$'s > 0.05). Both FDR corrected and uncorrected values for all analyses with CRF-WMI patterns and cognitive outcomes are presented in Table 2.

After adding TMT A as an additional covariate to control for processing speed effects on TMT B performance, expression of the CRF-AD ($\beta = -0.137$, $p = 0.045$, $FDRp = 0.130$, R^2 change = 0.011), CRF-RD ($\beta = -0.092$, $p = 0.248$, $FDRp = 0.340$, R^2 change = 0.001), and CRF-MD ($\beta = -0.095$, $p = 0.192$, $FDRp = 0.329$, R^2 change = 0.002) network patterns were no longer significant predictors of TMT B. These latter findings suggest that the associations between TMT B and expression of the CRF-WMI patterns were explained by the processing speed component of this executive function task. From the multiple linear regression results, only CRF-RD and CRF-MD network patterns were selected for follow-up mediation analyses with age and TMT A performance.

3.2 Simple Mediation Models

We sought to examine if the association of age with cognition in domains vulnerable to cognitive aging was mediated by the identified CRF-WMI patterns. For the processing speed measure, the mediation models showed that the influence of age on TMT A performance was significantly partially mediated by expression of the CRF-RD (Effect = 0.090, $SE = 0.041$, 95%

CI [0.023, 0.182; Figure 1A]) and CRF-MD (Effect = 0.104, *SE* = 0.040, 95% CI [0.036, 0.189], Figure 1B) patterns after we controlled for TIV, sex, education, vascular risk level, APOE ε4 carrier status, and WMH volume (Table 3). As shown in Figure 1, in the mediation models, age had a significant direct effect on TMT A performance, with greater age associated with longer completion time (i.e., worse performance). In the overall models for both CRF-RD and CRF-MD, older age predicted lower expression of the CRF-WMI patterns. In turn, lower expression of the network patterns then predicted slower completion time of TMT A, indicating poorer performance.

3.4 Serial Mediation Models

We then extended the significant simple mediation analyses to include global WMH volume as a mediator of age and pattern expression in the cognitive aging model to evaluate the potential impact of macrostructural white matter lesion load on CRF-WMI pattern expression in mediating the effects of cognitive aging for processing speed. Serial mediation models were limited to the CRF-WMI patterns and cognitive outcomes that were significant in the simple mediation models. In this case, age was included as the predictor, TMT A performance as the outcome, global WMH volume as mediator 1, and CRF-WMI pattern expression as mediator 2. These models revealed that the relationship between age and TMT A was partially serially mediated through WMH lesion load and CRF-RD pattern expression after controlling for TIV, sex, education, vascular risk level, and APOE ε4 carrier status (Effect = 0.150, SE = 0.073, 95% CI [0.012; 0.297]; Figure 2A), such that older age predicted greater WMH volume, which predicted lower expression of the CRF-RD pattern, which in turn predicted slower completion time of TMT A. Specifically, the model indicated significant direct and total effects of age on processing speed performance (Table 4). There was also a significant relationship between older age and greater global WMH volume as well as between greater WMH volume and lower CRF-RD network pattern expression, with lower expression of the network pattern then associated with poorer processing speed performance.

The relationship between age and TMT A was also significantly partially serially mediated through WMH lesion load and CRF-MD pattern expression after controlling for TIV, sex, education, vascular risk level, and APOE ε4 carrier status (Effect = 0.163, SE = 0.078, 95% CI [0.016; 0.324]; Figure 2B), such that increasing age predicted higher WMH volume, which was associated with lower expression of the network pattern, and less pattern expression predicted poorer performance on TMT A. This model also indicated significant direct and total effects of age on visuomotor processing speed. There was also a significant relationship between older age and greater WMH load as well as between greater WMH volume and lower expression of the CRF-MD network pattern. The lower expression of the network pattern then predicted poorer processing speed performance (Table 4).

4. Discussion

In the present study, we found that lower expression of regional patterns of CRF-related WMI for diffusion-weighted measures of RD and MD were associated with poorer processing speed performance and furthermore, that these patterns of CRF-WMI may mediate age-related decrements in processing speed in healthy older adults. The addition of WMH volume in these mediation models showed that older age predicting greater white matter lesion load was, in turn, associated with less expression of the regional CRF-related RD and MD patterns, which was also related to poorer processing speed performance. These results built upon our previous work that identified a set of multivariate network covariance patterns of CRF-related WMI by evaluating their associations with cognitive functions in the same healthy aging cohort. Together, these findings suggest a potential brain white matter pathway through which age-related macrostructural white matter lesion load impacts the effects of CRF on regional patterns of WMI to influence the association of age with processing speed performance in cognitive aging.

Although initial regression analyses revealed significant relationships between CRF-related patterns for AD, RD, and MD with executive function on the TMT B, these associations were not significant with correction for multiple comparisons after accounting for TMT A as a measure of the processing speed component of this executive function task. These results suggest that the CRF-WMI patterns may represent a process by which CRF may, in part, influence cognitive aging through modifying aspects of WMI important for efficiency of processing speed needed for higher-order cognition, such as executive function tasks. Notably, the direct and total effects of age on TMT A in the simple mediation models remained significant, indicating that the network pattern expression only partially explained the influence of age on visuomotor processing speed.

The serial mediation models added macrostructural white matter lesion load as an additional serial mediator to evaluate its role in a potential white matter pathway contributing to the effects of cognitive aging on processing speed. After we controlled for TIV, sex, years of education, vascular risk level and APOE ε4 status, we found that WMH volume mediated the relationship between age and both CRF-RD and CRF-MD pattern expressions. In turn, expression of these network patterns demonstrated significant indirect effects on age-related TMT A performance. Older age was associated with higher WMH load, which was associated with lower expression of the CRF-RD and CRF-MD network patterns. Lower expression of these patterns was then associated with poorer performance on TMT A. That the total and direct effects of age in the model remained significant, suggest that macrostructural lesion load and CRF-WMI pattern expression for RD and MD partially mediate the relationship between age and processing speed performance.

These results are consistent with previous findings in the literature suggesting that CRF preferentially impacts processing speed in aging and that the observed effects of CRF on executive function may be, in part, driven by underlying differences in processing speed performance (Newson & Kemp, 2006). Findings also support the processing speed theory of cognitive aging, postulating that variance in processing speed, specifically the rate of initially activating information (Salthouse, 1992), primarily explains observed differences in working memory and spatial abilities in healthy aging (Salthouse, 1996; Finkel et al., 2007). The CRF-RD and CRF-MD network patterns identified CRF-related enhancements in white matter tracts connecting anterior brain regions to one another (prefrontal body and genu of the corpus callosum) and anterior to posterior brain regions (superior longitudinal fasciculus) with relatively less enhancements in WMI in the corticospinal tract, middle longitudinal fasciculus, and acoustic radiation (Smith et al., in preparation). The findings suggest that WMI differences related to CRF in select white matter bundles may influence age-related differences in processing speed potentially via alterations in myelin degradation and tract integrity (Lu et al., 2011).

