

Associations Between Cardiorespiratory Fitness, Adiposity, and White Matter Integrity

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White matter (WM) is essential for transmitting neural signal between brain regions, and supporting healthy brain aging and cognitive function. Risk for WM deterioration is heightened in overweight and obesity, whereas increasing cardiorespiratory fitness may promote WM integrity. However, there is a lack of research comparing adiposity and cardiorespiratory fitness with WM. Further, it is not clear whether increasing cardiorespiratory fitness may outweigh the influence of excess adiposity on WM integrity in middle adulthood. In a sample of adults with overweight and obesity, we examined whether cardiorespiratory fitness and adiposity associate with WM integrity, both independently and jointly. We assessed WM pathways sensitive to cardiorespiratory fitness, adiposity, or both, and tested potential interactions.

Baseline data from 125 middle-aged participants ($M_{age} = 44.33 \pm 8.60$), with overweight or obesity ($M_{BMI} = 32.45 \pm 4.19$), were included in the study. Fitness was assessed via a submaximal graded exercise test. To quantify adiposity, whole body estimates of body fat % were calculated using dual-energy X-ray absorptiometry. Diffusion weighted images were acquired during an MRI protocol. We conducted whole-brain voxelwise analyses using the FMRIB's Software Library randomise function to examine main effects of adiposity and fitness, as well as the interaction term, on WM integrity.

After controlling for age, gender, and years of education, there were no significant main effects of adiposity or cardiorespiratory fitness on FA (all $p > .05$). There was a significant interaction ($p = .03$) such that with higher fitness levels, greater adiposity was associated with

higher WM integrity, whereas with lower fitness levels greater adiposity was negatively associated with WM integrity.

This pattern of findings was unexpected, and may be a function of the unique nature of the sample or related to the confounding effects of WM lesions or local inflammation. Future work may focus on accounting for the influence of WM lesions, and extending the analysis to older adults and patient populations.

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Preface

I would like to thank Dr. Kirk Erickson for providing me with funding from National Institutes of Health grant RO1 AG053952, which provided me with the opportunity to pursue this thesis study.

1.0 Introduction

The deterioration of neural tissue is evident in both healthy and pathological aging. Neural decay correlates with reduced cognitive ability (Isaac et al., 2011; Draganski, Lutti, & Kherif, 2013) and has been identified as a precursor to dementia (Morra et al., 2009). Deterioration of white matter (WM), a type of brain tissue responsible for connecting widely distributed neural networks, is particularly problematic as it hinders the transmission of information between cortical regions. Studies of WM microstructure link atrophy to reduced executive function (Kennedy & Raz, 2009) and reduced ability to adapt to aging-related cognitive decline (de Lange et al., 2016). Given the current lack of successful pharmaceutical therapies for neurodegeneration and related decline, it is critical to study and intervene on modifiable risk factors prior to the loss of cognitive abilities.

Risk of neural deterioration is heightened in overweight and obesity, conditions characterized by the excessive accumulation of fatty or adipose tissue. The prevalence of obesity among adults in the United States, alone, is 39.8% (Hales, Carroll, Fryar, & Ogden, 2017) and estimates of the associated economic burden extend beyond \$200 billion (Hammond & Levine, 2010). Mounting evidence links greater adiposity with reduced neural function and morphology (Driscoll et al., 2011; Jagust, Harvey, Mungas, & Haan, 2005). Accordingly, it has become increasingly important to investigate methods of offsetting the potentially harmful effects of excess adiposity on the brain. Recent work suggests that a promising intervention for adiposity-related neural decline is increasing cardiorespiratory fitness. Cardiorespiratory fitness facilitates better overall physiological health (Myers et al., 2015) by mitigating several of the consequences of obesity. Lee and colleagues (2012) investigated the effects of changes in fitness and fatness in

3,148 healthy adults over the course of six years. After controlling for confounds, they found that maintaining or increasing fitness lowered the risk of hypertension, metabolic syndrome, and hypercholesterolemia. Moreover, while fat gain heightened the risk of all three outcomes, maintaining or improving fitness reduced this effect. These results demonstrate that fitness may improve cardiovascular health and mitigate the influence of adiposity on cardiometabolic outcomes. This is notable since cardiovascular and metabolic risk are correlated with the structural integrity of the brain (Knopman et al., 2011; Marebwa et al., 2018). Yet, it remains unclear whether attenuating the negative effects of adiposity through fitness also extends to brain health.

On its own, fitness has been correlated with brain structure and function (Kramer & Erickson, 2007). Mounting evidence points to a beneficial association of fitness with WM health (Colcombe et al., 2003; Oberlin et al., 2016). Yet, research on the relationship between cardiorespiratory fitness and WM integrity remains limited and has focused primarily on healthy older adults. Furthermore, it is currently unknown if the possible benefits of cardiorespiratory fitness outweigh the influence of excess adiposity on WM integrity in middle adulthood. Thus, the purpose of the current investigation is to examine the associations between WM integrity and both cardiorespiratory fitness and adiposity in a sample of adults with overweight and obesity.

Specifically, our aims are the following:

Aim 1: Examine the association between cardiorespiratory fitness and WM integrity in a sample of obese and overweight adults, and identify regions that are sensitive to cardiorespiratory fitness, but not adiposity. Hypothesis 1: Higher cardiorespiratory fitness will be significantly associated with greater WM integrity in this sample, particularly in the corona radiata, inferior fronto-occipital fasciculus (IFO) and longitudinal fasciculi.

Aim 2: Examine the association between adiposity and WM integrity in a sample of obese and overweight adults, and identify regions that are associated with adiposity, but not cardiorespiratory fitness. Hypothesis 2: Higher percentage of whole-body adiposity will be significantly associated with lower WM integrity in this sample, particularly in the fornix.

Aim 3: Examine the associations between adiposity, cardiorespiratory fitness, and WM integrity in a sample of obese and overweight adults, and identify WM pathways that are sensitive to both adiposity and fitness. To account for the possibility of an interaction, we also aimed to test for interaction effects of adiposity and fitness on WM integrity. Hypothesis 3: Whole-body adiposity and cardiorespiratory fitness will both be associated with WM integrity in the corpus callosum and cingulum. Specifically, we predicted that cardiorespiratory fitness and adiposity would yield cumulative effects in these regions, such that individuals high in fitness and low in adiposity will have higher regional WM integrity than those with higher adiposity or lower fitness levels.

1.1 White Matter Microstructure

Human neural tissue is composed of grey matter and WM. Grey matter consists of neuronal cell bodies and their branching dendrites, while WM is primarily made up of axons and glia (Fields, 2008). Axons extend from cell bodies to transmit electrical signals between regions of grey matter. Glial cells form myelin, a fatty sheath that surrounds the axon and regulates the speed and strength of signal transmission. Accordingly, WM fiber bundles facilitate communication between widely distributed neuronal networks. Deterioration of WM structure, evident in advancing age, can lead to impaired neural connectivity and reduced cognitive ability.

Magnetic resonance imaging (MRI) provides a measure of brain morphology, including global and regional WM volume, as well as identification of WM hyperintensities (WMH). However, conventional MRI provides only a superficial depiction of WM, neglecting the intricate nature of fiber structure. Moreover, neuroimaging methodologies that are sensitive to WM microstructure reveal abnormalities that appear otherwise unremarkable in standard MR scanning (Pfefferbaum & Sullivan 2002). Diffusion weighted imaging (DWI) is a form of MRI that is uniquely sensitive to the diffusion properties of water molecules within brain matter (Le Bihan & Breton, 1985). As such, this modality allows researchers to analyze the microstructure of WM *in vivo*. Diffusion tensor imaging (DTI) is an approach used to model the neuroanatomical information attained by DWI, based on the mathematical principles of the diffusion tensor (Basser, Mattiello, & LeBihan, 1994). DTI provides information about the orientation, direction, and rate of water diffusion within different types of brain tissue. Fractional anisotropy (FA), a main outcome measure of DTI, quantifies the directional preference of water diffusion within a voxel. Diffusion within healthy WM tends to be anisotropic, or restricted in all directions except along the axon, whereas diffusion within the cerebrospinal fluid (CSF) is isotropic, or fully unrestricted. Isotropic diffusion within WM is thought to relate to demyelinated or damaged fiber structure, and reflects less WM microstructural integrity (Sullivan & Pfefferbaum, 2006). DTI also provides information about additional properties of WM organization, including mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD). MD inversely measures membrane density, with high values distinguishing CSF from both gray and white brain matter (Feldman, Yeatman, Lee, Barde, & Gaman-Bean, 2010). Low AD and high RD values are of interest because they tend to relate to damage to axonal structure and the breakdown of myelin, respectively (Song et al., 2003; Song et al., 2005). Nevertheless, FA is the most global index of WM microstructure,

combining information across all of the elements of DTI, and is equally consistent with fiber density, axonal structure, and myelination (Beaulieu, 2002).

