BODY-BRAIN DYNAMICS

- AN OBSERVATIONAL EPIDEMIOLOGICAL PERSPECTIVE

by

Qu Tian

B.Ed., Chengdu Sport University, China, 2007

M.S., University of Wisconsin – Milwaukee, 2010

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2013

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This dissertation was presented

by

Qu Tian

It was defended on

July 10, 2013

and approved by

Committee Chair:
Caterina Rosano, M.D., M.P.H.
Associate Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Committee Members:
Anne B. Newman, M.D., M.P.H.
Professor & Chair, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Robert M. Boudreau, Ph.D.
Assistant Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Howard J. Aizenstein, M.D., Ph.D.
Associate Professor, Department of Psychiatry
School of Medicine, University of Pittsburgh

Nancy W. Glynn, Ph.D.
Research Assistant Professor, Department of Epidemiology
Graduate School of Public Health, University of Pittsburgh

Kirk I. Erickson, Ph.D.
Assistant Professor, Department of Psychology
School of Arts and Sciences, University of Pittsburgh

Eleanor M. Simonsick, Ph.D.
Staff Scientist, Epidemiologist, Intramural Research Program
National Institute on Aging

BODY-BRAIN DYNAMICS
- AN OBSERVATIONAL EPIDEMIOLOGICAL PERSPECTIVE

Qu Tian, Ph.D.

University of Pittsburgh, 2013

ABSTRACT

Given the significant public health burden caused by brain abnormalities and dementia, there is a great need for identifying intervention strategies to delay the progression of brain abnormalities. Among the interventions tested, non-pharmacological approaches and lifestyle modifications have received great attention.

Indeed, the beneficial effects of physical activity on brain and mental health have been well documented. However, it remains unanswered how little and which component of physical activity could boost a change in brain structure and function, especially in adults aged 80 years and older who are at high risk of developing brain abnormalities and dementia. Answering these questions is important because structured aerobic exercise from current physical activity recommendations may not be feasible for older persons living with physical functional limitations and chronic diseases. It needs to be understood whether the positive effects of physical activity on brain health remain independent of health-related conditions. Another important question is which aspect of brain structure would benefit most from physical activity. For instance, brain areas in the frontal lobe are critical for executive function, a capstone for maintaining independence in late life, and the hippocampus is important for memory formation.

To answer these questions, I first critically reviewed the literature on the relationship between physical activity, fitness, and brain structure in older adults. Second, I collected

objectively measured physical activity data in a group of older adults with existing data on brain structure. I also analyzed existing data on physical activity, fitness, and brain structure obtained by advanced neuroimaging techniques.

The public health significance of my dissertation is to provide scientific recommendations of physical activity on preserving brain structural integrity in community-dwelling older adults. In conclusion, being exercise active and higher fitness are associated with greater microstructural integrity localized in the medial temporal lobe, independent of each other and health-related conditions. In a small subset of very old adults, more steps taken and longer duration of physical activity are associated with greater microstructural integrity in the superior longitudinal fasciculus connecting the frontal lobe and the lateral portion of the temporal lobe, independent of physical function and health-related conditions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

First, I would like to express my enormous gratitude for my advisor Dr. Caterina Rosano, for being a great mentor since I arrived at the University of Pittsburgh in 2010. Her wholesome commitment to mentoring me and her continuous encouragement allowed me to grow step by step and to become an independent scholar. She trusted my research ideas when I proposed to objectively measure physical activity for older adults in the Healthy Brain Project (PI: Dr. Rosano) during the first semester of my PhD studies. This proposal was fortunately funded by the 8th Annual Department of Epidemiology Small Grants program and made possible with her support. She always provided detailed feedback for my work and devoted her time to review my manuscripts. She introduced me to the brain lab directed by Dr. Howard Aizenstein and the multimodal neuroimaging training program to further solidify my training in Neuroimaging. She also introduced me to leading scholars and expanded my network. I am forever indebted to Dr. Caterina Rosano, my mentor and my role model.

I would like to acknowledge the excellent committee, Drs. Anne Newman, Robert Boudreau, Howard Aizenstein, Nancy Glynn, Kirk Erickson, and Eleanor Simonsick. Dr. Newman is committed to meet with me every time I ask for her guidance. She always asks me the questions that helped me to further improve my work. Dr. Boudreau is very gracious to meet with me regularly to critically review the statistics of the manuscripts. Dr. Aizenstein serves as

the brain lab director and provides me a friendly and supportive lab environment for my neuroimaging training. Dr. Glynn shares with me her valuable advice and guidance on the SenseWear Armband project and has been very supportive in coordinating my project with the Center for Aging and Population Health. Dr. Erickson has welcomed me to attend his lab meeting, trained me in the SenseWear Armband data pre-processing, and provided critical feedback on my manuscripts. Dr. Simonsick serves as a great external committee member. She communicates easily and has been available for conference calls and meetings. Her expertise has been a great resource and allows me to further the understanding of physical activity epidemiology.

I would like to thank my e-brain team- Dr. Timophy Hughes, Dr. Andrea Rosso, Karen Nunley, Ge Liu, and Anne Ritter. It is a pleasure working with this team; everyone is extremely helpful and supporting. I would like to thank the 8th Annual Department of Epidemiology Small Grants program and the Health, Aging and Body Composition Study participants, my colleague Brittney Lange-Maia and the staff at the Center for Aging and Public Health- Amy Schorr, Lori Cimino, and Sandy Burt. The SenseWear Armband project would not have been a success without their support and assistance.

I would like to thank my parents Wei Tian and Yan Gao and my family for their unending love and support, despite being a continent away. Finally, I would like to thank Thomas Sacco for his companionship, the joy he brings me, and his assistance proofreading my work throughout.

1.0 INTRODUCTION

"Exercise does more to bolster thinking than thinking does." - Reynolds, G., 2012 [83]

1.1 IMPORTANCE OF THE PROBLEM

Cognitive decline and structural abnormalities of the brain are important health outcomes among older persons. Alzheimer's disease is the most common among the dementia types in old age. According to recent data from the Alzheimer's Association, more than one in three adults aged 85 years and older is living with Alzheimer's disease today [84]. It is about 6 million adults in 2010 and estimated to grow up to 21 million by the year 2050 [84]. As of 2010, about 14.9 million family and friends devoted 17 billion hours of unpaid care to patients with Alzheimer's and other types of dementias. The estimated cost of health care was $183 billion in 2011. As the number of adults aged 85 years and older rapidly increases, a lot more pressure will add on the budgets of long-term care because this age group is at the highest risk of developing brain abnormalities and dementia [84]. Thus, it is crucial to develop strategies to prevent and delay the progression of brain abnormalities in older adults and potentially address this staggeringly costly condition of aging.

Physical activity is a so-called "best buy" for public health [1] and is a promising type of non-pharmacological intervention of cognitive decline for older adults [2]. More precisely,

specific areas of the brain which are important for information processing, mobility and mood, respond to physical activity or exercise training (see details in section 1.3). Experiments in animal models since the late 90's have shown that brain structure responds to aerobic exercise through increased neuro-regeneration [2]. More recently, the value of aerobic exercise on delaying brain atrophy is also being supported for well-functioning adults in their late-60s [2-4]. That this effect would be observed in older age is very remarkable, because brain volume is reduced as we age, especially in the prefrontal cortex and the medial temporal lobe. For example, the hippocampal volume declines about 1% annually in adults age 55 years or older, free of cognitive impairment, while other regions volume may remain stable until the late life [4]. The application of advanced precise neuroimaging methodologies is instrumental to define the response of these brain networks to physical activity. Precise and reliable neuroimaging measures can serve as biomarkers to quantify the association between physical activity and brain changes, help elucidate the underlying mechanisms and contribute to identify those adults who may be more likely to benefit from physical activity.