Although we found that older age was associated with greater global WMH lesion load, which was related to lower expression of the CRF-RD and CRF-MD patterns that predicted poorer visuomotor processing speed, our findings illustrate how greater levels of CRF may help to attenuate decrements in processing speed associated with age-related chronic small vessel vascular disease. Based on the differential mediating effects of white matter metrics, these results suggest that myelin degradation, to which RD is thought to be especially sensitive (Song et al., 2005), may be contributing to the observed effects of CRF-related WMI on cognition.

The present study has several limitations. First, the sample reflects a relatively small cohort of healthy older adults and was comprised mostly of non-Hispanic white individuals with high levels of education and relatively low levels of cardiovascular burden. Additionally, the current findings utilized cross-sectional data, which does not allow us to evaluate causality and change over time in the variables of interest.

Future research would benefit from studies with larger samples and inclusion of more ethnically diverse participants to increase generalizability of the current results. Studies including those with higher cardiovascular risk burden may also provide the ability to identify greater impacts of cardiovascular health on WMI and cognition consistent with previous research. While significant indirect effects were shown in mediation models with bootstrap resampling testing a hypothesized white matter pathway, the findings cannot address causal inferences or rule out the possibility of unmeasured confounding that might contribute to the observed results. Intervention studies assessing change in CRF on cognition and in longitudinal studies with neuroimaging and cognitive measures over time would be important to extend our findings and to help elucidate potential causal mechanisms of CRF, WMI, and cognition in healthy aging. Additional inclusion of participants with cognitive impairment and/or cardiovascular disease would also be beneficial to help clarify the role of CRF-related WMI in the context of pathological aging and the risk for dementia.

5. Conclusion

The expression of the CRF-related WMI patterns for RD and MD, characterized by enhanced integrity in areas connecting anterior brain regions to one another as well as anterior to posterior regions, may provide neuroimaging markers as part of a brain white matter pathway linking age related microvascular lesion load with expression of CRF-related regional WMI to influence the effects of cognitive aging on processing speed in healthy older adults. Observed findings only in patterns representing CRF-related RD and MD suggest that greater CRF may, in part, provide its protection from cognitive aging by mitigating age-related effects on myelin. These findings further suggest that CRF may have the potential to selectively attenuate detrimental effects of aging and macrostructural lesion load on processing speed through enhancement of WMI in tracts connecting anterior brain regions to one another. Future research is needed to address causality for the current findings and to evaluate how CRF-related WMI for RD and MD relate to longitudinal changes in cognition and dementia risk in the context of healthy and pathological aging.

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Appendix B: Table 1. Participant Characteristics

Variable	Mean ± *SD*
Age (years)	68.99 (10.41)
Education (years)	15.76 (2.58)
Sex (F/M)	78/89
MMSE score	28.98 (1.26)
VO_2max (ml/kg/min)	24.43 (5.40)
Total WMH volume (ml)	0.54 ± 0.44
Vascular risk level (H/L)	60/107
TMT A time (seconds)	32.81 ± 11.55
TMT B time (seconds)	76.91 ± 31.56
BSRT CLTR (words)	63.38 ± 38.00

Table 1. *SD* represents standard deviation. Details of participant characteristics have been reported previously (Smith et al., in preparation).

Key: F/M, Female/Male; MMSE, Mini Mental Status Exam; WMH, white matter hyperintensity; H/L, High/Low, High = two or more vascular risk factors, Low = less than two vascular risk factors; TMT, Trail Making Test; BSRT CLTR, Selective Reminding Test Consistent Long-Term Retrieval.

Appendix B: Table 2**.** Summary of multiple regression analyses for CRF-WMI patterns predicting cognitive performance

Variables	β	*B*	*SE*	*p*	*FDRp*[a]
TMT A					
CRF-AD	-0.124	-20.7	13.2	0.117	0.176
CRF-RD	-0.244	-33.0	12.0	0.006	0.024*
CRF-MD	-0.288	-43.4	11.8	0.0003	0.004*
CRF-FA	-0.203	-23.0	10.5	0.029	0.058
TMT B					
CRF-AD	-0.197	-90.1	35.8	0.013	0.036*
CRF-RD	-0.220	-81.2	33.0	0.015	0.036*
CRF-MD	-0.239	-98.3	33.0	0.003	0.018*
CRF-FA	-0.182	-56.3	29.0	0.054	0.093
CLTR					
CRF-AD	-0.011	-5.93	40.6	0.884	0.884
CRF-RD	0.093	41.7	37.3	0.265	0.340
CRF-MD	0.081	40.2	37.4	0.283	0.340
CRF-FA	-0.022	-8.21	32.4	0.800	0.873

Note. β represents the standardized coefficient and *B* the unstandardized coefficient. SE indicates t and standard error of natural log-transformed B, with adjustments for age, sex, years of education, TIV, vascular risk level, APOE ε4 status, and global WMH volume.
[a]*p*-value adjusted for multiple comparisons.
Key: AD, axial diffusivity; APOE, apolipoprotein E; CI, confidence interval; CLTR, Selective Reminding Test consistent long-term retrieval; CRF, cardiorespiratory fitness; FA, fractional anisotropy; FDR, false discovery rate; MD, mean diffusivity; RD, radial diffusivity; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity; WMI, white matter integrity.

*Not significant after TMT A added as additional covariate and *p* value adjusted for multiple comparisons, *FDRp*>0.0

Appendix B: Table 3. Total, direct, and indirect effects of age predicting processing speed performance through the CRF-WMI network patterns.

Variables	Indirect effects			Direct effects	Total effects
	Effect	*SE*	LLCI, ULCI	Effect	Effect
TMT A					
CRF-RD	0.090	0.041	**0.023, 0.182**	0.255*	0.346**
CRF-MD	0.104	0.040	**0.036, 0.189**	0.242*	0.346**

Note. Effect and SE represent standardized coefficients and standard error, respectively, with TIV, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume as covariates. LLCI and ULCI represent lower and upper bounds of a 95% bootstrap CI for the indirect effect. That zero does not lie within the 95% CIs indicates a significant mediation effect. Significant 95% CIs are bolded.

Key: APOE, apolipoprotein E; CI, confidence interval; CRF, cardiorespiratory fitness; MD, mean diffusivity; RD, radial diffusivity; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity.

* $p<0.05$, **$p<0.01$.

Appendix B: Table 4. Total, direct, and indirect effects of age predicting cognitive function sequentially through WMH volume and the CRF-WMI network patterns.