1.1.1 Healthy White Matter Development

DTI has allowed researchers to characterize healthy WM development across the lifespan. These analyses find the trajectory of WM growth to be best represented by an inverted U-shaped quadratic function (Bartzokis et al., 2001; Bartzokis et al., 2004). Westlye and colleagues (2010) corroborated these findings in a sample of 430 healthy individuals between the ages of 8 and 85. Examining global WM volume, they found that WM growth occurs at an accelerated pace from childhood into midlife, plateaus around the age of 40, and begins to decline after age 65. Interestingly, FA was observed to reach its peak earlier than did WM volume, around the third decade of life, after which it slowly declined until reaching a sharper drop around age 65. The observations of FA were largely stable across WM pathways, with the exception of the hippocampal cingulum bundles. These fibers showed less consistent decline later in life than the other WM tracts, a parallel finding to that of Hsu and colleagues' (2008) analysis of FA in the temporal lobes during aging. A number of possible underlying explanations may account for this inconsistency. For instance, it may be the case that the hippocampal cingulum bundles are simply more resistant to aging-related decline than other tracts. Another possibility is that changes in the FA of these fibers are dependent upon differences in lifestyle factors that the study had not accounted for, such as aerobic exercise and dietary habits. These possibilities indicate the importance of exploring the regional influence of lifestyle factors on WM microstructure. Furthermore, changes in WM microstructure are important to characterize, as investigations utilizing FA find that aging-related losses in WM integrity (Madden et al., 2012) are even more

pronounced in the presence of cognitive deficits (Medina et al., 2006) and dementia (Kuczynski et al., 2010). The integrity of WM is also associated with cognition, particularly processing speed and executive functioning (Madden et al., 2008; Turken et al., 2008, Vernooj et al., 2009), and reduced WM integrity relates to impairment in these cognitive domains.

1.2 Adiposity and White Matter Integrity

Recent studies of microstructural changes in WM have shown reduced integrity in multiple pathways as a function of obesity (Kullmann, Schweizer, Veit, Fritsche, & Preissl, 2015). Body mass index (BMI), calculated as weight (kg) divided by height (m²), is one of the most common metrics used for indexing body fat, with a measurement of 30.0 or higher accepted as an indicator of obesity and 25.0 to 29.9 corresponding to overweight. A study (Ryan & Walther, 2014) of healthy elderly women, found that BMI inversely correlated with FA in the temporal lobe WM and the corticospinal tract. Studies of both older (Bettcher et al., 2013) and younger adults (Xu, Li, Lin, Sinha, & Potenza, 2013; He et al., 2015) support an inverse relationship between BMI and FA in the corpus callosum, cingulum, and fornix. Furthermore, high adiposity appears to advance the effects of aging on WM deterioration. Tract-based measurements of FA show age-related decline in the fibers of the corpus callosum, especially in the genu WM (Sullivan, Adalsteinsson, & Pfefferbaum, 2005). Correspondingly, in a lifespan sample of 103 adults between the ages of 21 and 86, Stanek and colleagues (2011) correlated increasing BMI with lower WM integrity in the genu, splenium, and fornix. Deterioration was especially heightened in the splenium and body of the corpus callosum in older adults with high BMI. In all, BMI appears to consistently associate with FA in the corpus callosum and nearby medial WM pathways including the fornix and

cingulum, regions essential for transmitting signals between the temporal and frontal lobes (Jones, Christiansen, Chapman, & Aggleton, 2013; Nowrangi & Rosenberg, 2015), and implicated in memory and executive function (Grambaite et al., 2011). Notably, these are the same cognitive domains previously mentioned as being markedly sensitive to declines in WM integrity. The associations between BMI and cingulum FA is also notable considering Westlye and colleagues' (2008) finding that the hippocampal cingulum shows less consistent reductions in later adulthood than do other pathways. This suggests that the fibers of the cingulum bundles may be particularly sensitive to individual lifestyle differences, and experience considerable harm related to elevated BMI.

It is important to note that the majority of studies examining associations between obesity and related factors with WM have primarily focused on BMI. BMI is a useful metric for quantifying obesity within and across populations (Shah & Braverman, 2012), particularly because it is inexpensive and simple to calculate (Burkhauser & Cawley, 2008). However, BMI is limited as a measure of adiposity by its failure to distinguish between lean muscle and fat mass, as well as additional factors such as water retention. Thus, an individual with high muscle mass and little accumulation of fatty tissue may have the same BMI as an individual with little muscle mass and high adiposity, despite the latter of the two resulting in much higher risk for obesity-related health problems. While previous investigations have successfully linked BMI to brain outcomes, more accurate measurements of adiposity include whole body estimates of body fat composition, such as dual-energy X-ray absorptiometry (DEXA). Several investigations (Burns, Johnson, & Watts, 2010; Burns, Johnson, Watts, Swerdlow, & Brooks, 2010; Karlsson et al., 2013) have utilized measures of body fat composition to link adiposity to cortical thickness and WM volume, but few have applied these approaches to WM microstructure. A small number of studies have linked other

measures of adiposity, including waist-to-hip ratio combined with BMI (Verstynen et al., 2013), and waist circumference (Allen, Muldoon, Gianaros, & Jennings, 2016), to global FA. Still, investigations using metrics of adiposity other than BMI are few in number and do not illuminate regional effects. Thus, it will be important to determine whether previously established patterns of WM microstructural changes with high BMI are maintained when utilizing whole body measurements of body fat.

1.2.1 Adiposity is Related to Cognition

The impact of adiposity on WM health is also reflected in its effect on cognition. In a systematic review of 30 studies, Smith, Campbell, and Trollor (2011) reported that obesity was consistently related to cognitive deficits in children, adolescents, and adults, but not older adults. The authors reasoned that the lack of consistent findings in elderly populations may be due to the inadequacy of BMI in distinguishing between fat and muscle mass, especially as body composition changes during aging. In addition, weight loss in older adults may be confounded by fat and muscle loss due to illness, which itself can impair cognitive performance. Still, there is robust evidence for poor cognitive performance related to adiposity in all other age groups. In a study of 2,000 school-aged children and adolescents, Li, Jackson, and Zhang (2008) found that after adjusting for age, gender, and socioeconomic status (SES), overweight correlated with poor performance on tests of working memory and attention, as well as global cognition. In adults, one prospective study (Wolf et al., 2007) found that increases in waist-to-hip ratio from early to late midlife predicted worse executive functioning. Furthermore, recent work (Zhang et al., 2018) showed that WM integrity mediates the relationship between adiposity and cognition in otherwise healthy adults, particularly with regard to processing speed and executive function. Altogether, it appears that

adiposity imparts a harmful effect on both WM integrity and cognitive function. These findings, considered alongside reports (Whitmer, Gunderson, Barrett-Connor, Quesenberry, & Yaffe, 2005; Singh-Manoux et al., 2018) that indicate an increased risk of dementia in obesity, underscore the importance of intervention.

1.2.2 Potential Mechanisms Underlying Associations Between Adiposity and White Matter

Several mechanistic theories have been put forth to explain the effect of adiposity on the brain. Rosano, Marsland, and Gianaros (2012), as well as Verstynen and colleagues (2013), postulated that obesity partly influences WM microstructure as a consequence of adiposity-induced systemic inflammation. Specifically, central adipose tissue is a potent source of proinflammatory cytokines (Coppack, 2001), including interleukin-6 (IL-6), interleukin 1 beta IL-1 β , and tumor necrosis factor alpha (TNF- α). These cytokines are able to pass the blood-brain barrier and elicit an inflammatory response within the microglia of the central nervous system (CNS). In an activated state, microglia show an increased capacity to bind with oligodendrocytes, a form of glial cell (Mosley & Cuzner, 1996). Additionally, activated microglia release toxic substances, such as nitric acid, which induce the death of oligodendrocytes (Zajicek, Wing, Scolding, & Compston, 1992), consequently degrading myelin structure.