One of the limitations of the field is that it is not known what type, component, or amount of physical activity would lead to stronger benefits to prevent or delay age-related cognitive decline and brain abnormalities. Specifically, these relationships have not been quantified in very old adults or using precise neuroimaging methodologies and an objective measure of physical activity. Identifying the optimal dose and type of physical activity becomes even more critical for very older adults in their eighties as these adults have a number of chronic medical conditions that could affect not only brain health directly, but also the ability to engage in physical activity effectively and safely. It is important to remember that 80% of adults age 65 and older have at least one chronic health condition, and that about one in four has limited activities due to chronic

health conditions. Overall, 42% of adults aged 65 years and older have a functional limitation, and about 10% need long-term care. As longevity gradually increases, adults at the age of 65 are estimated to live with functional disability for 7.5 years of the remaining 17 years [85]. As explained below, the 'ability' to safely participate in physical activity depends on overall physical health, including muscle strength, joint and cardiovascular health. Unfortunately, most studies examining the relationship between physical activity and brain health have examined well-functioning older adults in mid- to late-60s, while largely overlooking adults older than 80. Moreover, few studies have included careful characterization of the health profile of the participants. Given that the brain is susceptible to cardiovascular diseases and risk factors which also limit the ability to participate in physical activity, it is extremely important to have longitudinal measures of exposure to these risk factors and conditions when examining the relationship between physical activity and brain health. Only very recently, intervention trials of physical activity have begun to include adults aged 80 years and older (e.g. Lifestyle interventions and independence for Elders Pilot (LIFE-P) study; [5]). While initial findings indicate that it is feasible for adults this age to adhere to a physical activity program of moderate intensity, the effects on the spatial distribution of brain abnormalities have not been carefully quantified in the context of other health-related conditions.

1.2 CONCEPTUAL MODEL

I propose a conceptual model that includes these important components (Figure 1) and an approach that is based on the application of cutting-edge neuroimaging methodologies in a

carefully characterized cohort of very old adults with retrospective data on cognitive and physical performance and health-related conditions since 9 years prior.

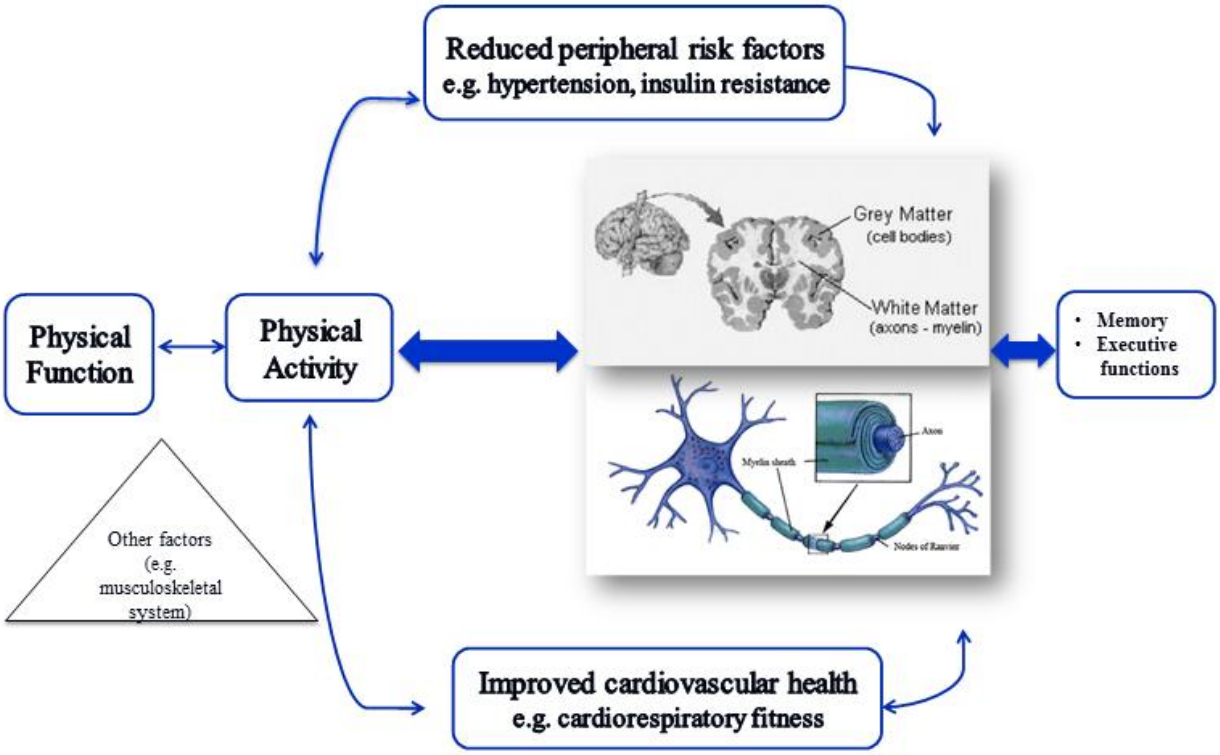

Figure 1: The conceptual model describing the relationships between physical activity, cardiorespiratory fitness, and brain structure.

1.3 PHYSICAL ACTIVITY

"The exercise program should be modified according to an individual's habitual physical activity, physical function, health status, exercise responses, and stated goals" [6].

1.3.1 Definitions

In epidemiologic and sports science research, the ***dose*** of physical activity comprises three dimensions: duration (e.g. minutes, hours), frequency (e.g. times per week) and intensity (e.g. low, moderate, vigorous/high). The intensity of physical activity is often objectively expressed in metabolic equivalents relative to a resting level (MET) [7] and the subjective intensity is expressed as the rating of perceived exertion using the Borg Rating of Perceived Exertion [86]. The ***type*** of physical activity refers to whether physical activity includes aerobic or non-aerobic (muscular-strengthening) activities. Of note, the type is different from the ***domain*** of physical activity, which is related to household, transport, leisure-time, occupation and community activities. In this document, the terms physical activity and exercise are used depending on the context after careful examination of the source material.

The measurements of physical activity can be generally grouped into two categories: subjective measures (including behavioral observation, questionnaires), and objective measures (including heart rate, calorimetry, and motion sensors) [8]. Self-report questionnaires or surveys have been commonly used in most epidemiologic studies to report the type and frequency of activities. The larger proportion of these activities is from leisure-time physical activity. Based on self-report information, estimates of energy expenditure (or *cost* of physical activity) are then derived and can be presented as physical activity doses. Using self-report population data on physical activity, compelling evidence indicates that higher doses of physical activity are associated with reduced risks of mortality and chronic diseases [9]. However, self-report questionnaires or surveys inevitably bring in reporting bias, social desirability bias and their reliability is related to the level of cognitive function [10]. Furthermore, population questionnaires or surveys are mostly related to leisure-time physical activity and do not cover all

types of bodily movements. Specifically, these measurements will overlook very low intensities of physical activity. Since very old adults are primarily involved in low-to-moderate intensity activities, self-reported measures may underestimate their physical activity level. In contrast, objective measures of physical activity, such as pedometer, accelerometer, and the SenseWear Armband, provide a valid estimation of free-living physical activity and are very sensitive to detect all types of bodily movements, including the lowest values. In 2003, the objective measure of accelerometry was first applied in the National Health and Nutrition Examination Survey to collect population data on physical activity [11].

1.3.2 Determinants

Participation in physical activity is influenced by physiological, psychological, and environmental factors. Specifically, the ability to participate in physical activity relies on physical fitness and the integrity of the cardiorespiratory systems as well as on physical performance (e.g. endurance, flexibility, range of motion, and balance [12]). In turn, a decrease in physical activity participation contributes to the decline in physical performance [13] and also to a decrease in physical fitness, including cardiovascular diseases, obesity or type 2 diabetes. In sum, a thorough understanding of the interrelationships between physical activity, fitness, and performance is crucial to identify those older adults who may have the greatest potential to gain benefits from engaging in physical activity intervention program for brain health.