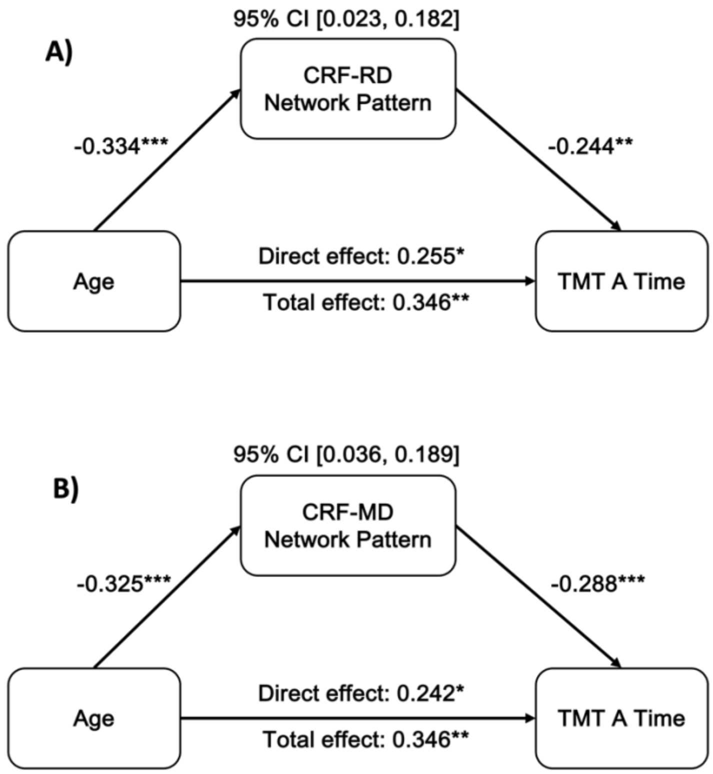
Variables	Indirect effects			Direct effects	Total effects
	Effect	*SE*	LLCI, ULCI	Effect	Effect
TMT A					
CRF-RD	0.150	0.073	**0.012, 0.297**	0.255*	0.405***
CRF-MD	0.163	0.078	**0.016, 0.324**	0.242*	0.405***

Note. Effect and SE represent standardized coefficients and standard error, respectively, with TIV, sex, years of education, vascular risk level, and APOE ε4 status as covariates. LLCI and ULCI represent lower and upper bounds of a 95% bootstrap CI for the indirect effect. That zero does not lie within the 95% CIs indicates a significant mediation effect. Significant 95% CIs are bolded.

Key: APOE, apolipoprotein E; CI, confidence interval; CRF, cardiorespiratory fitness; MD, mean diffusivity; RD, radial diffusivity; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Appendix B: Figure 1

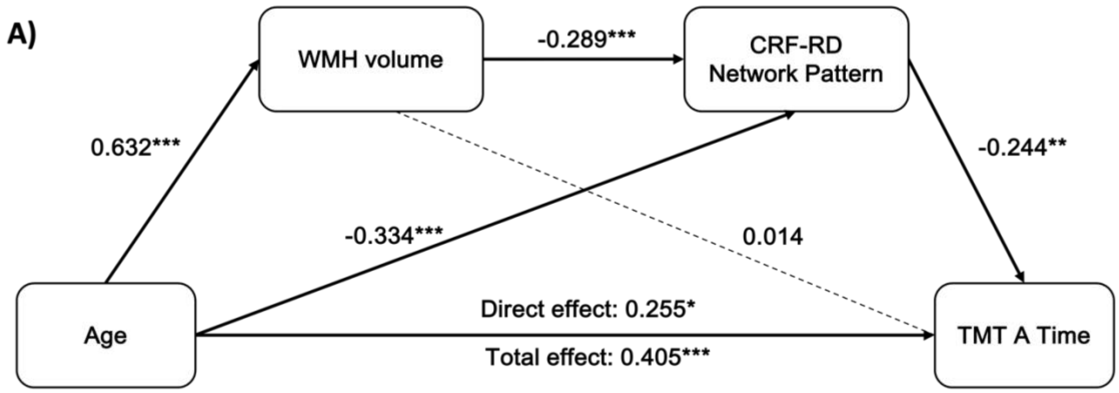


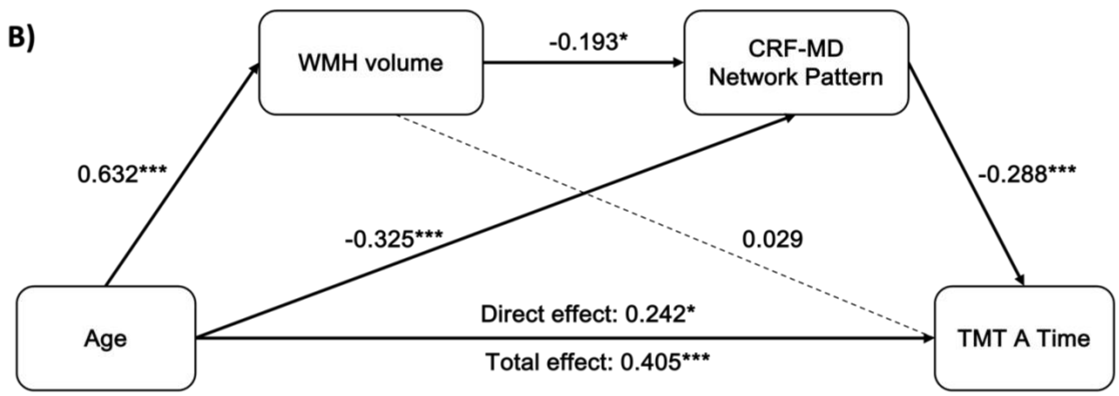
Appendix B: Figure 1. Boxes and paths indicate hypothesized variables and their associations. Black solid lines indicate statistically significant paths. Standardized path coefficients with TIV, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume as covariates are presented. (A) Mediation model of CRF-RD network pattern expression on the association between age and TMT A completion time. In this model, zero did not lie within the 95% CIs, indicating a significant mediation effect. (B) Mediation model of CRF-MD network pattern expression on the association between age and TMT A completion time. In this model, zero did not lie within the 95% CIs, indicating a significant mediation effect. Standardized path coefficients with TIV, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume as covariates are presented.

Key: APOE, apolipoprotein E; CI, confidence interval; CRF, cardiorespiratory fitness; MD, mean diffusivity; RD, radial diffusivity; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity.

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$

Appendix B: Figure 2





Appendix B: Figure 2. Boxes and paths indicate hypothesized variables and associations. Black solid lines indicate statistically significant paths, whereas gray dotted lines indicate non-significant paths. Standardized path coefficients with TIV, sex, years of education, vascular risk level, and APOE ε4 status as covariates are presented. **A)** Total WMH volume and CRF-related RD network pattern sequentially mediating the relationship between age and TMT A completion time. **B)** Total WMH volume and CRF-related MD network pattern sequentially mediating the relationship between age and TMT A completion time.