Although much of the data has been derived from animal models, studies in human subjects lend support to the theory. Bettcher and colleagues (2015) repeatedly assessed systemic inflammation and WM integrity in a sample of 276 older adults over a span of six years. After controlling for demographic variables and vascular risk factors, they found that more rapid decreases in C-reactive protein (CRP), a marker of systemic inflammation, associated with higher FA in the dorsal and temporal SLF and uncinate fasciculi. Bettcher and colleagues (2013) also

assessed the combined influence of inflammatory and vascular factors on the association between body mass and WM. BMI was independently related to FA in the fibers of the cingulate and genu. The combination of vascular risk factors, including smoking and hypertension, and inflammatory markers, such as IL-6, mediated the association between BMI and FA in the fornix and middle-posterior regions of the corpus callosum. The authors suggested that these regions may be particularly sensitive to the vascular and inflammatory factors that mediate the relationship between body mass and WM. These findings lend support to previous studies (Jurgens, Amancherla, & Johnson, 2012; Kern et al., 2012) that find the hippocampus and fornix, which serves as a major hippocampal output tract, to be markedly sensitive to inflammation.

1.3 Cardiorespiratory Fitness and White Matter

Alongside studies that establish a link between adiposity and WM, research has shed light on the salutary relationship between cardiorespiratory fitness and WM integrity. Cardiorespiratory fitness refers to the body's ability to take in and deliver oxygen to working muscles during periods of sustained activity, and it can be improved by engagement in aerobic exercise (Manley, 1996). Aerobic exercise is a form of physical activity that increases cardiovascular workload by engaging large muscle groups over extended periods of time. To meet the energy demands of prolonged physical exertion, aerobic exercise leads to elevated oxygen consumption, and habitual engagement in aerobic exercise increases overall oxidative capacity. Accordingly, cardiorespiratory fitness is often objectively measured as the maximal oxygen intake level of the cardiorespiratory system (VO_{2max}), and is reflective of habitual aerobic exercise.

Studies of the relationship between fitness level and WM microstructure find that higher fit individuals have higher FA in widespread tracts that interconnect the frontal, temporal, parietal, and occipital gray matter, as well as both cerebral hemispheres. Namely, Johnson, Kim, Clasey, Bailey, and Gold (2012), as well as Tarumi and colleagues (2015), linked fitness to FA in the body and genu of the corpus callosum, internal and external capsules, corona radiata, and superior longitudinal fasciculi (SLF). Further, Marks and colleagues (2007) reported a relationship between fitness and FA in the unicate fasciculus and cingulum, which interconnect frontal and medial-temporal brain regions. Tseng and colleagues (2013) compared WM in 10 sedentary older adults and 10 older master athletes. They found that master athletes had higher FA in the right IFO, right superior corona radiata (SCR), both SLF hemispheres, and the left inferior longitudinal fasciculus (ILF). Analyses of the relationship between fitness and WM integrity have also been conducted in memory impairment. In a study of 22 patients with memory deficits and a high risk of progression to dementia, as identified by the presence of biomarkers of Alzheimer's disease (AD), Teixeira and colleagues (2016) reported a positive correlation between FA and VO₂max in the longitudinal fasciculus, fronto-occipital fasciculus, and corpus callosum. Likewise, in a recent study of 37 older adults in the early stages of AD, Perea and colleagues (2016) reported that higher fitness correlated with higher FA in the right IFO, a WM pathway connecting frontal, temporal, and occipital cortices. These effects have been replicated across numerous other investigations (Burzynska et al., 2014; Hayes, Salat, Forman, Sperling, & Verfaellie, 2015; Opel et al., 2019). Altogether, these studies suggest that fitness may be diffusely protective of WM integrity, even in the context of neuropathology.

Exercise interventions, which allow for causal inference, provide further evidence of the role of fitness in preserving WM integrity. Exercise interventions typically consist of at least one

exercise group and at least one control group. To control for the biasing effects of socialization, standard exercise trials are designed to ensure that each group receives comparable levels of social engagement. A one-year intervention (Voss et al., 2013) in 70 sedentary, but otherwise healthy, older adults found that increased cardiorespiratory fitness after exercise training was related to higher frontal and temporal WM integrity. Parallel findings were reported in an eight-month trial (Schaeffer et al., 2014) with overweight children. Moreover, in a recent trial with children between the ages of 7 and 9, participation in a 9-month exercise program was associated with increased WM integrity in the genu of the corpus callosum of the intervention group, but not the control group (Chaddock-Heyman et al., 2018). Overall, higher aerobic fitness is related to higher FA in various regions connecting frontal, temporal, and occipital cortices. While the positive association is strong across studies, there is somewhat of a lack of consensus with regard to regional specificity. This limitation may be accounted for by the small sample sizes found in a number of these studies, a challenge associated with aerobic fitness testing, which tends to be expensive and contains a level of participant burden. Interestingly, it is also not yet known how longevity and magnitude of fitness differentially relates to WM and so, regional integrity may vary as a function of these factors.

1.3.1 Cardiorespiratory Fitness is Related to Cognition

Fitness has also been found to support cognitive function. In a longitudinal study of healthy older adults (Barnes, Yaffe, Satiriano, & Tager, 2003), low baseline cardiorespiratory fitness related to steeper declines in global cognitive performance, measured at baseline and at a 6-year follow-up. Participants with lower baseline cardiorespiratory fitness also performed worse than their higher fit counterparts on all cognitive tests administered at follow-up, especially measures

of global cognition, attention, and executive function. An exercise intervention (Colcombe et al., 2005) with older adults found that after six months, individuals in the physical activity group exhibited increased performance on the Rockport 1-mile walk test (Kline et al., 1987), a measure of cardiovascular fitness that is highly concordant with estimates of cardiorespiratory fitness. Furthermore, individuals in the physical activity group demonstrated enhanced speed and accuracy on a test of working memory when compared to a stretching and toning group. In addition, a recent pilot exercise trial randomized 101 participants to a control group, or one of three aerobic exercise groups: 75 minutes of exercise per week (min/wk), 150 min/wk, and 225 min/wk. They reported that attention improved as a function of exercise, regardless of dose, and there was a dose-response relationship between exercise and visuospatial processing. Other studies (Liu et al., 2012; DeFina et al., 2013) have suggested that higher fitness relates to a lower risk of later life dementia. Although the causal direction of this relationship is unclear, overall, it is apparent that increased fitness relates to neurocognitive advantages.

1.3.2 Potential Mechanisms Underlying Associations Between Fitness and White Matter

With regard to mechanisms, elevated aerobic fitness promotes the delivery and utilization of oxygen throughout the cardiovascular system. Animal models (Van Praag, Shubert, Zhao, & Gage, 2005; Ding, Vaynman, Akhavan, Ying, & Gomez-Pinilla, 2006) demonstrate that exercise-related improvements in oxidative capacity upregulate neuronal growth factor expression, including insulin-like growth factor I (IGF-I) and brain-derived neurotrophic factor (BDNF). Heightened levels of these growth factors are associated with neurogenesis and increased cortical thickness throughout gray matter (Erickson et al., 2010; Erickson et al., 2011; Morel, León, Uriarte, Reggiani, & Goya, 2017) and recent evidence suggests that this may also be the case for

WM. For instance, Maillard and colleagues (2016) examined WM and BDNF in 557 middle-aged adults, finding a subtle but significant positive relationship between levels of serum BDNF and FA. Likewise, Feeney and colleagues (2017) found that in a sample of individuals with diffusely low FA related to traumatic brain injury (TBI), higher levels of serum IGF-I were associated with greater increases in splenium FA over time. These findings converge with the animal literature, which indicates that BDNF serves to downregulate the action of myelin-associated glycoprotein (MAG), an inhibitor of axonal growth (Ghiani, Ying, de Vellis, & Gomez-Pinilla, 2007). Accordingly, exercise reduces the action of MAG in rats, but the effect disappears when the action of BDNF is blocked. Other animal work has demonstrated that voluntary exercise precedes an increase in BDNF and induces enhanced axonal regrowth following sensory nerve injury (Molteni, Zheng, Ying, Gómez-Pinilla, & Twiss, 2004). Taken together, these findings suggest that aerobic fitness may improve WM integrity by promoting the expression of neuronal growth factors.