Physical Activity or Fitness? Although physical activity and physical fitness are strongly inter-related, there are profound differences between the two [8, 14]. Physical activity is a behavior while physical fitness is a set of physiologic attributes. Physical activity and physical fitness can be differentially determined by an individual's health profile, such as age, sex,

genotypes, clinical and subclinical diseases, and social and environmental factors [15-17]. Thus, it is possible that some individuals with higher physical activity participation are less physically fit whereas others with less physical activity participation are more physically fit. Major determinants of physical activity include physical fitness, intention, behavioral skills, commitment, and reinforcement [18]. The major determinants of cardiorespiratory fitness include age, sex, body mass index, and physical activity level [19]. Studies have shown only a moderate association of 0.1 to 0.4 between self-reported physical activity and cardiorespiratory fitness [16, 20-22] and an association of 0.15 to 0.37 between objectively measured physical activity and cardiorespiratory fitness [23, 24]. Lower physical activity and fitness predict heart disease risk [14] and all-cause mortality [25] independently of each other.

Glossary of Terms

Physical Activity: Any bodily movement produced by skeletal muscles that results in energy expenditure above the resting level [26].

Free-living Physical Activity: All activities under free-living conditions, such as walking, stair climbing, walking while carrying a load [27].

Exercise: Physical activity that is planned and structured, with the purpose to improve or maintain physical fitness or generate other health benefits [26]. Examples: walking, jogging, running, cycling, swimming, and dancing.

Physical Fitness: A set of attributes that people have or achieve related to the ability to perform physical activity. Physical fitness can be classified into health-related fitness, which includes

cardiorespiratory fitness, and performance- or skilled-related fitness [26].

Health-related fitness is the ability to perform daily activities and it is associated with a low risk of disease conditions and complications resulting from physical inactivity, such as cardiovascular disease, obesity, type 2 diabetes, and mental health. It contains muscular strength and endurance (muscular fitness), body composition, flexibility, and cardiorespiratory fitness (the ability of the circulatory and respiratory systems to supply fuel during sustained physical activity and to eliminate fatigue products after supplying fuel). Performance- or skilled-related fitness is the ability associated with adequate athletic performance, including agility, balance, coordination, speed, power, and reaction time (neuromotor fitness) [26].

Physical function: The capacity of an individual to carry out the physical activities of daily living. Physical function reflects motor function and control, physical fitness, and habitual physical activity and is an independent predictor of functional independence, disability, morbidity, and mortality [7]. Examples: activities of daily living.

Physical Performance: The ability to carry out a physical task or sport at a desired level. It depends on both skills and physical fitness. It is also considered as an objective instrument for assessing physical function. It can be used in the context of daily activities and exercise/sport. Examples: gait speed and lower extremity function [26].

1.3.3 Guidelines

In 2008, the first-ever physical activity guidelines from the federal government of the United States were issued for Americans [87]. The rationale underlying these guidelines is based on an abundance of scientific literatures since the 1996 Surgeon General's report on physical activity and health. Some key information is conveyed to the public for all adults including elderly aged 65 years and older, especially the importance for reducing the risk of cardiovascular diseases. The main points are: 1) getting started in any physical activity is beneficial, 2) approaching a minimum of 150 minutes/week moderate-intensity aerobic activity is recommended, 3) the more, the better (dose-response relationship), and 4) single bouts of activities as short as 10 minute can be accumulated as physical activity [87]. These guidelines were supported by a systematic evidence review with most studies examining adults aged 45 to 60 years old [88] and many of the studies reviewed had applied one single variable of physical activity, mostly walking. Because the prevalence of chronic medical conditions substantially increases with age, these conditions may not allow older adults to achieve a minimum of 150 minutes/week moderate-intensity aerobic activity. Thus, older adults are guided to be "as physically active as they can". To date, it remains unclear the minimum effective dose of physical activity (duration, frequency, and intensity) and the type of physical activity to prevent detrimental health effects in very old adults who are limited in physical function.

1.4 PHYSIOLOGICAL RESPONSES TO PHYSICAL ACTIVITY

Definitions [91, 92]**:**

Maximal oxygen uptake: the maximum capacity of an individual's body to transport and use oxygen during incremental exercise, which reflects the physical fitness of the individual.

Maximal oxygen uptake = cardiac out x A-vO_2 difference

Cardiac output: the total blood volume ejected from the left ventricle of the heart per minute.

Cardiac output = heart rate x stroke volume

A-vO_2 difference: the difference in oxygen level between the arterial and mixed venous blood.

A single episode of exercise modifies the homeostasis of multiple physiological systems, including cardiopulmonary, musculoskeletal, and neurohormone systems. Regular physical activity and exercise training lead to chronic adaptation of these physiological systems to minimize the initial homeostatic modifications [90]. Specifically, long-term adaptation of physiological systems refers to changes in the structure and functions of these systems. A brief discussion of how physical activity and exercise affects cardiovascular, respiratory and skeletal muscular systems is provided. Two major types of exercise are discussed: aerobic or cardiovascular endurance exercise (e.g. walking, jogging, running, cycling, swimming, dancing, and in-line skating) and resistance exercise (e.g. strength-developing exercise).

1.4.1 What are the physiological responses to exercise/physical activity in cardiovascular and respiratory systems?

The cardiovascular and respiratory systems primarily provide the body with oxygen and nutrients and remove carbon dioxide and waste products from metabolic activities [94]. During exercise, the cardiovascular and respiratory systems metabolize the carbohydrates and fats stored in the working muscles and the liver [94]. The capacity of the cardiopulmonary system increases as the muscular workload increases till it reaches the maximal capacity that an individual's body transports and uses oxygen. The maximal capacity mainly depends on age, sex, body mass index, and physical activity level [19].

Physiological response to episodes of exercise (Figure 2): There is a proportional increase in oxygen uptake as the work rate increases during a single bout of acute exercise. Maximal oxygen uptake is a function of cardiac output and the arterial-mixed venous oxygen (A-vO_2) difference [94]. The cardiac output increases linearly with the work rate and reaches a maximum. The A-vO_2 difference increases with the work rate, from 5 ml/100 ml of oxygen delivery at rest to 15 to 16 ml/100 ml of blood at a maximal work rate [28].

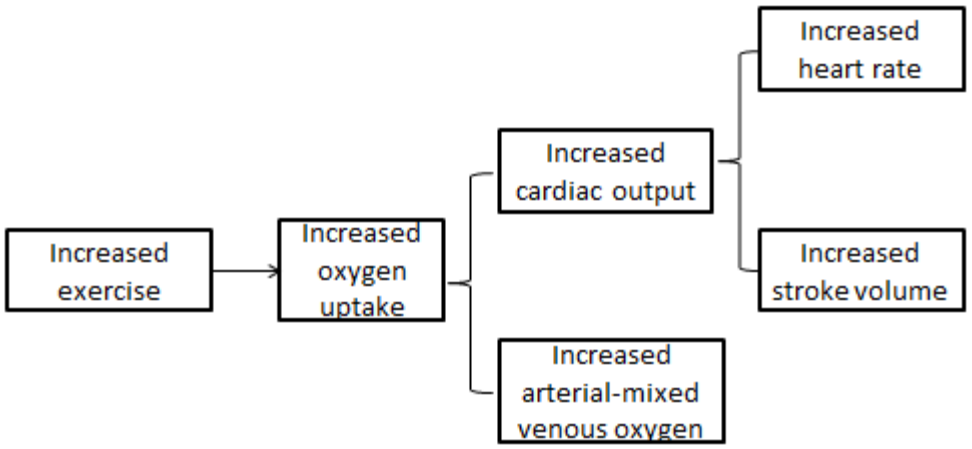

Figure 2: Physiological responses to a single bout of exercise in the heart.