Key: APOE, apolipoprotein E; CI, confidence interval; CRF, cardiorespiratory fitness; MD, mean diffusivity; RD, radial diffusivity; TMT, Trail Making Test; TIV, total intracranial volume; WMH, white matter hyperintensity.

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$

APPENDIX C

Moderating Effects of Cardiorespiratory Fitness on the Association of Free Water with Cognition in Healthy Aging

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Abstract

Objective: Free water (FW) is a neuroimaging marker of white matter that has been associated with cognitive decrements in healthy and pathological aging. The present study sought to evaluate whether differences in cardiorespiratory fitness (CRF) moderate the association of FW with cognitive aging.

Method: A cohort of 162 healthy older adults were evaluated with magnetic resonance imaging scans to assess global FW, measures of processing speed, executive function, and verbal memory, and a graded exercise treadmill test of CRF. Multiple regression analyses investigated main and interactive effects of FW and CRF in predicting cognitive outcomes, after controlling for demographic and other vascular health factors, as well as self-reported physical activity (PA).

Results: After controlling for total intracranial volume, age, sex, years of education, vascular risk level, APOE ε4 status, and total white matter hyperintensity volume, analyses revealed that greater FW was associated with poorer processing speed and executive function performance, but only at a low level of CRF. There was no interaction of FW by CRF for memory performance. The moderating effects remained significant after additionally controlling for PA levels.

Conclusions: These results suggest that, among healthy older adults, having higher levels of CRF, distinct from PA, may attenuate the relationship between decrements in brain white matter and associated cognitive aging for processing speed and executive function, but not for aspects of memory. Future research is needed to evaluate interactive effects of FW and CRF on cognitive decline in the context of healthy and pathological aging.

Word Count: 246

Key words: Cognitive aging, free water, cardiorespiratory fitness, processing speed, executive function

Introduction

While some cognitive domains can remain relatively stable into old age, others, including processing speed, executive functions, and memory, show preferential vulnerability, even in the absence of pathological processes, like Alzheimer's disease (AD; Alexander et al., 2012b; Craik & Salthouse, 2008; Park & Reuter-Lorenz, 2009). Structural brain differences assessed by magnetic resonance imaging (MRI) may, in part, underly observed reductions in cognitive performance associated with healthy aging (Kennedy & Raz, 2009; Laukka et al., 2013). One metric acquired from diffusion MRI (dMRI), extracellular free water (FW) measured within normal-appearing white matter, has been shown to be particularly sensitive to age-associated differences in cognition and brain structure (Maillard et al., 2019), potentially more so than conventional dMRI metrics of microstructural white matter integrity (Chad et al., 2018; Maillard et al., 2019) and macrostructural MRI white matter hyperintensity (WMH) lesion load (Duering et al 2018; Dumont et al., 2019; Maillard et al., 2019). Higher FW levels have shown significant associations with poorer processing speed (Duering et al., 2018), executive function (Berger et al., 2022; Gugger et al., 2023; Gullett et al., 2020; Gustavson et al., 2023; Maillard et al., 2022a; Pappas et al, 2024), and memory (Gullett et al., 2020) in cognitively unimpaired cohorts. Increased FW was also found to be associated with more severe dementia symptoms in patients with AD, combined AD with cerebrovascular disease, and vascular dementia. Additionally, poorer executive functioning was associated with higher FW in all dementia subgroups (Ji et al., 2017). While the pathophysiology that leads to increased FW is not fully understood (Chad et al., 2018), it has been suggested that this dMRI white matter marker may reflect several key biological processes that lead to neural degradation, including atherosclerosis secondary to poor vascular health (Maillard et al., 2017), cerebral atrophy, neuroinflammation or injury (Gugger et al., 2023; Ji et al, 2017), and alterations in blood-brain barrier permeability (Pasternak et al., 2012; Maillard et al., 2017; Pappas et al., 2024).

Although there is evidence suggesting that FW differences may occur in white matter before other markers of small vessel disease, such as WMH lesions (Duering et al., 2018), it remains unknown whether FW associations with cognitive aging are impacted by cardiorespiratory fitness (CRF), an important indicator of vascular health that reflects a combination of genetic and modifiable lifestyle factors (Bouchard et al., 2015; Sarzynski et al., 2022; Zadro et al., 2017). Elevated CRF has been associated with better brain and cognitive aging outcomes (Angevaren et al., 2008; Erickson et al., 2011; Kramer et al., 1999; Kramer & Colcombe, 2018; Weinstein et al., 2012; Wendell et al., 2014), even when controlling for differences in physical activity (PA) (Burzynska et al., 2014; Erickson et al., 2014; Raichlen et al., 2020; Voss et al., 2016). Elucidating how modifiable factors, like CRF, are associated with FW alterations in white matter may help identify new vascular health pathways to support the development of interventions designed to prevent or diminish the effects of cognitive aging and the risk for dementia.

The present study sought to investigate the potential interactive effects of CRF with global FW in white matter on cognitive performance in a cohort of healthy community-dwelling older adults. We hypothesized that higher levels of FW would be associated with worse cognitive performance in age-sensitive domains, including processing speed, executive functioning, and memory in those with low levels of CRF, after we controlled for differences in demographic and other common vascular and health risk factors; and that this association would be mitigated by elevated CRF levels. We also sought to determine if such interactive effects of CRF with FW were distinct from differences in engagement in PA by additionally adjusting for self-reported habitual activity levels in this healthy aging cohort.

Methods

Participants

Healthy adults (N =162; Age = 68.9 ± 10.5 years) ages 50 – 88, underwent cognitive testing, cardiopulmonary exercise testing to assess CRF, and brain MRI scans. The sample was cognitively unimpaired, as indicated by the Mini Mental Status Exam (MMSE = 29.0 ± 1.24; Folstein et al., 1975), and included 76 females (42.0%) and predominantly (93.2%) non-Hispanic White participants. To exclude significant neurological, medical, and psychiatric disorders, participants underwent an extensive medical screen, and a physical and neurological examination performed by a neurologist (GAH) who specializes in aging and dementia. Participants were excluded if they had a MMSE score less than 26 or a Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960) score greater than 9. The present participants comprise a subsample of a larger cohort of 210 older adults, including only those who were able to complete a treadmill test of CRF and obtain diffusion-weighted MRI scans of adequate quality. Fourteen participants were excluded due to inability to complete VO_2max testing, 16 participants were excluded for not undergoing neuroimaging, and 18 participants were excluded based on poor data quality for the MRI scans. Sixty participants (33.1%) were determined to have high cardiovascular risk based on endorsement of two or more available risk factors: history of cardiac arrest, hypertension, high cholesterol, diabetes, and historical or current smoker status (Bangen et al., 2014; Boutzoukas et al., 2022; see Table 1). Participants completed the Physical Activity Questionnaire (PAQ; Voorrips, et al., 1991) to provide a total score reflecting their self-reported habitual engagement in physical activities. All procedures were approved by the Institutional Review Board at the University of Arizona and all participants provided informed written consent.