Alternately, another possible mechanistic pathway underlying the benefits of cardiorespiratory fitness on WM integrity is through an effect of exercise on inflammatory pathways. A number of studies found that plasma concentrations of the inflammatory cytokine IL-6 increase substantially during bouts of exercise (Nielsen, Secher, Christensen, & Pedersen, 1996; Pederson & Hoffman-Goetz, 2000; Pederson, Steensberg, & Schjerling, 2001). They demonstrated that as exercise intensity increased, particularly in the amount of muscle groups recruited, levels of serum IL-6 increased as well, and suggested that the likely sites of IL-6 production during exercise are contracting limb and skeletal muscle cells. These results seem somewhat counterintuitive due to the heightened levels of IL-6 found in obesity. However, the authors indicated that there may exist a difference in the effects of acute increases in IL-6, demonstrated during exercise, and those of chronically elevated IL-6 that are characteristic of obesity. One

possible explanation is that muscle-derived IL-6 operates in a different manner than IL-6 produced by adipocytes, and may have a beneficial effect on metabolic function (Lyngsø, Simonsen, & Bülow, 2002; Wallenius et al., 2002; Hall et al., 2003; Keller et al., 2003). This line of research suggests that systemic IL-6 may actually be a consequence, rather than a cause, of chronic metabolic disorders, including obesity, and may function to downregulate the action of other aspects of metabolic dysregulation, such as TNF- α (Febbraio & Pederson, 2002). Accordingly, studies in animals have shown that levels of TNF- α are elevated in IL-6 knockout mice (Matthys, Mitera, Heremans, Van Damme, & Billiau, 1995). Along a similar vein, other studies in mice have shown that aerobic exercise leads to a decrease in a number of inflammatory markers including TNF- α (Lowder, Padgett, & Woods, 2006; Vieira et al., 2006; Cook et al., 2016). However, evidence of an inflammatory-regulating effect of exercise has not been well-established in humans (Starkie et al., 2003) and more work is needed to demonstrate whether cardiorespiratory fitness influences brain health through immune mechanisms. Still, the current evidence highlights the possibility that cardiorespiratory fitness may impact WM integrity by mitigating the negative effects of elevated adiposity.

1.4 Adiposity, Fitness, and White Matter Integrity

Overall, the current view in the obesity literature is that excess adiposity is related to accelerated decay of WM microstructure (Kullmann, Schweizer, Veit, Fritsche, & Preissl, 2015) and impairment in related cognitive modalities, including executive function (Wolf et al., 2007). It has been suggested that the connection between excess adiposity and WM degeneration is mediated by increased activity of inflammatory cytokines secreted by fatty tissue (Verstynen et

al., 2013). These cytokines set off an inflammatory chain reaction that results in the death of oligodendrocytes, cells which are primary components of WM. In contrast, evidence from observational and intervention studies (Johnson, Kim, Clasey, Bailey, & Gold, 2012; Voss et al., 2013) indicates that aerobic fitness may protect WM integrity and related cognitive function. One mechanism by which this may occur is through the upregulation of neuronal growth factors, induced by increased oxidative capacity (Van Praag, Shubert, Zhao, & Gage, 2005). Proliferation of growth factors, such as BDNF, leads to increased genesis of new neurons, thereby increasing the quantity of cells that constitute WM. Another perspective suggests that increased cardiorespiratory fitness, attained through engagement in aerobic exercise, actually promotes the action of muscle-derived IL-6, which may inhibit other inflammatory cytokines that harm WM (Pedersen & Hoffman-Goetz, 2000). The question remains as to whether fitness or adiposity has a more robust influence on WM integrity and if the effects are general or regionally specific. In addition, the dynamics of these associations are not well understood. For instance, it is not known if the associations of cardiorespiratory fitness and adiposity on WM integrity are additive, or if the variables interact such that one offsets the impact of the other.

In the past, weight loss has been proposed as the most effective therapy for obesity and related morbidity (Dixon, 2010). More recent research suggests that increasing fitness, achieved through engagement in physical activity, may be another route to mitigate the adverse effects of obesity. Studies comparing the associations of adiposity and fitness with cardiovascular health suggest that this is the case (Lee et al., 2012). A number of these investigations report a surprising effect, in which individuals with overweight or obesity and high cardiorespiratory fitness tend to have better cardiovascular outcomes than their low-fit, normal weight counterparts (Lavie, De Schutter, & Milani, 2015). In one such study, McAuley and colleagues (2012) reported that among

9,563 adult men, those with low cardiorespiratory fitness experienced an increasing risk of cardiovascular events and all-cause mortality as a function of BMI. Among participants with high fitness, there was no significant difference in risk in any of the BMI categories. Moreover, a meta-analysis of 10 studies (Barry et al., 2014) demonstrated that mortality rates in low-fit individuals are double that of fit individuals, regardless of BMI. These studies emphasize the importance of aerobic fitness in playing a protective role against the impact of obesity on cardiovascular health. However, the influence of aerobic fitness in offsetting the consequences of obesity has received little attention with regard to brain structure, in general. One recent study (Boyle et al., 2015) compared the impact of physical activity, rather than fitness, and BMI on gray matter volume, and found that greater amounts of self-reported walking correlated with greater brain volume, even when controlling for BMI. Others report conflicting results, suggesting that the associations between self-reported physical activity and gray matter volume is not maintained after accounting for BMI (Ho et al., 2010). Seemingly, fitness has the potential to promote brain health. However, thus far, there has been very little research addressing whether the benefits of cardiorespiratory fitness extend above and beyond the effects of altering body fat, in the context of the brain. In addition, crucial details concerning how fitness and adiposity differentially influence distinct brain regions, and WM pathways, remain unknown.

Through a mechanistic lens, increasing fitness appears to be a plausible means of promoting WM integrity. Whether by potentially promoting axonal growth within WM, or by inducing an anti-inflammatory response, elevating fitness may be protective against adiposity-induced WM degeneration. Presently, few studies have examined the ability of fitness to mitigate the impact of adiposity on WM. One investigation (Marks, Katz, Styner, & Smith, 2010) compared the effects of VO₂max on WM with BMI and abdominal girth in 15 participants. They revealed

that while both measures of adiposity negatively correlated with FA in the right posterior cingulum, higher fitness levels were related to increased FA in the left middle cingulum. Moreover, fitness explained 28.5% of the variance in left middle cingulum FA. This suggests that adiposity and fitness both relate to WM microstructure, but in distinct brain regions. Nevertheless, given the very small sample size in this study, more research is needed to establish whether the benefits of fitness can attenuate the damage of obesity on WM. Understanding the relative impact of these factors on brain health is meaningful from a medical and public health perspective, and may better inform the development of targeted interventions and prioritization of health guidelines, particularly for those at risk for later life cognitive decline.

1.5 Current Study

The purpose of the current investigation was to examine associations between fitness, adiposity, and FA, and to evaluate whether fitness offsets the impact of adiposity throughout the brain and within specific WM pathways. Furthermore, as a strength of the current study, we assessed adiposity using DEXA, the current gold standard for measuring body fat. We focused this study on young to middle aged adults, for several reasons. First, as the majority of previous investigations that independently studied WM in obesity and fitness have focused on older adults, there is a gap in the current literature with regard to young and midlife adults. This is important from a preventative perspective, as both fitness and adiposity may require earlier intervention to substantially promote healthy WM aging. In addition, as outlined by lifespan studies, volumetric WM growth reverses in the sixth decade of life, whereas FA begins to decline several decades earlier. These findings highlight a need to investigate the impact of lifestyle on FA in younger

populations. Importantly, we aimed to focus this study on both overweight and obese adults, a sample for whom research elucidating the impact of fitness level and extent of adiposity on brain health is particularly applicable. Given that obesity is thought to accelerate the process of brain aging, it is valuable to extend the scope of this research to overweight and obesity in early adulthood and midlife.

To account for unanticipated relationships between adiposity, fitness, and WM that may arise in this unique sample of obese and overweight adults, we utilized a whole brain analysis of WM FA. Nevertheless, studies in related samples suggest that adiposity and fitness associate with FA in certain WM pathways more so than others. Based on the most consistent associations found in the literature, we hypothesized that $VO_{2submax}$, alone, would be significantly associated with FA in the corona radiata, longitudinal fasciculi, and IFO (**Hypothesis 1**), while body fat percentage, alone, would be significantly associated with FA in the fornix (**Hypothesis 2**). Furthermore, in line with previous reports, we hypothesized that both fitness and adiposity would be associated with WM in the corpus callosum and cingulum (**Hypothesis 3**). These hypothesized relationships are modeled in **Figure 1**. Mechanistic theories suggest that while the action of adiposity on WM integrity is through elevated systemic inflammation, cardiorespiratory fitness promotes heightened WM integrity by upregulating neuronal growth-factors and axonal genesis. Given these distinct mechanistic pathways, we predicted that the influence of fitness and adiposity in the corpus callosum and cingulate would be additive. However, as previous literature also suggests the possibility of a regulatory effect of aerobic exercise on inflammatory cytokines, we also planned to examine whether cardiorespiratory fitness and adiposity interact in any of these potentially significant WM pathways. Altogether, testing these associations will elucidate on the

plausibility of enhancing cardiorespiratory fitness to mitigate WM microstructural deterioration in adults with obesity or overweight.