Within **the vascular** system, arteries play an important role in conducting oxygenated blood to tissues that are metabolically active. The delivery of the blood is affected by the blood pressure and the resistance in the arteries and arterioles (Figure 3). During a single bout of acute exercise, the flow of blood increases substantially as the work rate increases. More blood is distributed in the working muscles and the skin with increased cardiac output and redistributed blood flow in the body [Phys]. Mean arterial pressure increases with the work rate in which the systolic blood pressure plays a dominant role because the diastolic blood pressure remains unchanged at the nearly resting level [91, 92, 93]. Patients with hypertension have greater increase in systolic blood pressure and they may also exhibit increased diastolic blood pressure compared to healthy individuals [90]. There is a substantial increase in blood pressure during acute resistance exercise which is mainly due to considerable force generated from the muscle mass [94]. The coronary circulation also changes with the work rate during a single bout of acute exercise. The coronary blood flow is closely correlated with the demand in myocardium oxygen and both increase during acute exercise [94]. The myocardial oxygen consumption is mainly determined by heart rate, myocardial contractility, and wall stress, which all increase during a single bout of vigorous exercise [95].

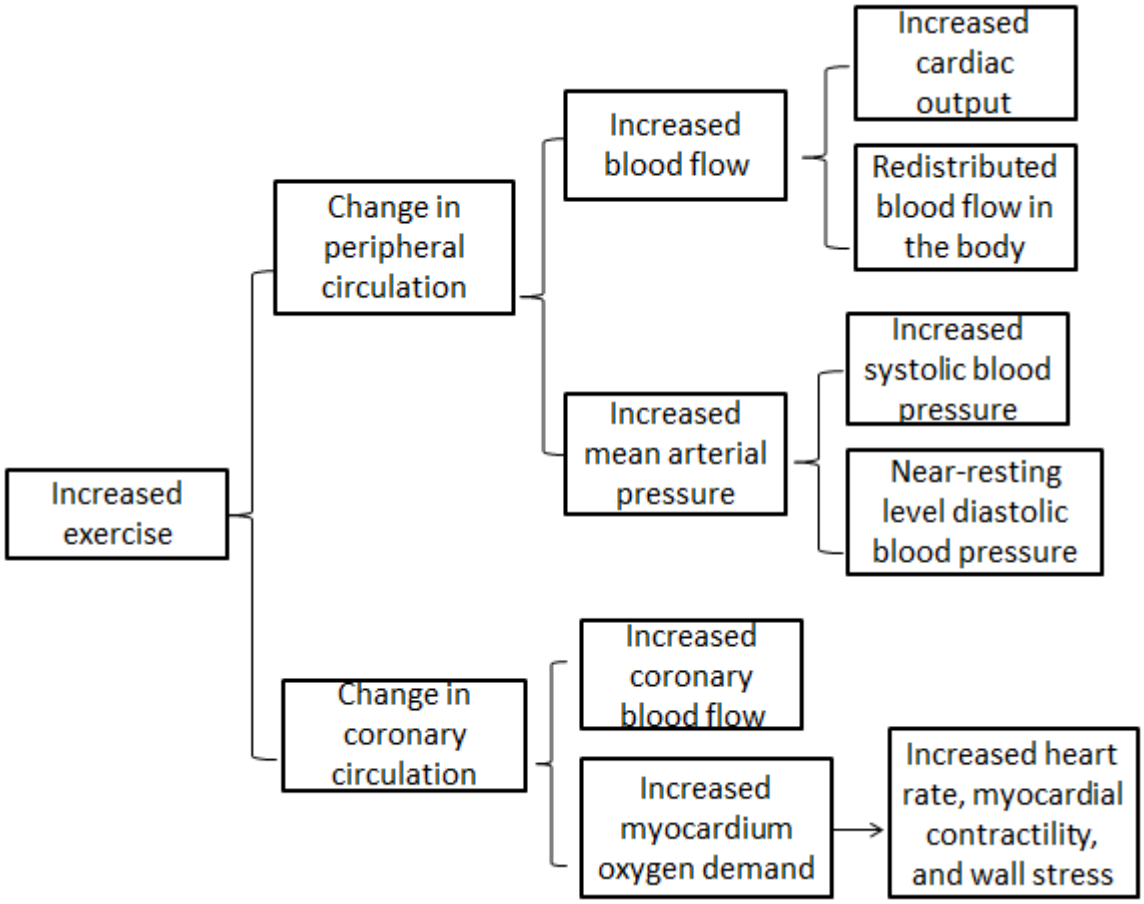

Figure 3: Physiological responses to a single bout of exercise in the circulatory system.

In the **respiratory system**, the primary change during acute exercise is the immediate increase in pulmonary ventilation. This increase in pulmonary ventilation is stimulated by the respiratory centers in the motor cortex with feedback from the working muscles and joints [94]. The pulmonary ventilation further increases during the prolonged exercise due to the increased CO_2 production, hydrogen ions (H+), and body and blood temperatures [94].

Chronic adaptation to regular physical activity: Exercise training, in particular cardiovascular/aerobic type, increases cardiorespiratory fitness (i.e., the capacity of the body to use oxygen) and improves cardiopulmonary function [29]. Exercise training improves cardiorespiratory fitness by enhancing the efficiency of cardiovascular and respiratory systems,

such as increasing blood volume at each stroke, enlarging heart muscles, increasing the number of small arteries in skeletal muscles and increasing the amount of oxygen to supply body tissues [29]. Exercise also has a favorable effect on the dilation capacity of blood vessels and on the vascular wall permeability [29]. Specifically, the major change is the increased cardiac output at or near maximal work rate. The stroke volume increases at rest, submaximal exercise, and maximal exercise, while heart rate decreases at rest and submaximal exercise but relatively stable during maximal exercise [94]. Thus, the increase in stroke appears dominant in the cardiac output increase. Several factors account for the increased stroke volume, including increased plasma volume, improved heart structure, increased number of capillaries and declined arterial blood pressure [94]. First, the increased plasma volume prompts a greater amount of blood volume returning to the right heart and the left ventricle and also leads to the increased end-diastolic volume. Second, regular physical activity increases the size of cardiac muscle fibers (e.g. hypertrophy). The hypertrophy leads to greater muscle mass of the ventricles and allows the heart to pump more forcefully at each stroke. The thickness of the posterior and septal walls of the left ventricle is also increased by regular physical activity and it leads to a more efficient contraction of the left ventricle and more blood will be emptied from the left ventricle. Third, regular physical activity increases the number of capillaries and the capacity of blood flow in the skeletal muscles, which are associated with a decreased resistance of peripheral system. Thus, the left ventricle can contract more efficiently with a lower peripheral resistance.

In the respiratory system, the major adaptation from regular physical activity is the increase in pulmonary blood flow. Consequently, there is an increase in tidal volume and respiration rate as well as an increase in pulmonary diffusion at maximal work rate. All converge to increase the maximal rate of pulmonary ventilation.

1.4.2 What are the physiological responses to exercise/physical activity in skeletal muscular system?

The primary function of the **skeletal muscular system** is to navigate the bodily movement during the exercise.