Cognitive Testing

One test from each cognitive domain susceptible to aging (i.e., processing speed, executive function, and memory) were included in the analyses (Alexander et al., 2012b). Processing speed was assessed with time to complete the Trail Making Test A (TMT A), a sequencing task that requires visual scanning abilities and measures visuomotor speed by connecting randomly placed numbered circles in numerical order (Reitan, 1956). Executive function was assessed by performance on the Trail Making Test B (TMT B), which provides a measure of cognitive flexibility and set shifting abilities by connecting alternating numbers and letters in ascending numerical and alphabetical sequences, respectively (Reitan, 1956). To remove potential confounding processing speed effects on the executive function required for TMT B, the standardized residual values of TMT-B, after statistically removing performance on TMT A, was used as the executive function outcome (Crowe, 1998). Memory was assessed by long-term retrieval (LTR), the number of words recalled without reminding, on the 12-item/12-trial version of the Buschke Selective Reminding Test (BSRT; Buschke, 1973). One participant did not complete memory testing (N=161), but did have available the other cognitive tests, exercise testing, and MRI scans.

Cardiopulmonary Exercise Testing

Participants completed a graded treadmill exercise test based on the modified Naughton treadmill protocol, with gradual increases in speed and incline over time during exercise (Berry et al., 1996; Strzelczyk et al., 2001). Standard techniques of open-circuit spirometry were used to measure respiratory gas exchanges. Full details of the exercise testing have been described previously (Smith et al., in preparation; Song et al., 2023). Consistent with the American College of Sports Medicine guidelines (Pescatello et al., 2014), maximal oxygen consumption (VO_2max [ml/kg/min]) was reached when two of the following three criteria were met: (1) respiratory exchange ratio of 1:1 ($VCO_2:VO_2$) or higher; (2) plateau in VO_2 with increase in workload; and (3) heart rate within ten beats of age-predicted maximum.

Genetic testing

Apolipoprotein E (APOE) genotyping was performed at the Translational Genomics Research Institute (TGen, Phoenix, AZ, USA) and was determined using restriction-fragment-length polymorphisms (RFLP) from venous blood samples, which has been described in previous studies (Van Etten et al., 2021; Song et al., 2023). Briefly, high molecular weight DNA were extracted, assayed, and then amplified using AmpliTaq Gold Fast PCR Master Mix (Applied Biosystems: Thermo Fisher Scientific, Waltham, MA). Samples were then evaluated for identifying banding patterns of the six common APOE genotypes according to previously published methods (Addya et al., 1997). In the present cohort, there were 48 APOE ε4 carriers (29.6%) and 114 non-carriers (70.4%).

Magnetic Resonance Imaging

T1-weighted Spoiled Gradient Echo (SPGR) MRI scans (slice thickness = 1.0mm, TR = 5.3ms, TE = 2.0ms, TI = 500ms, flip angle = 15°, matrix = 256x256, FOV = 25.6cm) and T2 Fluid-Attenuated Inversion Recovery (FLAIR) scans (slice thickness = 2.6mm, TR = 11000ms, TE = 120ms, TI = 32250ms, flip angle = 90°, matrix = 256x256, FOV = 25.0cm) were acquired on a 3T GE Signa scanner (HD Signa Excite, General Electric, Milwaukee, WI). Diffusion-weighted images (DWI) in 51 directions with 8 B0 images (b = 1000 s/mm2, slice thickness = 2.6mm, TR = 12500ms, TE = 70ms, flip angle = 90°, matrix = 128 x 128, FOV = 25cm, and 58 slices) were also acquired during the same MRI session.

Diffusion MRI Processing and Free Water Estimation

Pre-processing of raw dMRI involved motion and eddy current correction using FSL (Smith et al., 2004), with an appropriate adjustment of the gradient vector matrix to account for head motion. Susceptibility induced EPI distortion was corrected using BrainSuite's non-rigid registration-based INVERSION method (Bhushan et al., 2015). Fractional anisotropy (FA) maps were generated using FSL's *dtifit* tool for use in subsequent template registration.

Next, the diffusion signal in each voxel was fitted to a two-compartment model: a tissue compartment, which was modeled using a diffusion tensor, and an extracellular FW compartment, which was modeled as an isotropic diffusion tensor with a constant diffusion coefficient of water at 37°C (Pasternak et al., 2009). These FW fraction maps were estimated using a protocol developed by the MarkVCID consortium (https://markvcid.partners.org/; Maillard et al., 2022a; 2022b). The resulting FW values, which range from 0 to 1, represent the fractional contribution of FW in each voxel. We modified the image registration in the original MarkVCID protocol to instead use the Symmetric Registration framework implemented under Advanced Normalization Tools (ANTs; Avants et al., 2008; Tustison et al., 2021), as ANTs has demonstrated highly accurate and reproducible registration, and has been suggested to outperform other contemporary image registration algorithms (Klein et al., 2009; Avants et al., 2011).

In this procedure, the FA maps from each participant were warped to the standard FSL FA template space (FMRIB 1-mm FA template) using the linear and the non-linear SyN registration in ANTs. Next, these resulting transformation parameters were applied to each participant's corresponding FW map. Subsequently, a whole brain white matter mask, generated by thresholding the standard FSL FA template at 0.3 (Maillard et al., 2022b), was applied to each participant's template space FW map to eliminate potential spatial overlap with cerebrospinal fluid regions. Visual inspection was performed for each FW map to ensure good quality spatial registration and appropriate overlap of the white matter mask. Finally, the global mean FW across the masked region for each participant was extracted using the *fslstats* tool in FSL.

White Matter Hyperintensity Segmentation

Global WMH maps were generated using the T1 and T2-FLAIR scans with the automated lesion segmentation toolbox (LST; Schmidt et al., 2012) for the Statistical Parametric Mapping software (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK). An optimization approach (Van Etten et al., 2021; Franchetti et al., 2020) was adopted to determine the appropriate kappa threshold (0.35) for LST for this cohort of healthy older adults. Voxel volumes from the resulting lesion probability maps were summed to compute the total WMH volume in milliliters (ml) and were log-transformed for data analyses (Van Etten et al., 2021; Franchetti et al., 2020). Total intracranial volume (TIV) was computed with Statistical Parametric Mapping (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK) software for use a covariate in analyses by summing the native brain space T1 brain scan segments of gray matter, white matter, and cerebrospinal fluid (Alexander et al., 2012a).