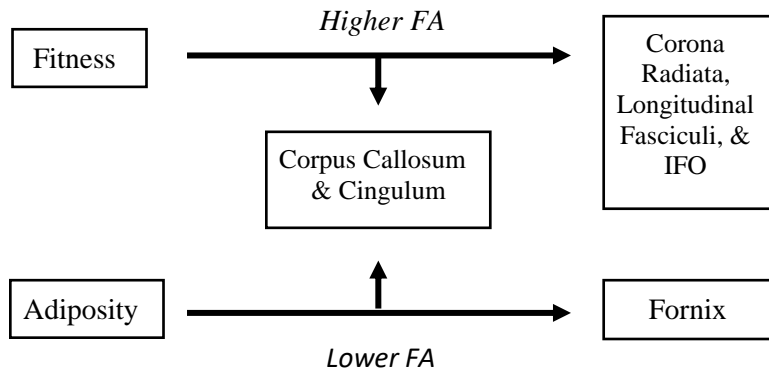


Figure 1. Hypothesized relationships between adiposity, fitness, and regional FA

1.5.1 Secondary Aims

A major strength of the proposed analysis is our use of DEXA as a measure of adiposity, as this technique quantifies body fat without the added confound of skeletal and muscle tissue weight. Yet, the majority of previous studies examining the influence of adiposity on brain health have used BMI as a metric of body fat. To assess for consistency between previous research and the current investigation, our secondary goal is to compare the associations between adiposity, fitness, and WM integrity, when adiposity is quantified by both BMI and body fat percentage. Thus, the secondary aims of this study were: (1) to examine the strength and direction of the relationship between adiposity, as indexed by BMI alone, and WM integrity, and identify pathways of significance; and (2) to identify WM pathways that are significantly associated with both adiposity, as indexed by BMI, and $VO_{2submax}$. In these regions, we also planned to assess for any potential interaction in the influence of adiposity, as indexed by BMI, and $VO_{2submax}$ on WM integrity. To address these aims, we conducted an additional whole brain analysis of WM,

substituting BMI for body fat percentage. Ultimately, we expected that the results of the secondary analysis would be consistent with the primary hypotheses.

2.0 Methods

2.1 Participants

One hundred and twenty-five participants were recruited from a parent study (PI: Jakicic) that investigated the effects of a 12-month dietary and physical activity intervention. Participants were informed of the neuroimaging ancillary study (PI: Erickson) during a baseline session of the parent study. Eligible participants interested in the neuroimaging component were required to have enrolled in the ancillary study prior to commencing the intervention.

Participants were between the ages of 22 to 55 years, with BMI indices in the obese and overweight range (25.0 – 39.9 kg/m²). Recruitment occurred in the Pittsburgh community via newspaper, radio advertisements, and direct mailing. Potential participants underwent an initial phone screening that assessed for eligibility. Participants were required to provide written consent prior to inclusion in the parent and ancillary study.

2.1.1 Exclusionary Criteria

Participants were excluded if they had: history of bariatric surgery; current medical condition that could affect body weight (e.g., cancer, diabetes mellitus); current cardiac conditions that increase risk of a cardiac event (e.g., congestive heart failure); resting systolic blood pressure higher than 159 mmHg or resting diastolic blood pressure higher than 89 mmHg; eating disorder; alcohol or substance abuse; current treatment for psychological disorders, psychotropic medication within the past 12 months, or hospitalization for depression within the past five years; report of

exercise for more than three days per week for more than 20 minutes per day in the last three months; report of weight loss greater than 5% or participation in a weight reduction diet in the past 3 months; contraindication to MRI (e.g., metal implant, claustrophobia); history or presence of neurological disorder (e.g., dementia, stroke); history of developmental pathology; traumatic brain injury; and left-handedness. Individuals who were pregnant, breastfeeding, or planning to become pregnant during the duration of the intervention were excluded from the study. Individuals with a substantial history of welding work or tattoos with metal components (e.g., iron oxide) were subject to additional safety screening prior to participation. In addition, participants were required to obtain consent from a physician to engage in both the exercise intervention and in fitness testing.

2.2 Study Protocol

The present study is cross-sectional in nature and relied solely on the baseline measurements of the parent exercise intervention. Participants attended multiple baseline assessment visits. At these sessions, participants provided demographic information and had height and weight measured by intervention staff. To assess body fat percentage, participants underwent DEXA scanning. Participants also completed $VO_{2submax}$ cardiorespiratory fitness testing, a less invasive variant of VO_{2max} testing that is terminated at approximately 85% of maximal oxygen capacity. Lastly, participants underwent an MRI protocol that included a DTI sequence.

2.3 Instruments

2.3.1 Demographics

Basic demographic information including age, race, ethnicity, gender, and education, as well as medical history were collected during the baseline session with a brief questionnaire.

2.3.2 Body Mass Index Calculation

Height and weight measurements were used to calculate BMI using a calibrated stadiometer, as weight (kg) divided by height (m²).

2.3.3 Body Composition Assessment

Baseline measures of adiposity were assessed using whole body composition DEXA. DEXA utilizes x-rays to create full body, two-dimensional images of skeletal and soft tissue structure which can then be used to calculate percent body fat. Participants were scanned for about 5 minutes in a supine position, which was adjusted to include all body parts in composition measurements.

2.3.4 Cardiorespiratory Fitness Testing

Cardiorespiratory fitness, indexed by $\text{VO}_{2\text{submax}}$, was assessed during a graded submaximal exercise test conducted on a motor-driven treadmill. Participants were instructed to walk at a speed slightly faster than normal walking pace, and at grade that increased every 2

minutes by 2%, while oxygen uptake was recorded at 30 second intervals. Heart rate and blood pressure were monitored by a trained nurse and cardiologist. $VO_{2submax}$ was calculated as the level of oxygen uptake when 85% of age-predicted maximum heart rate was reached. Testing lasted between 5 to 15 minutes depending on oxygen uptake level. Following exercise testing, the participants were given a recuperation period of 10 minutes.

2.3.5 DTI Acquisition

Diffusion weighted images were acquired during an MRI protocol [echo time (TE) = 96ms, repetition time (TR) = 11100ms] in a standard a Siemens Verio 3-Tesla magnet with a 32-channel transmit-receive head coil. Foam inserts were positioned inside of the head coil to restrict head motion. Fifty 2.4mm³ slices were imaged along the anterior-posterior commissure. T2-weighted acquisition was followed by six repetitions of a 56-direction diffusion-weighted echo planar scan (b-value = 2,000 s/mm²).

2.4 DTI Processing

DTI data was acquired in order to test the effects of cardiorespiratory fitness and body fat on WM integrity, as well as whether cardiorespiratory fitness attenuates the influence of high body fat on WM integrity.

During initial preprocessing, DTI images were qualitatively assessed for signal loss and artifact. DTI images were then processed utilizing several features of FMRIB Software Library (FSL; Image Analysis Group, FMRIB, Oxford, UK; Smith et al., 2004). Data for each participant

underwent standard eddy correction, a process that registers the brain image to a reference in order to adjust for field distortions. Next, we used the Brain Extraction Tool (BET; Smith and Nichols, 2009) to skull strip the images of non-brain tissue. Diffusion tensor values, including FA, were calculated for each voxel with the DTIfit function.

The FA data was then processed using FSL's tract-based spatial statistics toolbox (TBSS; v1.2; Smith et al., 2006). TBSS algorithms aligned FA images across multiple subjects into a standard space (Smith et al., 2006). The process then zeroed the end slices of the FA images to remove likely outliers. Next, images were aligned into the standard FSL FA brain template (FMRIB58_FA), which was pre-registered to MNI152 space. This process involved a non-linear transformation of FA images for each participant to the FSL template. Then, TBSS generated a mean FA image and a skeleton of major WM tracts (**Figure 2**) across all participants, thresholded at 0.2 (Smith et al., 2007). This is a standard thresholding value, used to ensure the inclusion of major WM tracts in the skeleton. Lastly, the normalized FA image for each participant was aligned onto the skeleton, resulting in the final image file containing all of the processed FA data that was then fed into voxelwise statistics processing.