Physiological response to episodes of exercise: In the skeletal muscular system, the main physiological response to a single bout of acute exercise is involved with three basic energy systems: the ATP-PCr system, the glycolytic system, and the oxidative system [94]. During vigorous exercise for a short amount of time, ATP-PCr system is the primary energy system to provide energy at very high rates. Usually, ATP and PCr stores are used up in 20 seconds but will sustain in order to complete the exercise. During acute exercise from 30 seconds to 2 minutes, such as 100-800-meter track races, the glycolytic energy system is the primary system only using glucose from blood plasma and glycogen in muscle and the liver, to produce ATP in the absence of oxygen [30]. This energy system produces lactate. The production of lactate stops when enzymes stops functioning at the PH value of approximately 6.6 [94]. The blood lactate levels maintain at the near-resting level during the low-intensity exercise, and it starts to increase above the resting level as the intensity increases. The starting point at which the blood lactate concentration increases above the resting level is the lactate threshold. During low-intensity exercise for a long time, such as long-distance running, the oxidative energy system is the primary system using oxygen in the mitochondria to produce ATP.

Chronic adaptation to regular physical activity: Regular aerobic physical activity at low- and moderate-intensity levels leads to an increase in slow-twitch fibers [31]. Fast-twitch type ‘b’ fibers usually transit to fast-twitch type ‘a’ fibers which have a higher oxidative capacity [31]. The activity of oxidative enzymes and the size and number of mitochondria also increase [32].

The amount of oxygen in the muscle fibers substantially increases due to the increased myoglobin content in the muscle. The increased number of capillaries in skeletal muscles offers a higher capacity of blood flow in the skeletal muscles. Regular aerobic physical activity also improves the muscle's capacity to store glycogen [33]. The capacity to mobilize free-fatty acids from fat depots and to oxidize fat increases which leads to greater utilization of fat as an energy source in the body. The increased capacity to metabolize fat also results in increased muscle enzymes for fat oxidation [89]. Resistance training increases both the size and number of myofibrils in both fast-twitch and slow-twitch muscles fibers and leads to a consequence of fiber hypertrophy. The fiber hypertrophy and increased recruitment of muscle fibers from resistance training result in muscular strength and endurance and influences fat-free mass and body composition.

1.4.3 Effect of age on cardiorespiratory responses to physical activity

As explained in the introduction, age can have a substantial impact on the ability to participate in physical activity and how the body responds to physical activity and exercise.

Physiological functions decline with older age and this decline will primarily influence an individual's ability to participate in physical activity. For example, both cardiac output and stroke volume decline with age. Cardiac output decreases 1% annually since early adulthood and stroke volume could decrease by 30% before the age of 85 [85]. Age-related pulmonary function decline adds 20% burden to the respiratory muscles, including decreased elasticity of lungs, greater stiffness of the chest walls, and weakened respiratory muscle strength. In addition, number of alveoli and capillaries decreases and the pulmonary vital capacity also decline with age. Specifically, the vital capacity of the lungs, defined as maximal amount of air that an individual can exhale from lungs at the end of a maximal inhalation, decreases by up to 50% by

the age of 70. Age-related changes in vasculature elasticity and permeability and cardiac output can worsen or precipitate into clinically overt cardiovascular conditions, such as hypertension, stroke and peripheral vascular disease.

Age-related decline rate of maximal exercise capacity (VO_2max) is 1% per year since the age of 30 and about 0.5% per year for athletes [85]. Many physiological factors may account for this change, including changes in body composition and cardiovascular function. Notably, age-related decline in exercise capacity can be preserved or improved through exercise training. Exercise training may enhance the sensitivity to circulating catecholamines and reduce peripheral vascular resistance, and the two converge to increase stroke volume [85]. It has been shown that moderate-intensity exercise in older adults can increase exercise capacity by 20% to 30%, which is comparable to observed increase in young adults [85].

In response to the same absolute rate of work, older adults also have lower stroke volume and higher heart rate in order to maintain cardiac output compared to young and middle-aged adults. It shows that blood pressure or submaximal heart rate during exercise may be the major determinant to explain the individual differences in response to regular physical activity or exercise training [15]. Therefore, the amount of improvement in physical fitness in older adults depends on baseline physical activity level, genetics, environmental conditions, and more importantly health profiles.

2.0 REVIEW OF RELEVANT LITERATURE

2.1 WHAT DO WE KNOW ABOUT PHYSICAL ACTIVITY AND BRAIN STRUCTURE

The intriguing relationship between physical activity/exercise and brain health has been examined in both animal and human studies (for review, see Kaliman et al. 2011 [34]). It has been known that exercise triggers neurogenesis in the brain by inducing growth factor cascades and stimulating synaptic plasticity [35-42]. Exercise enhances synaptic plasticity by changing neuronal structures and stimulates the production and expression of growth factors, such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF). These growth factors play an important role in the structural change in the brain. BDNF can expand neural structures while VEGF triggers the production of the new blood vessels. Exercise-induced BDNF expression in hippocampus plays an essential role in improving cognitive function in aged brains. Exercise also modifies peripheral levels of the neurotransmitters that are important for cognition and mood, such as serotonin, adenosine, norepinephrine, γ-Aminobutyric acid (GABA), and opioidergic release [43]. These findings suggest that exercise-induced transmitters may mediate cognitive performance changes related to physical activity. Notably, neurotransmitters are also regulated by BDNF.

Besides this direct biological cascade underlying exercise-induced brain structural changes, it's also proposed that exercise may promote brain health by improving cardiovascular health and by reducing peripheral risk factors. The improvement in cardiac output related to physical activity may also lead to increased cerebral blood flow and enhanced cerebrovascular function. Further, exercise can protect against brain dysfunction and neurodegeneration also by reducing cardiovascular conditions/diseases outside of the central nervous system, especially hypertension, insulin resistance, obesity and type 2 diabetes [44]. All these three pathways (cell proliferation via growth factors release, cerebrovascular function via enhanced cardiovascular health, and the reduction of peripheral cardiovascular conditions/diseases) can converge to improve brain health and delay the onset, progression and/or clinical manifestations of the underlying neurodegenerative events [29]. This conceptual model illustrates the important role of cardiovascular health in the relationship between exercise and brain health (Figure 1). The key biomarker of cardiovascular health hereby examined is the cardiorespiratory fitness.

The beneficial effects of exercise have been shown consistently in observational and intervention studies [4]. Findings are more provocative among older adults as this population may potentially gain larger benefits from engaging in physical activity (e.g. larger space for improvement). Observational studies show that higher doses of physical activity measured by either self-report questionnaires or the objective measure of accelerometer are associated with higher performance scores in cognitive tests among older adults [45, 46] and are also associated with less cognitive decline over time [45, 47]. There is also strong evidence that higher doses of physical activity are associated with a reduced incidence of cognitive impairment [48] and predict a lower risk of dementia [49, 50]. Even higher doses of midlife leisure-time physical activity predict a reduced risk of dementia in later life [51].

2.1.1 Synopsis of the relationship between physical activity and brain structure

Neuroimaging studies indicate that higher doses of physical activity are associated with greater gray matter volume of the hippocampus, prefrontal and cingulate cortices [52], greater white matter of the corona radiata extending into the parietal-occipital junction [53], and greater white matter integrity [54]. Longitudinal data suggest that higher doses of physical activity predict greater gray matter volume of frontal, occipital, entorhinal, and hippocampal regions over time [55]. As reviewed thus far, most neuroimaging evidence shows that the association of physical activity with brain structures is focalized in hippocampal areas, important for memory, and in fronto-parietal regions important for processing speed (see Table 1).

Table 1: Articles on the association between physical activity and brain structure in adults aged 65 years or older.