Statistical Analyses

In SPSS (v22, Chicago, Illinois, USA), we performed block-wise multiple regression analyses to investigate the interactive effects of FW and CRF on cognition while controlling for demographic and common vascular health characteristics. With the model adjusted for TIV, age, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume (block 1), FW (block 2), CRF (block 3), and the interaction term of FW by CRF (block 4) were entered. Separate models were then run to assess potential interaction effects on individual hypothesized tests of processing speed, executive function, and memory to limit Type 1 error.

Significant interaction effects were then probed using both simple slopes (Aiken et al., 1991) and Johnson–Neyman (J-N) analyses (Hayes, 2018; Johnson & Neyman, 1936). Specifically, we tested the significance of the simple slopes between the predictor (FW) and the outcome (cognitive performance) at specific conditional values (i.e., the mean, and 1 *SD* above and below the mean) that represent “average”, “high”, and “low” values on the moderator (CRF), respectively (Aiken et al., 1991). We additionally applied the J-N analysis approach with 10,000 bootstrap iterations to identify the range of CRF values within which the negative associations of FW with cognitive performance were statistically significant (Johnson & Neyman, 1936). Continuous variables that constituted an interaction term were mean centered prior to analyses. J-N analyses were performed using the PROCESS macro v4.2 (Hayes, 2018) for SPSS 28.0 (IBM Corp., Armonk, NY).

To assess if potential interactive effects of FW and CRF were distinct from effects of self-reported PA on cognition, PAQ total score activity levels were added to the regression models as a separate, additional covariate in a new block 2. PAQ total was also added as a covariate when probing significant interactive effects with J-N analyses. These values were natural log transformed for normalization before PAQ was added as a covariate.

Results

While initially controlling for TIV, age, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume (block 1), the main effects of FW (block 2) and CRF (block 3) on cognitive performance were assessed. FW did not significantly predict TMT A performance when added (B = 56.8, SE = 34.2, p = 0.098, F change = -0.362, R^2change = 0.009) while CRF did demonstrate a significant main effect (B = -0.438, SE = 0.203, p = 0.033, F change = -0.006, R^2change = 0.019) with greater CRF associated with better TMT A performance. When analyzing main effects on executive function, neither FW (B = 4.85, SE = 3.24, p = 0.136, F change = -0.002, R^2change = 0.008) nor CRF (B = -0.023, SE = 0.019, p = 0.240, F change = -0.105, R^2change = 0.002) significantly predicted residualized TMT B performance with all covariates. Verbal memory performance as measured by LTR on the BSRT was also not significantly predicted by FW (*B* = 103.2, *SE* = 87.2, *p* = 0.238, F change = -1.30, R^2change = 0.002) or CRF (*B* = 0.635, *SE* = 0.525, *p* = 0.228, F change = -1.27, R^2change = 0.002) with all covariates in the model (Table 2).

As shown in Table 2, the interaction term of FW by CRF significantly predicted performance in processing speed and executive function, while accounting for TIV, age, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume: TMT A (*B* = -9.93, *SE* = 4.46, *p* = 0.027, F change = 0.053, R^2change = 0.020), TMT B residual (*B* = -0.880, *SE* = 0.425, *p* = 0.040, F change = 0.246, R^2change = 0.020). The direction of these interaction effects indicated that higher levels of FW were associated with slower completion times on TMT A and TMT B residuals (i.e., worse performance) only at lower values (i.e., below the normal range) of CRF. In contrast, the interactive effect of FW by CRF did not significantly predict memory performance (*B* = 11.8, *SE* = 11.6, *p* = 0.312, F change = -0.870, R^2change = 0.00).

When the significant interaction effects for cognition were probed, simple slopes analyses revealed a significant relationship of elevated FW with greater TMT A completion time (i.e., slower performance) only at low (-1 *SD*) CRF values (*B* = 91.9, *SE* = 37.2, *p* = 0.015), and no significant relationship at moderate (mean; *B* = 37.9, *SE* = 34.2, *p* = 0.270) or high (+1 *SD*; *B* = -16.2, *SE* = 46.2, *p* = 0.727) CRF values. Similarly, higher FW was significantly associated with greater residualized completion time on TMT B, with the inverse association only significant at a low level of CRF (*B* = 7.97, *SE* = 3.56, *p* = 0.027) and not significant at moderate (*B* = 3.18, *SE* = 3.29, *p* = 0.335) or high (*B* = -1.60, *SE* = 4.42, *p* = 0.718) CRF values.

The J-N analyses confirmed these results and further identified CRF values where significant associations of FW with each of the cognitive measures were observed. As shown in Figure 1, higher FW was significantly associated with slower TMT A performance when CRF was below 21.6 ml/kg/min (27.3 percentile). The strength of this association increased as CRF decreased from 21.6 to 14.1 ml/kg/min, and was no longer significant when CRF was at or above 21.6 ml/kg/min. When residualized TMT B performance was examined, the results followed the same pattern: there was a significant association of elevated FW with poorer TMT B performance, as indicated by greater completion time, when CRF was below, but not at or above, 20.7 ml/kg/min (27.3 percentile). Within this region of significance, the lower the CRF, the stronger the association of FW with processing speed and executive function performance (Figures 1 & 2).

Additionally, the interactive effects of FW and CRF for processing speed and executive function remained significant after additionally controlling for PAQ (B = -9.15, SE = 4.45, p = .041 for TMT A; B = -0.939, SE = 0.426, p = .029 for TMT B residuals).

Discussion

The current study evaluated CRF as a moderating health/lifestyle factor for the association between higher global FW in brain white matter and poorer cognitive performance in a generally healthy cohort of older adults. It was expected that having high levels of FW would be associated with poorer age-sensitive cognitive performance on measures of processing speed, executive function, and memory with low levels of CRF, but that this association would be mitigated in those with higher CRF levels, after controlling for demographic and other vascular health factors. We found significant associations for higher levels of FW with poorer performance on measures of processing speed and executive function, but only in those with low levels of CRF, and not in those with moderate or higher levels. In contrast, there was not interaction effect for FW with CRF on memory performance in this cohort. Together, these findings support the mitigating effects of CRF in reducing the relation of this neuroimaging marker of white matter structural degradation with age-sensitive cognitive measures of processing speed and executive function, but not for verbal episodic memory. Moreover, the observed moderating effects of CRF on the relation of FW to cognitive performance was not appreciably affected after we controlled for self-reported activity levels, suggesting that the attenuating benefits of greater levels of CRF for processing speed and executive function were distinct from engagement in habitual PA.

More specifically, the relationship between FW and processing speed was moderated by CRF such that greater FW predicted slower TMT A performance only at a low level of CRF. Similarly, higher FW only predicted poorer TMT B performance with the processing speed component statistically removed at the lowest values of CRF. These results suggest the potential beneficial effect of high levels of CRF on structural brain differences in white matter associated with visuomotor processing speed and executive function performance.