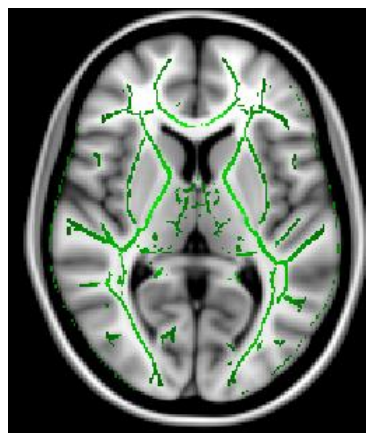


Figure 2. Example skeleton of major WM pathways created by TBSS

2.5 Statistical Analysis

We conducted a whole-brain voxelwise analysis using the FSL randomise function to examine the main effects of body fat and fitness, as well as the interaction term, on FA. Randomise examined the correlation between an explanatory variable (EV), such as VO_{2submax}, and the outcome, FA, by testing the t-statistic for each voxel against the null distribution, which was generated with a sequence of 5000 permutations. All EVs were demeaned prior to being input into the regression matrix. This is a requirement of the randomise function for both categorical and continuous variables. We also applied the Threshold-Free Cluster Enhancement (TFCE) technique at a threshold of $p < .05$ in order to correct for multiple comparisons. Age, gender, race, and years of education were included as covariates for all group level analyses. To identify all significant pathways, we overlaid the statistically-derived images of p-values at each voxel, generated by the randomise function for each main effect and for the interaction, onto the Johns Hopkins University (JHU) 1mm International Consortium of Brain Mapping (ICBM) DTI-81 atlas of WM pathways (**Figure 3**). We then extracted average FA values from significant clusters and export the data into the IBM Statistical Package for the Social Sciences (SPSS; SPSS Inc., 2011) and used the SPSS PROCESS macro for post-hoc modeling. Figures were produced using the R (R Core Team, 2014) package ggplot2 (Wickham, 2009).

Aim 1: To test our first hypothesis, we calculated the regression model of the association between body fat percentage and FA, as well as the regression model of the main effect of VO_{2submax} on FA. We identified significant pathways that corresponded solely to the association between VO_{2submax} and FA.

Aim 2: To test our second hypothesis, we again utilized the estimated regression model of the association between body fat percentage and FA, as well as the regression model of the

association between $VO_{2submax}$ and FA. We identified significant pathways that corresponded solely to the association between body fat percentage and FA.

Aim 3: To test our third hypothesis, we assessed the regression model of the association between body fat percentage and FA, as well as the regression model of the main effect of $VO_{2submax}$ level on FA. We then identified significant WM pathways that were simultaneously associated with body fat percentage and $VO_{2submax}$. In addition, to test the possibility of an interaction effect, we estimated the multiplicative regression model of the interaction term, calculated as the product of $VO_{2submax}$ and body fat percentage. We then identified significant pathways that specifically corresponded to the association between the interaction term and FA.

Lastly, in order to investigate our secondary aims, we utilized the same statistical procedures used to test our three primary hypotheses, with the substitution of BMI for body fat percentage.

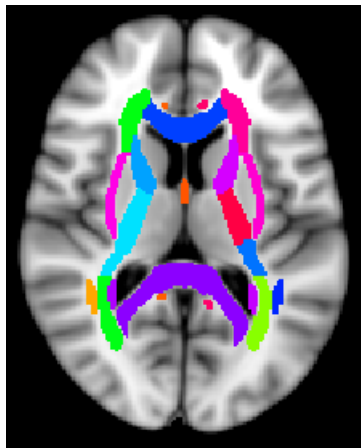


Figure 3. JHU ICBM DTI-81 white matter labels atlas

3.0 Results

3.1 Demographics

An initial 125 participants were enrolled in the neuroimaging ancillary to the parent study. During the preprocessing stage of the DTI analysis, one participant was excluded due to excessive artifact in their imaging data. For the 124 remaining participants, **Table 1** summarizes demographic characteristics. The distribution of the sample was generally skewed, with a greater proportion of female participants (79%) and individuals aged 40 or older (74.2%). In addition, the great majority of the sample identified as Caucasian/White (72.6%) or African American/Black (20.2%). **Table 2** summarizes the associations between body fat percentage, VO₂submax, BMI, and demographic characteristics. Pearson correlations revealed that male participants ($r = -.5; p < .0001$) had higher fitness levels and lower body fat percentages ($r = .5; p < .0001$) compared to women. Body fat percentage and BMI were positively correlated with each other ($r = .5; p < .0001$). Age, race, and education did not correlate with VO₂submax, BMI, or body fat percentage in this sample (all $p > 0.5$).

Table 1. Demographic characteristics

N	124
Gender (%)	
Female	79
Male	21
Years of Age (<i>M, SD</i>)	44.33 ± 8.60
Years of Education (<i>M, SD</i>)	16.40 ± 2.63
Race (%)	
Asian/Asian American	2.4
African American/Black	20.2
Caucasian/White	72.6
Multiracial	4.8
DEXA Body Fat % (<i>M, SD</i>)	0.43 ± 0.06
VO ₂ submax (<i>M, SD</i>)	22.74 ± 4.37
BMI (<i>M, SD</i>)	32.45 ± 4.19

Table 2. Associations among demographic characteristics and explanatory variables

		Body Fat %	BMI	VO ₂ submax
Gender	r	.67***	.05	-.49***
Age	r	-.06	-.12	-.12
Education	r	-.09	-.16	.10
Body Fat %	r	-	.49***	-.56***
BMI	r	-	-	-.24**

*p < .05, **p < .01, ***p < .001

3.2 Primary Results

Our primary aims involved assessing the association between VO_{2submax} and FA, as well as body fat percentage and FA, and examining potential pathways of overlap. To address the three primary hypotheses, we conducted whole-brain voxelwise analyses using the FSL randomise function to test the association between VO_{2submax} and FA, as well as the association between body fat percentage and FA.

3.2.1 Hypothesis One

Our first hypothesis was that cardiorespiratory fitness would be significantly associated with FA, such that higher VO_{2submax} would relate to higher FA values. We further predicted that given the most consistent findings in the literature, VO_{2submax}, but not adiposity, would be significantly related to FA in the corona radiata, longitudinal fasciculi, & IFO. Whole-brain voxelwise analyses did not reveal any significant main effects between VO_{2submax} and FA that met our cluster thresholding ($p > 0.05$). These results were contrary to our hypothesis in that there was no significant relationship between VO_{2submax} and FA across the brain, or within any specific regions of interest in this sample.

3.2.2 Hypothesis Two

Our second hypothesis was that adiposity would be significantly associated with FA, such that higher adiposity would relate to lower FA values. We further predicted that given the most consistent findings in the literature, adiposity, but not VO_{2submax}, would be significantly related

to FA in the fornix. Whole-brain voxelwise analyses did not reveal any significant main effects between body fat percentage and FA that met our cluster thresholds ($p > 0.05$). These results did not lend support to our hypothesis that there would be an association between body fat percentage and FA across the brain, or within any specific regions of interest in this sample.

3.2.3 Hypothesis Three

Our third hypothesis was that both cardiorespiratory fitness and adiposity would significantly associate with FA in certain WM pathways, specifically in the corpus callosum and cingulum. We first predicted that the effects of $VO_{2submax}$ and body fat percentage in these regions would be additive, such that the net influence of these variables on FA would vary as a result of a relative negative effect of higher body fat percentage and a relative positive effect of higher $VO_{2submax}$. However, whole-brain voxelwise analyses revealed that neither $VO_{2submax}$, nor body fat percentage, showed significant main effects (both $p > 0.05$) with FA. We had also posited that there may exist an interaction between $VO_{2submax}$ and body fat percentage. To test this, we estimated the multiplicative regression model of the interaction term ($VO_{2submax} * \text{body fat percentage}$). Whole-brain voxelwise analyses revealed a significant interaction between $VO_{2submax}$ and body fat percentage in several WM clusters ($p = 0.03$).

To model the data, we utilized the JHU 1mm ICBM DTI-81 WM labels atlas to extract significant clusters that overlapped with previously defined WM fiber tracts. Clusters of significance primarily corresponded to the genu of the corpus callosum (gCC), however, there was also apparent overlap with the body of the corpus callosum (bCC), both the left and right anterior corona radiata (aCR-L; aCR-R), and left superior corona radiata (sCR-L). **Table 3** summarizes

average FA values in the whole brain cluster, along with each significant region of interest. **Figure 4** depicts the overlap between the WM atlas and significant clusters.