Authors, year	Study design	Sample	Age	Measures of PA	Neuroimaging measures	Covariates	Findings
Rovio et al. 2010	Prospective cohort (mean follow-up=20.9 years)	N=75 (31 control, 23 MCI, 21 dementia)	Mean age=73 for the active group, 72 years for the sedentary group at the time of MRI	Two levels of PA (active=leisure PA ≥ twice/week; sedentary=leisure PA<twice/week) by a self-administered questionnaire	MRI at 1.5 T. WML severity, total brain volume (GMV+WMV), GMV, and WMV	Age, sex, dementia or MCI dx, education, follow-up time, systolic BP, total serum cholesterol, BMI, apoE ε4, and smoking.	Higher PA-greater GMV (localized in middle frontal gyrus) and larger total brain volume after covariates adjustments.
Erickson et al. 2010	Prospective cohort	N=299 cognitively normal at the time of MRI	Mean age=78 at the time of MRI	The number of blocks walked over 1 week by a self-reported questionnaire	MRI at 1.5T. GMV 9 years after PA assessment	Total intracranial volume, gender, BMI, race, WM grade, MRI infarcts, ventricular grade, time to walk 15 feet, education	Higher PA-greater GMV in frontal, temporal, occipital regions, entorhinal cortex and hippocampus after covariates adjustments.
Ho and Caji et al. 2010	Cross-sectional	N=226 cognitively normal	Mean age=77.9	The number of kilocalories of energy expended per week estimated by a self-report questionnaire	MRI at 1.5T. Brain volumes	Age, sex, and education	Higher PA-greater WMV in the corona raiata extending to the parietal-occipital junction after covariates adjustments.
Bugg and Head, 2011	Cross-sectional	N=52 cognitively normal	Mean age=69 (ranged from 55-79 years old)	Two levels of PA (low vs. high) by a self-ported PA in past 10 years	MRI at 1.5T. GMV in the prefrontal, parietal, temporal, occipital, neostriatal, and medial temporal regions	None	Higher PA-greater GMV in superior frontal cortex.
Benedict et al. 2012	Cross-sectional	N=331 cognitively healthy	Mean age=75	Four levels of PA (very low, low, medium, and high) by a self-reported	MRI at 1.5T. Brain volumes	Sex, education, serum low density cholesterol, mean arterial blood	Higher PA-greater total brain volume, WMV, and GMV in a cluster in

Table 1 Continued

				questionnaire		pressure, abdominal visceral fat volume, plasma glucose concentrations	bilateral precuneus after covariates adjustments.
Gow et al. 2012	Cross-sectional	N=691 cognitively normal	Mean age=72.5 at the time of MRI	The amount of PA levels (rated on 6-point scale based on self-reported information)	MRI and DTI at 1.5T. Brain atrophy, GMV, NAWM, WML, FA and MD in white matter tracts	Age, IQ at age 11, social class, hypertension, cardiovascular disease, stroke	Higher PA-greater GMV and NAWM, and less atrophy after covariates adjustments.

Abbreviations: PA=physical activity; GMV=gray matter volume; WMV=white matter volume; DTI=diffusion tensor imaging; IQ=intelligence quotient.

2.1.2 Gap in knowledge 1: accurate measures of physical activity and brain structure

Little empirical data exist on the association between dimensions of free-living physical activity (duration, intensity and frequency) and brain structure in very old adults. Little concrete information is available on the intensity, duration, frequency, or type of physical activity that could be undertaken safely and effectively by very old adults. Most studies have used composite measures of physical activity exposure that combine activity type, duration, and frequency from the self-report questionnaires. Findings from this work are somewhat mixed. For example, one study reported that high, not moderate activity level was associated with reduced cognitive decline [47], while another showed that self-report moderate or high activity were associated with lower risk of incident cognitive impairment over two years [45]. I am aware of only one study focusing on physical activity dimensions and it indicates that change in physical activity intensity, not duration, is associated with 5-year cognitive decline in processing speed among middle-aged adults [56].

Additionally, prior studies have mostly relied on subjective measures of physical activity, such as self-reported measures of 'presence' of physical activity and studies have yielded inconsistent findings. Only one study using the objective measure of physical activity (ActiCal accelerometer) examined the association with cognition in an older population [46]. This study indicates that objectively measured physical activity, not self-reported, was associated with cognition. This study supports the notion that the objective measure of physical activity might be more sensitive in collecting lower intensity levels of physical activity as compared to self-report measures. However, this study did not address the dose-response relationship of free-living physical activity with cognition. Of note, a major recommendation of the National Institutes of

Health Cognitive and Emotional Health Project [57] and of the Centers for Disease Control and Prevention and Alzheimer's Associations sponsored Healthy Brain Initiative: A National Public Health Roadmap to Maintaining Cognitive Health, released in June 2007 is the need to use objective measures to precisely quantify the effect of physical activity on cognition and to objectively formulate prescriptions of physical activity for reducing the risk of cognitive decline and improving cognitive function.

Another major gap in knowledge in this line of research is accurate measures of brain structure. Most of prior studies examining the association of physical activity with brain structures rely on volumetric measures of the brain structure. A few studies performed multimodal neuroimaging analyses at the macro- and micro-structural level. Investigating the relationship of physical activity with brain macro- and microstructures may explain which aspect of brain function would benefit the most from free-living physical activity. For example, physical activity could be beneficial for those frontal-parietal- subcortical networks, also known as watershed areas, particularly susceptible to changes in blood oxygenation levels due to the cardiorespiratory fitness nature of free-living physical activity [58]. These networks are the key to support information processing and executive function [59, 60] and are two domains important for maintenance of cognitive function.

Table 2 summarizes the multimodal measures of brain structure that I have learnt and used during my PhD studies. They include gray matter volume and white matter hyperintensities (WMH) and also the integrity of the tissue at the micro-structural level, mean diffusivity for normal appearing gray matter regions and fractional anisotropy for normal appearing white matter tracts. The measures of both macro- and micro-structure has been previously validated and applied in aging research to detect tissue abnormalities in the brain [61, 62].

Table 2: Brain MRI measures for gray and white matter (continuous variables). Volumes of white matter hyperintensities (WMH) are normalized by total brain volume.

Level of Precision	MRI sequence	Characteristic Measures	Units (Direction)
Macro-structure	T1-weighted	Gray matter volume	cm^3 (higher is better)
	T2-FLAIR	Volume of WMH	cm^3 (higher is worse)
Micro-structure	Diffusion Tensor Imaging (DTI)	Mean Diffusivity of gray matter	Unitless (higher is worse)
		Fiber Alignment of white matter	Unitless (higher is better)

WMH refers to regions within the white matter with high intensity on T2 weighted MRI images. Since the white matter is where the connecting tracts reside, their presence reflects connectivity abnormalities. The presence of WMH is common in normal aging. The prevalence of WMH is about 11-21% at age 64 and 94% in adults aged 82 [63, 64]. Previous research has shown that WMH is associated with the deterioration of cognitive function [65]. The presence of WMH is an important marker of small vessel disease, especially in older persons [66]. WMH is also seen in some neurological diseases and psychiatric disorders, such as major depression and bipolar disorders [67, 68].

Diffusion tensor imaging (DTI) is a measure of molecular diffusion in brain tissues due to its high sensitivity of detecting the motion of molecules. It mainly reflects the interactions of water molecules with other barriers, such as fibers and macromolecule. The integrity of the micro-structure is measured separately for normal appearing gray matter (mean diffusivity) and white matter (fractional anisotropy) [69, 70]. Mean diffusivity is related to the cellular size and cell membrane integrity and lower values of mean diffusivity in the gray matter indicate higher tissue integrity [70]. The fractional anisotropy reveals the orientation of cellules within the tracts and higher values of fractional anisotropy in the white matter indicate higher connectivity integrity [69]. More specifically, component diffusivities may provide more detailed information on the integrity of axons and the myelin sheaths. For instance, axial diffusion (AD) and radial

diffusion (RD) are indices of the eigenvalue of the primary axis and the average rate of the two perpendicular eigenvalues, respectively. They are thought to reflect axonal differences (i.e. axonal damage or loss), and variation of the myelination, respectively. DTI has been used to examine the severity of myelin degeneration in multiple sclerosis [71] and to distinguish Alzheimer's disease from vascular and other types of dementia [72]. It is a promising tool to investigate microscopic details of brain tissues non-invasively. Different indices derived from DTI may provide us important information on the brain's integrity from different biological perspectives. Both global (FA and MD) and component (AD and RD) markers may give us a comprehensive view about which aspect of the brain microstructure may occur with change in physical activity levels and where in the brain we may observe the change.