That these interaction effects remained significant after additionally controlling for self-reported PA levels, suggests distinct effects of CRF separable from self-reported engagement in habitual PA alone. CRF levels and trainability may be determined by multiple factors, including demographic characteristics like sex and age, lifestyle behavioral factors like PA and smoking, and genetic components (Bouchard et al., 2015, Zeiher et al., 2019).

Our results suggest the possibility that below average levels of CRF may not afford the benefits of moderate to high CRF levels for brain white matter and associated decrements in processing speed and executive function due to cognitive aging. We used a global measure of FW in white matter, which did not allow for the evaluation of regional differences in FW and how they may be differentially influenced by CRF in its relation to cognition. Previous research has implicated frontal brain regions with decrements in both processing speed and executive functions, whereas age-related alterations in episodic memory have been most notably associated with differences in medial temporal lobe structures, especially in regions of the hippocampus. Future research should evaluate how regional differences in FW are moderated by CRF to influence the impact on specific cognitive processes in healthy and pathological aging.

Recent literature has suggested detrimental effects of sedentary lifestyle behaviors on cognition and dementia risk (Raichlen et al. 2023; Zou et al., 2024). Although high levels of sedentary behavior over an extended period may lead to lower CRF, our current findings suggest that the moderating effects of CRF on brain white matter, as measured by FW, and associated cognitive aging may not be explained simply by low levels of PA, since the observed interactive effects of CRF remained significant after additionally adjusting for self-reported engagement in physical activities.

Notably, the interactions between FW and CRF for cognition were also significant while controlling for vascular risk level, APOE ε4 status, and global WMH volume. This suggests that greater CRF may positively impact processing speed and executive functions distinctly from the adverse effects of common vascular health and AD-related risk factors (Maillard et al., 2019). It has been suggested that CRF may attenuate age-related cognitive differences via increased delivery and upregulation of neurotrophins as well as by increased cerebral perfusion, synaptogenesis, and angiogenesis (Maass et al., 2015; Stimpson et al., 2018; Tsai et al., 2016). FW may be sensitive to detrimental effects on brain structure in the white matter due to poor vascular health as well as enhancements or neural protection in the functional brain networks associated with higher CRF (Raichlen et al., 2016).

It is important to note that our study has several limitations. First, analyses utilized cross-sectional data, which do not allow for the interpretation of the current findings in terms of casual inferences or differences in cognitive decline. Future research would benefit from including longitudinal assessments of CRF, cognition, and a range of brain imaging biomarkers to evaluate interactive effects over time and to help determine causality. In this regard, interventions targeting CRF would help to clarify the extent to which CRF and its subsequent effects on brain and cognitive aging are modifiable and causally related. Additionally, the current cohort was comprised of mostly White, highly educated individuals with relatively low vascular risk burden and average CRF. Especially given evidence of differential associations of FW across ethnic groups in pathological aging processes (Ofori et al., 2024), further work would benefit from assessing the impact of CRF on neuroimaging and cognitive measures in samples with greater diversity in racial/ethnic representation, activity and CRF levels, health status, and cognition (Maillard et al., 2017).

Future research would also benefit from validation of CRF associations with other measures of neuroimaging and biomarkers of cognitive aging to elucidate potential mechanisms underlying the observed effects. There is evidence that different types of physical activity (Liu et al., 2022) and sedentary behavior (Wanders et al., 2021) have distinct associations with cognitive aging. Future work would benefit by additionally considering differential contributions of daily activities with varying levels and time spent in different intensities of PA. Further studies may also benefit from inclusion of regional FW values to enhance specificity when assessing CRF effects on brain and cognitive aging (Ji et al., 2017; Ofori et al., 2019; Gullett et al., 2020).

Conclusion

In a cohort of healthy older adults, higher levels of FW only predicted worse processing speed and executive functioning at low levels of CRF. This interaction effect was not observed for verbal memory performance. These findings suggest that moderate and higher levels CRF may help attenuate the association of FW with processing speed and executive function. These effects were distinct from differences in PA levels, suggesting a separable influence of CRF on brain white matter and associated cognitive aging for processing speed and executive function, but not verbal episodic memory. Global FW elevation may be an important biomarker for evaluating impacts and potential neural benefits of CRF as an intervention to preserve cognition in aging with modifiable lifestyle factors.

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Competing Interests

The authors have no actual or potential conflicts of interest.

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Appendix C: Table 1. Participant Characteristics

Variable (N=162)	
Age [years; M (SD)]	68.9 (10.5)
Sex (F/M)	76/86
Education [years; M (SD)]	15.7 (2.57)
MMSE score [M (SD)]	29.0 (1.24)
VO_2max [ml/kg/min; M (SD)]	24.5 (5.44)
Vascular risk level (H/L)	60/102
APOE ε4 Status (Y/N)	48/114
Total WMH volume [ml; M (SD)]	0.571 (0.453)
Free water fraction[a] [M (SD)]	0.212 (0.039)
PAQ total score [M (SD)]	2.36 (0.554)
TMT A time [seconds; M (SD)]	32.4 (11.2)
TMT B residualized time [seconds; M (SD)]	0.001 (0.980)
BSRT LTR [words; M (SD)][b]	85.9 (31.3)

Table 1. Means (standard deviations) for the sample. F/M, Female/Male; Y/N, Yes/No; MMSE, Mini Mental Status Exam; APOE, apolipoprotein E ε4; H/L, High/Low, High = two or more vascular risk factors, Low = less than two vascular risk factors; WMH, white matter hyperintensity; PAQ, Physical Activity Questionnaire; TMT, Trail Making Test; BSRT LTR, Buschke Selective Reminding Test long-term retrieval.

[a]The isotropic fraction of total diffusion in normal-appearing white matter.

[b]N=161

Appendix C: Table 2. Summary of multiple regression analyses for FW and CRF interactive effects on cognition.

Variables	β	*B*	*SE*	*p*
TMT A				
FW	0.183	56.8	34.2	0.098
CRF	-0.213	-0.438	0.203	0.033*
FWxCRF	-0.906	-9.93	4.46	0.027*
TMT B residuals				
FW	0.179	4.85	3.24	0.136
CRF	-0.127	-0.023	0.019	0.240
FWxCRF	-0.919	-0.880	0.425	0.040*
LTR[a]				
FW	0.119	103.2	87.2	0.238
CRF	0.110	0.635	0.525	0.228
FWxCRF	0.069	11.8	11.6	0.312

Note. β represents the standardized coefficient and *B* the unstandardized coefficient. SE indicates t and standard error of natural log-transformed B, with adjustments for age, sex, years of education, TIV, vascular risk level, APOE ε4 status, and global WMH volume.

[a]n=161

Key: APOE, apolipoprotein E; CI, confidence interval; LTR, Selective Reminding Test long-term retrieval; CRF, cardiorespiratory fitness; FW, free water; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity.

*p<0.05.

Appendix C: Figure 1. Conditional effects of FW on TMT A by CRF.