We extracted average FA values and utilized the SPSS PROCESS macro to model the relationships among body fat percentage, VO₂submax, and FA in each of the significant regions of interest, as well as in the general cluster of significance as a whole. The results of these interaction analyses were consistent with the results of the whole-brain voxelwise analyses conducted in FSL, and showed a significant interaction between VO₂submax and body fat percentage in the whole brain cluster and each region of interest. These statistics are shown in **Table 4**. We then used the R package ggplot2 to create interaction plots depicted in **Figure 5**. The interaction plots illustrated an effect such that the association between body fat percentage and FA differs as a function of fitness level. As expected, among individuals with low levels of fitness, higher body fat percentage was associated with lower FA. However, contrary to our hypothesis, among individuals with high levels of fitness, higher body fat percentage was associated with greater FA. This effect was consistent in each of the significant WM pathways, and in the larger whole brain cluster of significance, as well.

Table 3. FA in whole brain cluster and regions of interest (*M ± SD*)

Whole Brain Cluster	.58 ± .03
Genu (Corpus Callosum)	.70 ± .03
Body (Corpus Callosum)	.65 ± .04
Anterior Corona Radiata (Right)	.50 ± .04
Anterior Corona Radiata (Left)	.44 ± .04
Superior Corona Radiata (Left)	.45 ± .05

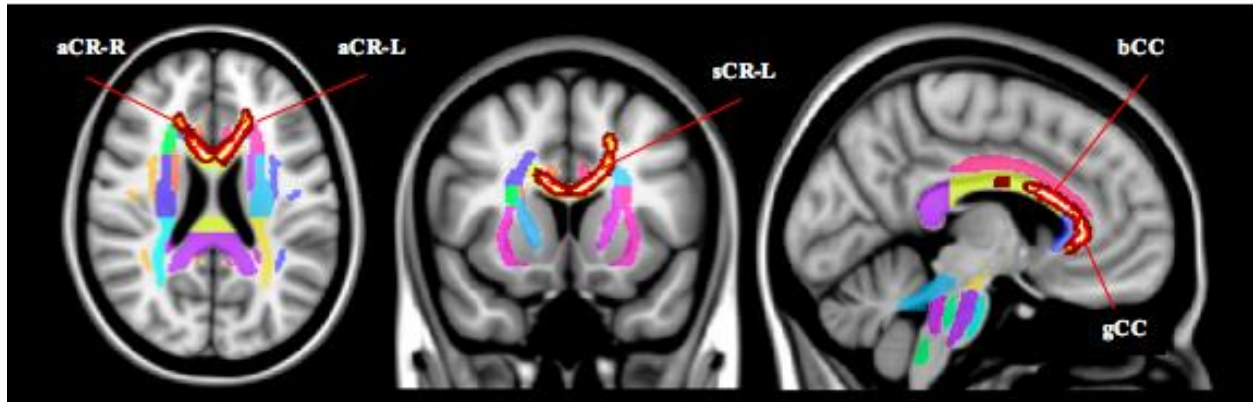


Figure 4. Significant clusters for the interaction of VO₂submax and body fat % on FA
 Clusters of significance (red outline; $p < .05$) overlaid on the JHU 1mm ICBM DTI-81 WM atlas. Corresponding WM pathways included: genu of the corpus callosum (gCC), body of the corpus callosum (bCC), both right and left anterior corona radiata (aCR-R; aCR-L), and left superior corona radiata (sCR-L).

Table 4. Tests of highest order, unconditional interaction effects (SPSS PROCESS)

	ΔR_2	$\Delta F(1, 117)$	b	s.e.	$t(117)^*$
Whole Brain Cluster	.10	16.27***	.04	.01	4.03***
Genu (Corpus Callosum)	.07	10.16**	.03	.01	3.18**
Body (Corpus Callosum)	.08	11.41**	.04	.01	3.38**
Anterior Corona Radiata (Right)	.06	8.80**	.04	.01	2.97**
Anterior Corona Radiata (Left)	.10	15.29***	.04	.01	3.91***
Superior Corona Radiata (Left)	.07	9.51**	.05	.02	3.08**

* $p < .05$, ** $p < .01$, *** $p < .001$

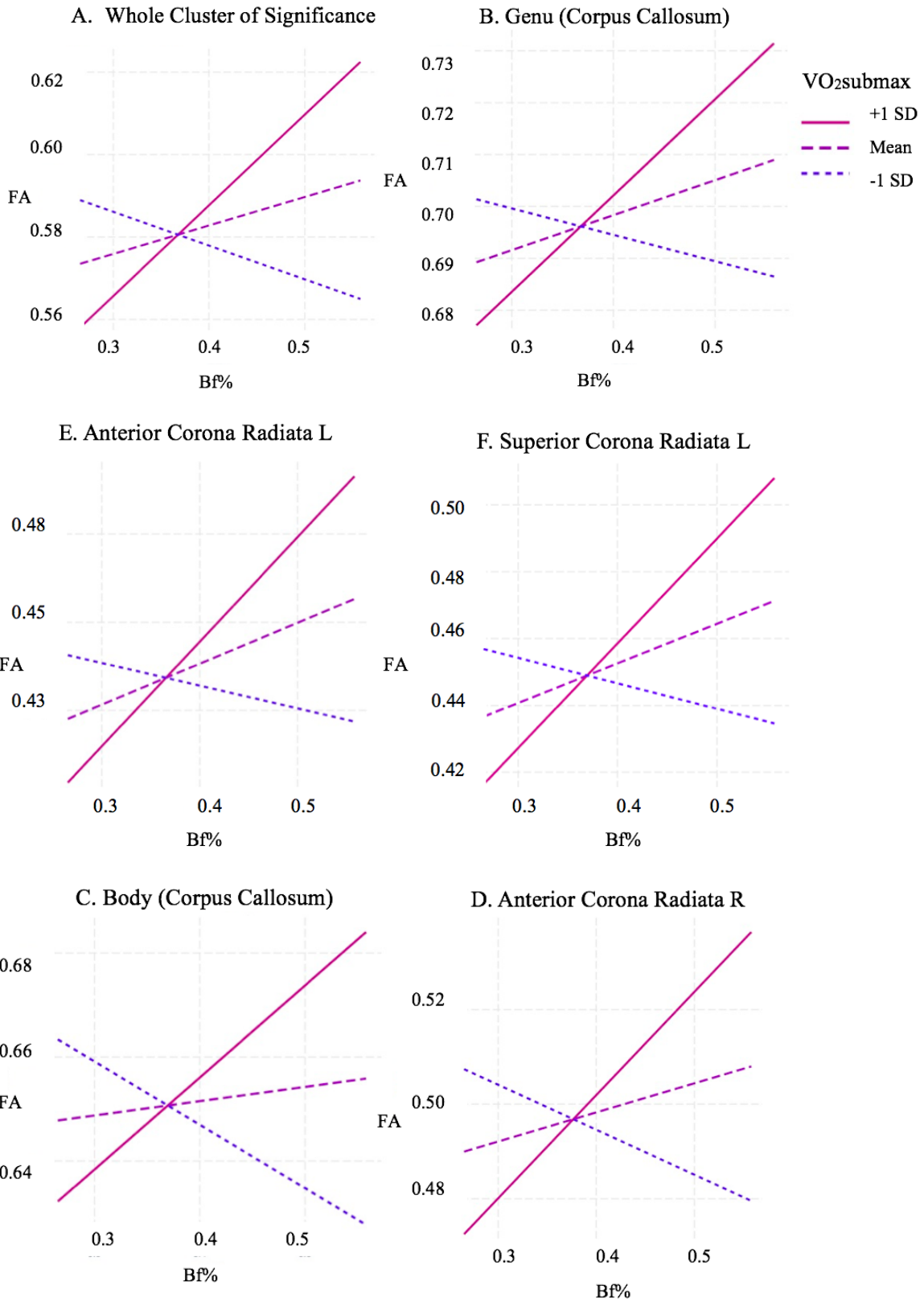


Figure 5. Plots A–F depict interactions between body fat % and VO₂submax on FA

Plots created using the R ggplot2 package demonstrate the interaction between body fat % and VO₂submax on FA in the full voxelwise significance cluster as a whole (A), the genu (B) and body of the corpus callosum (C), and the right (D) and left (E) anterior corona radiata, and left superior corona radiata (F). To facilitate graphical representation of the interaction, fitness level is partitioned into categories based on standard deviations from the mean VO₂submax level: dotted blue represents -1 SD; dashed purple represents the mean; and solid red represents +1 SD.