To address the gap in knowledge, **paper #1** quantified the cross-sectional associations of physical activity dose, duration, and intensity by the SenseWear Armband (SWA) with brain structures in adults aged 80 and older. In December 2010, I obtained initial departmental funding to objectively quantify physical activity in participants of the Healthy Brain Project (PI: Dr. Caterina Rosano) who were receiving the follow-up brain MRI and extensive neuropsychological evaluations in 2010-2012. Among various objective measures of physical activity, I chose the SWA because it provides good accuracy and reliability both at rest and during active physical activity at light, moderate, and high intensities [73, 74]. The SWA collects a variety of physiologic data through portable multiple sensors (a three-axis accelerometer, heat flux sensor, skin temperature sensor, near-body ambient temperature sensor, and galvanic skin response sensor). The unique combination of multiple sensors of SWA overcomes the limitations of other objective assessment. It is suggested for use in clinical and non-clinical research for its good accuracy, moderate price, and the ease of use in measuring physical activity [75]. In

addition, SWA can detect various types of activities, using the pattern detection algorithms based on the physical signal. Data collection started in Mary 2011 and was finished in September 2012, a total of 174 SWA were offered to participants seen at the clinical visit or home visit. Of these 174, 134 participants worn the armband, 7 were ineligible, 30 refused, and 3 were missing devices. Data with on-body time ≥ 3 days were usable for analysis. Of 134, 96 had concurrent MRI data. With these data, I tested the hypothesis that higher PA dose, duration, and intensity, is associated with greater structural integrity of brain white matter in adults aged 80 years and older. I also tested the hypothesis that these associations would be attenuated by physical function and health-related conditions known to be related to both physical activity participation and brain health. Because of its cross-sectional design, this analysis cannot provide direct information on causality.

2.2 WHAT DO WE KNOW ABOUT THE MODERATING EFFECTS OF PHYSICAL FUNCTION AND HEALTH-RELATED CONDITIONS ON THE ASSOCIATION OF PHYSICAL ACTIVITY WITH BRAIN STRUCTURE?

As described in section 1.2, it is important to consider the interrelationships between physical activity, fitness, and physical function within the context of ageing in quantifying the associations between physical activity and brain structure in very old adults. Physical function is not only a reflection of both physical activity and physical fitness (including cardiorespiratory/aerobic fitness), but also a reflection of an individual's health profile, such as chronic disease conditions. Although some of previous studies have accounted for health-related

conditions, none of the studies have extensively addressed the potential confounder or moderating effect of physical function and/ or health-related conditions.

2.2.1 Gap in knowledge 2: moderating effects of physical function and health-related conditions

To address this gap in knowledge, **paper #2** quantified the longitudinal association of physical activity with microstructural integrity of the brain and the moderating effects of physical function and chronic disease conditions known be to related to both physical activity participation and brain health.

2.3 WHAT DO WE KNOW ABOUT THE RELATIONSHIP BETWEEN CARDIORESPIRATORY FITNESS AND BRAIN STRUCTURE AND THE CONTRIBUTION OF PHYSICAL ACTIVITY TO THIS RELATIONSHIP?

"In the old, fitness will enhance quality of life (beyond just activities of daily living). In the oldest old, fitness will help with maintaining independence." [85].

Cardiorespiratory fitness is a major indicator of free-living physical activity and a more important marker of cardiovascular health. It also refers to aerobic fitness, aerobic capacity, or cardiorespiratory endurance. It measures the capacity of cardiovascular and respiratory systems to deliver oxygen to skeletal muscles during sustained exercise. Cardiorespiratory fitness is mainly determined by the level of free-living physical activity. It is well known that exercise training, in particular aerobic exercise, strengthens cardiopulmonary function by increasing the

capacity to transport oxygen to working muscles. It also ensures delivery of oxygen and of other nutrients to brain regions with increased cerebral blood flow. Thus, improvements in cardiovascular function and/ or fitness may serve as one underlying pathway between physical activity and brain health. Cumulative evidence shows that higher levels of cardiorespiratory fitness are cross-sectionally associated with higher scores on cognitive tests [58] and preserve cognitive function over time [60]. Neuroimaging studies also indicate that higher levels of cardiorespiratory fitness are associated with greater gray matter of the dorsolateral prefrontal cortex, medial-temporal lobe, and parietal cortex [76-78] and also with higher white matter integrity of the corpus callosum [79-81].

2.3.1 Synopsis of the relationship between cardiorespiratory fitness and brain structure

Table 3 summarizes the literature on the association between cardiorespiratory fitness and brain structure in older adults with a focus on observational and intervention studies.

Table 3: Articles on the association of cardiorespiratory fitness (CRF) and brain structure in adults aged 65 years or older.

Author, year	Study design	Sample	Age	Measures of CRF	Neuroimaging measures	Covariates	Findings
Colcombe et al. 2003	Cross-sectional	N=55 cognitively normal	Mean age=66.5 (ranged from 55 to 79)	VO_2max by graded maximal exercise test (GXT)	MRI at 1.5T. GM and WM atrophy	Caffeine, HRT, smoking, alcohol, education, and hypertension	An age by VO_2max interaction for GMV in prefrontal, superior parietal, middle/inferior temporal cortices and WMV in anterior tracts and in transverse tracts running between the frontal and the posterior parietal lobes after covariates adjustments. No main effect of fitness.
Colcombe et al. 2006	Intervention (6 months)	N=59 older adults plus 20 younger controls	Mean age=66.5 (ranged from 60-79 years)	VO_2max by GXT	MRI at 1.5T. Brain volumes	None	Aerobic exercise- increased GMV in the frontal lobes and WMV in the anterior WM tracts
Marks et al. 2007	Cross-sectional	N=28 cognitively normal (13 younger; 15 older)	Younger=24; older=70	Estimated VO_2max based on age, sex, BMI, self-reported physical activity	DTI at 4T. FA maps from white matter	Age and sex	Higher CRF- higher FA in the uncinate fasciculus and cingulum
Burns et al. 2008	Cross-sectional	N=121 (64 non-demented; 57 early AD)	Mean age=73.5 (Non-demented=72.7; early AD=74.3)	VO_2max by GXT	MRI at 3T. Brain atrophy	Sex, dementia severity, PA, physical frailty	For early AD only, higher CRF- greater whole brain volume and WMV after adjustment for age.
Honea et al. 2009	Cross-sectional	N=117 (56 non-demented; 61 early AD)	Mean age=73.8	VO_2max by GXT	MRI at 1.5T. Brain atrophy	Age, sex, and education	For early AD only, higher VO_2max- greater WM in bilateral inferior parietal cortex and WM in hippo region and GM in parahippocampal region
Erickson et al. 2009	Cross-sectional	N=165 cognitively normal	Mean age=66.5 (ranged from 59-81)	VO_2max by GXT	MRI at 3T. Hippocampal volume (adjusted for ICV)	Age, sex, education, and ICV	Higher CRF- greater hippocampal volume.
Erickson et al. 2011	Intervention (1-year)	N=120	Mean age=67.6 for aerobic exercise group	VO_2max by GXT	MRI;Hippocampal volume	None	Aerobic exercise training- greater hippocampal volume