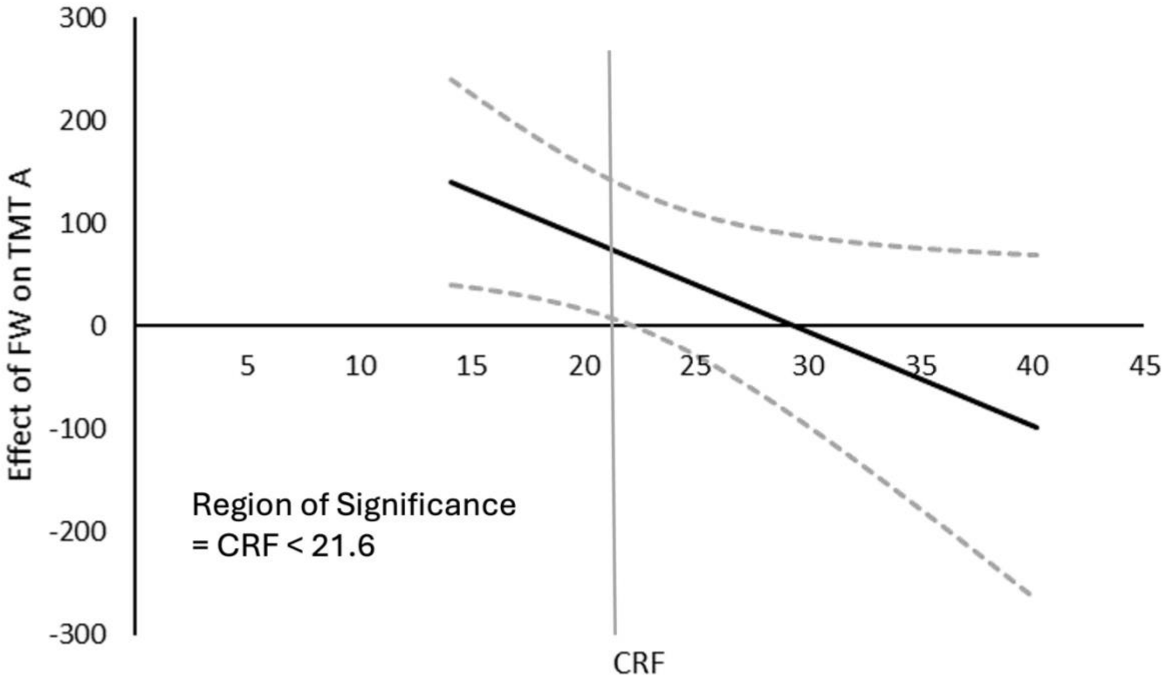

Figure 1. Conditional effects of FW on TMT A along the range of CRF. Effect indicates unstandardized regression coefficient with TIV, age, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume as covariates. The dotted lines indicate 95% CIs, while the gray vertical line represents the value at which the effect of FW on TMT A performance transitions between statistically significant and not significant. Greater FW was significantly associated with higher TMT A scores (i.e., slower completion time) when CRF was below, but not at or above, 21.6 ml/kg/min. Within the region of significance, the strength of the positive association between FW and TMT A performance decreased as CRF increased.

Key: APOE, apolipoprotein; CI, confidence interval; CRF, cardiorespiratory fitness; FW, free water; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity.

Appendix C: Figure 2. Conditional effects of FW on TMT B residuals by CRF.

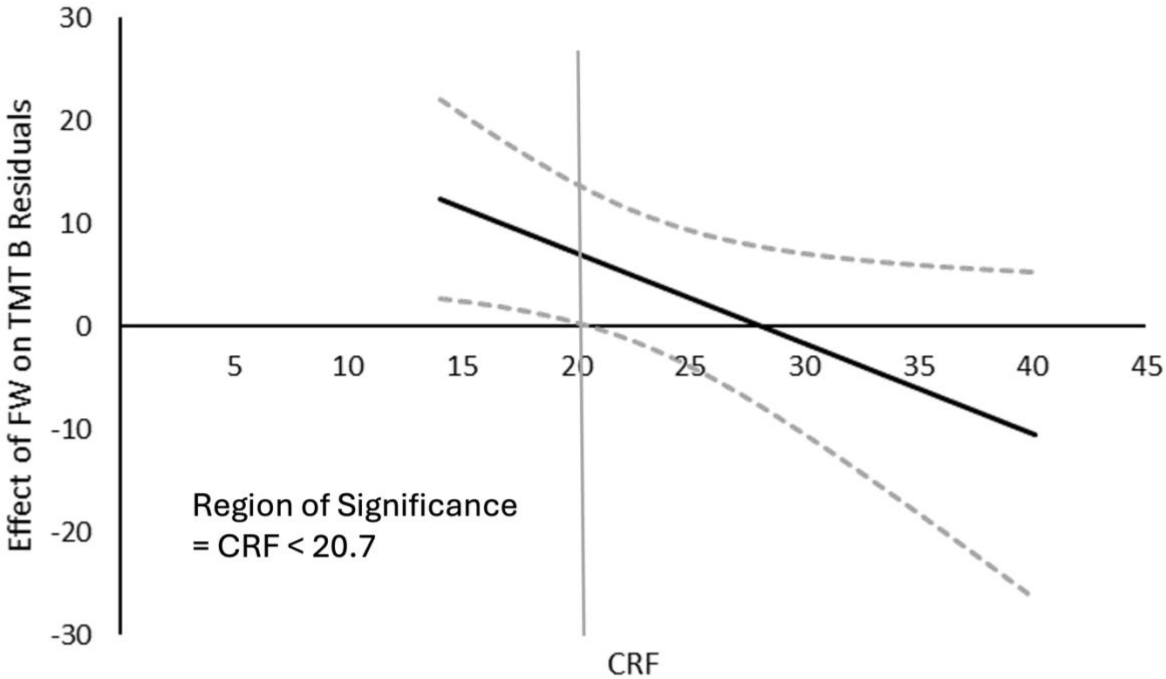

Figure 2. Conditional effects of FW on TMT B residuals along the range of CRF. Effect indicates unstandardized regression coefficient with TIV, age, sex, years of education, vascular risk level, APOE ε4 status, and global WMH volume as covariates. The dotted lines indicate 95% CIs, while the gray vertical line represents the value at which the effect of FW on TMT B residuals transitions between statistically significant and not significant. Greater FW was significantly associated with higher TMT B residualized scores (i.e., slower completion time) when CRF was below, but not at or above, 20.7 ml/kg/min. Within the region of significance, the strength of the positive association between FW and TMT B residuals decreased as CRF increased.

Key: APOE, apolipoprotein; CI, confidence interval; CRF, cardiorespiratory fitness; FW, free water; TIV, total intracranial volume; TMT, Trail Making Test; WMH, white matter hyperintensity.