3.3 Secondary Results

As a majority of previous studies comparing the impact of cardiorespiratory fitness and adiposity on WM integrity utilized BMI rather than DEXA, we aimed to assess for consistency between previous work and the current investigation. Thus, the secondary aims of this study were to: (1) to examine the relationship between adiposity, as indexed by BMI alone, and WM integrity, and identify significant pathways; and (2) to identify WM pathways that are significantly associated with both adiposity, as indexed by BMI, and VO₂submax. To address these aims, we conducted the same whole brain voxel-wise analyses used to test the primary aims, substituting BMI for body fat percentage. Whole-brain voxelwise analyses did not reveal any main effects between BMI and FA ($p > 0.05$). As this null finding was also evident in the relationship between VO₂submax and FA in this sample, we were not able to assess for potential regional overlaps. To test for a potential interaction effect, we estimated the multiplicative regression model of the interaction term (VO₂submax * BMI). Whole-brain voxelwise analyses did not reveal any significant interaction between BMI and FA ($p > 0.05$).

4.0 Discussion

The primary aim of this study was to shed light on the relationships between cardiorespiratory fitness, adiposity, and WM integrity in a large sample of cognitively healthy adults with overweight and obesity. Given previous findings, we hypothesized that cardiorespiratory fitness and adiposity would be independently associated with WM integrity in a number of pathways. Specifically, we predicted that cardiorespiratory fitness, but not adiposity, would exhibit a positive association with WM integrity in the corona radiata, longitudinal fasciculi, and IFO. In contrast, we hypothesized that adiposity, but not cardiorespiratory fitness, would exhibit an inverse association with WM integrity in the fornix. We also hypothesized that both adiposity and cardiorespiratory fitness would be associated with WM integrity in a number of overlapping regions, particularly the corpus callosum and cingulum.

Although a large body of literature suggests that the action of cardiorespiratory fitness and adiposity on WM health operates through independent mechanisms, other evidence indicates that these variables impact WM through shared pathways. Accordingly, a number of scenarios plausibly characterize the associations between adiposity, cardiorespiratory fitness, and WM integrity in regions of overlap. Given the larger body of current evidence, we first hypothesized that we would find main effects of both cardiorespiratory fitness and adiposity in the corpus callosum and cingulum, indicating that the impact of these variables is independent and cumulative. In this scenario, increasing fitness may contribute to improved WM integrity, even in individuals exposed to the detrimental effects of high adiposity. Alternately, owing to the possibility that there may exist an interaction between adiposity and cardiorespiratory fitness in their influence on WM integrity, we also predicted that we may find a moderating effect of

cardiorespiratory fitness. In this scenario, we expected that any detriment of adiposity would be moderated by cardiorespiratory fitness level, such that the negative consequences of high body fat composition on WM integrity would not be apparent in those who are highly fit.

Contrary to our hypotheses, voxelwise whole-brain analyses failed to reveal any significant main effect relationships between cardiorespiratory fitness, indexed by $\text{VO}_{2\text{submax}}$, and whole brain FA. This contradicts previous findings, as most published reports examining cardiorespiratory fitness and physical activity have found significant effects on WM integrity (Sexton et al., 2016). However, the majority of these studies have focused on older adults. Although lifespan studies of WM integrity report that FA tends to peak and then slowly decline after the third decade of life, the participants in the current study ranged in age from 22 to 55 years of age. Thus, it is possible that this age range is still too early to detect fitness-related alterations in WM integrity.

Likewise, we also failed to find significant main effect associations between adiposity, measured using DEXA estimates of body fat percentage, and whole brain FA. Notably, few previous investigations that utilized DEXA reported a significant relationship between body fat percentage and WM integrity. Instead, the bulk of previously reported findings included BMI as a metric of adiposity. This, coupled with our null main effect results, indicates that body composition may simply not relate to WM integrity. Thus, to examine the consistency of our findings with previous work, we conducted additional voxel-wise analysis substituting body fat percentage for BMI. However, the results of our secondary voxel-wise analyses were similar to our primary DEXA main effects analyses, revealing that BMI did not significantly relate to FA in our sample. These results contradict a number of previously published findings, pointing to the likelihood that the unexpected outcomes of this study are a function of the unique nature of our sample.

Further, voxel-wise whole-brain analyses revealed significant interaction effects between body fat percentage and VO_2submax on FA that corresponded to a number of WM pathways. These included the genu and body of the corpus callosum, as well as the bilateral anterior corona radiata, and left superior corona radiata. However, post-hoc modeling of the associations did not corroborate our predictions. Specifically, we found that in all of the regions of significance, higher body fat percentage was related to lower FA only in low fit individuals. Unexpectedly, higher body fat percentage was related to higher FA in high fit individuals. In contrast to our predictions, and previous findings, these results appear to suggest that higher adiposity may actually be beneficial for WM integrity in highly fit adults with overweight and obesity.

Given the breadth of previous findings that elevated adiposity has a detrimental effect on brain structure, function, and overall health, it seems surprising that adiposity would be protective in fit adults. A few recent studies of dementia risk reported a paradoxical effect of overweight and obesity that reduced risk of dementia in elderly participants (Atti et al., 2007; Fitzpatrick et al., 2009). The authors noted that high BMI may play a different role in older age than in early life. For instance, individuals with overweight or obesity who live to older adulthood without experiencing fatal cardiovascular complications may have elevated health status to begin with. In addition, weight gained later in life or after menopause may not have the same harmful impact on health as long-term adiposity throughout midlife. Thus, it seems unlikely that such a protective effect of adiposity would be apparent in a young to mid-life adult sample. Furthermore, studies focusing on brain structure and WM health have not yet reported such findings.

There are a number of possible explanations for the lack of anticipated findings in this study. First, it may be possible that in a sample with overweight or obesity, variations in body fat percentage and VO_2submax are not sufficient to influence substantial differences variations in FA.

While this study was unique in its focus on young to mid-life adults with overweight and obesity, the sample did exhibit a somewhat restricted range of BMI and body fat percentage. This limited our ability to find significant effects of adiposity that may only be evident when comparing high levels of adiposity to those that are in the low range. Likewise, previous work has linked overweight and obesity to lower levels of cardiorespiratory fitness and engagement in physical activity (Olds et al., 2011; Ostojic, Stojanovic, Stojanovic, Maric, & Njaradi, 2011). Thus, it is also possible that the $VO_{2submax}$ range was restricted in this sample, when compared to healthy adults.

The demographic distribution of participants in this study was also skewed with regard to several variables. First, there was a much greater proportion of female participants than male participants. Females tend to have lower fitness and are more susceptible to adiposity than males, and this was the case in our sample. Gender also appears to influence WM integrity, with higher FA values in the splenium of the corpus callosum in males (Inano, Takao, Hayashi, Abe, & Ohtomo, 2011). Interestingly, Mueller and colleagues (2011) reported that increased BMI was significantly related to decreased WM integrity in the corpus callosum, in female participants only. Accordingly, the gender skew in our sample may have further contributed to restricted variability in the data. Moreover, we did not survey participants about menopause status, and were not able to use this variable as a covariate in the current investigation. It is not yet known how menopause may influence structural brain connectivity, and so this may have contributed to additional noise in the data.

Lastly, an important consideration is that lesions in WM, known as white matter hyperintensities (WMH), are common in aging-related brain pathology. WMH are microstructurally similar to healthy appearing WM and have been shown to confound FA results

in previous investigations. Svärd and colleagues (2017) reported that WMH load significantly alters WM integrity estimates in the dorsal and ventral cingulum, the superior longitudinal fasciculus, and the corticospinal tract. The authors noted that without adjusting for WMH, high WMH load may result in an inaccurately low estimate of WM integrity in healthy participants and an overestimation in patient populations. Although some reports show that WMH are also found at increased rates in obesity (Kim, Seo, Kwak, & Kim, 2017), we were not able to image for WMH in the current study. Thus, unknown WMH load may have significantly confounded the results in the current study, and future investigations comparing the influence of fitness and adiposity on WM integrity should adjust for WMH.

Ultimately, this study sought to examine the relationship between adiposity, cardiorespiratory fitness, and WM integrity in adults with overweight and obesity. There were several limitations in the current study that may have led to a number of null findings and unexpected results. Still, this work was the first to examine the nuances of fitness and adiposity and their relationship to WM integrity in adults with overweight and obesity, a population that is at increased risk for pathological brain aging and increased need for effective intervention. Accordingly, it is essential for future investigations to continue to examine the influence of adiposity and fitness on the brain prior to the development of later-life brain pathology. This work is particularly important as rates of obesity and dementia are projected to continue increasing, and has significant implications for early intervention trials and the development of targeted and effective changes to public health policy.

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