Table 3 Continued.

			and 65.5 to stretching and toning group				
Marks et al. 2011	Cross-sectional	N=15 cognitively normal (8 active, and 7 sedentary)	Mean age=66	VO_2max by GXT	DTI at 3T. FA maps from white matter	Age and sex	Higher VO_2peak- higher FA in the middle and posterior cingulum and lower MD in the middle cingulum after adjustment for age and sex.
Sen et al. 2012	Cross-sectional	N=121 (64 non-demented and 57 early AD)	Mean age=65 (ranged from 44-83)	VO_2max by GXT	MRI at 3T. WML and BPF	Age, sex, HTN, diabetes, smoking status, total cholesterol, BMI, treatment by beta and CA channel blockers	In men only, higher VO_2max- Less WML after covariates adjustment
Johnson et al. 2012	Cross-sectional	N=26 cognitively normal	ranged from 60-69	VO_2max by GXT	DTI at 3T. FA from white matter tracts, RD, AD, and MD	Age, sex, and SES	Higher CRF (1-min HR, treadmill time, VO_2peak)- Higher FA in the corpus callosum body extending to genu; Higher CRF (VO_2peak and time)- lower RD in the corpus callosum; No associations with AD.
Voss et al. 2012	Intervention (3 times 40-min exercise/ week for 1-yr)	N=35	Mean age=65	VO_2max by GXT	DTI at 3T		Exercise intervention group- Greater FA in white matter tracts of the frontal and temporal lobes
Alosco et al. 2013	Cross-sectional	N=69 patients with heart failure	Mean age=68	Physical fitness by 2-min step test	MRI at 1.5T	Age, sex, education, LVEF, BDI-II, hypertension, diabetes, and ICV	Higher PF – reduced GM atrophy and cortical thickness.

Table 3 Continued.

Abbreviations: PA=physical activity; HRT=hormone replacement therapy; GMV=gray matter volume; WMV=white matter volume; WML=white matter lesions; BPF=brain parenchymal fraction; DTI=diffusion tensor imaging; FA=fractional anisotropy; MD=mean diffusivity; RD=radial diffusivity; AD=axial diffusivity; HR=heart rate; ICV=intracranial volume; BMI=body mass index; LVEF=reduced ventricular ejection fraction; BDI-II=Beck depression inventory-II.

As the synopsis table shows above, data on the association between cardiorespiratory fitness and brain microstructure are sparse. I focused on the literature examining the relationship between fitness and brain microstructure in older adults and submitted a systematic review paper for the course EPIDEM 2183 in fall 2012. Details of the systematic review are provided below.

Settings and study population: A literature search in Ovid MEDLINE and PsychINFO that included the following criteria: (1) population-based cohort or intervention studies; (2) fitness as an independent variable and brain microstructure as the outcome; and (3) examining older adults aged 65 years or older. A total of four studies fulfilled the criteria and were selected for review. All four studies were conducted in the United States [79-82]. Of these four, three were cross-sectional observations [54, 79-81] and one was an intervention study [82].

Measures of Brain Microstructure: All four studies investigated white matter integrity using Diffusion Tensor Imaging (DTI). Fractional anisotropy (FA) and mean diffusivity (MD) are two commonly used indices of white matter integrity derived from DTI. Higher FA values indicate greater microstructural integrity, while higher MD values indicates increased diffusion which suggest tissue breakdown and increased water components in the brain. Two examined the microstructure from the whole brain [79, 82], the other two studies selected regions and tracts of interest as *a priori* [80, 81]. Consistent findings derived from these four studies are that higher cardiorespiratory fitness or physical activity is associated with higher FA from the white matter. Associations with other component diffusivities of myelin integrity (axial or radial diffusivity) were not significant. This may indicate that physical activity or cardiorespiratory fitness has an impact on white matter integrity as indexed by FA, but not MD or component diffusivities. It is also possible that associations would exist but were not detected in these studies due to small

sample sizes and different analytic approaches. A potential change in the brain microstructure may also depend on health profiles of participants and the dosage of physical activity regimen.

Methods to Quantify Brain Microstructure Four studies quantified white matter integrity using predefined region of interest approach, while Johnson et al.'s study [79] performed the whole-brain analysis using voxel based morphometry. In addition, Voss et al. conducted both region-of-interest approach and voxel-based morphometry [82]. Marks et al. focused on anterior, dorsal, and posterior segments of the cingulum [80]. Voss et al. focused on primary lobes of the telencephalon, including the prefrontal, temporal, parietal, and occipital lobes [82]. In Marks et al.'s study, seven regions were predefined for analyses [80], but there were no descriptions of what these seven regions were referred to in the article.

Measures of Fitness Two of the four studies measured cardiorespiratory fitness using graded maximal exercise testing on a treadmill [79, 81], one study estimated cardiorespiratory fitness using a non-exercise regression equation, and another study calculated a composite score of cardiorespiratory fitness by averaging VO_2max from graded maximal exercise test and Rockport one-mile test. In Voss et al.' study, the composite measure of cardiorespiratory fitness might provide a more accurate estimation since measurements of graded maximal exercise and Rockport walk test were highly correlated with each [82]. Although graded maximal exercise test was considered to be the gold standard measure of cardiorespiratory fitness, the composite measure of cardiorespiratory fitness may provide higher accuracy especially in older adults. It is because the graded maximal exercise test may not be feasible in very old adults due to limited physical function and cardiovascular conditions. The efforts made in the graded maximal exercise test may vary based on individual's health profiles.

Summary of findings Three observational studies provide empirical evidence that higher cardiorespiratory fitness is associated with higher FA in overall white matter, and with selected regions and tracts, including the cingulum, uncinate fasciculus, and corpus callosum. Voss et al.'s intervention study showed that one-year aerobic exercise training increased white matter integrity in the temporal and prefrontal lobes using predefined region of interest approach. As regards to the component diffusivity, Johnson et al.'s study found an association of greater cardiorespiratory fitness with less RD, not AD in healthy older adults [79], whereas Voss et al.'s study did not observe a change of the brain's integrity with either RD or AD after one-year aerobic exercise training [82]. The associations of cardiorespiratory fitness with FA in the cingulum and uncinate fasciculus remained significant after adjustment for age and sex [80, 81].

Conclusions and Future directions To sum up, greater cardiorespiratory fitness was associated with higher FA in regions and tracts important for memory and executive control function in healthy older adults, as shown in these four studies. These reviewed studies have several strengths in their methodology. Three studies used graded maximal exercise testing, which is considered to be the gold standard measurement, to quantify cardiorespiratory fitness. All studies examined the brain's integrity using fractional anisotropy, and two included component diffusivities, radial diffusion and axial diffusion. However, all four had small sample sizes of no more than 35 participants and limited measures of potential confounders. Future studies in a large sample size are warranted to examine the associations of cardiorespiratory fitness with brain integrity in very old adults who are at risk for developing cognitive impairment and dementia. Further, examining potential confounders or moderators can provide detailed information on individualized prevention or treatment of physical activity. Accurate

quantification on cardiorespiratory fitness is essential to define the pattern of the relationship and sheds light on future intervention studies.

2.3.2 Gap in knowledge 3: population measures of cardiorespiratory fitness in very old adults

Besides the limitation in using the macroscopic measures of the brain structure, one major gap in knowledge in this line of research is the safe and valid measure of cardiorespiratory fitness in very old adults. Prior studies have mostly examined young old adults in their mid-60s applied the graded maximal exercise tests, which poses safety risk for very old adults aged 80 years and older. Graded maximal exercise test may not be feasible in adults aged 80 years and older due to their increased functional limitations and prevalent chronic disease conditions with age. Thus, it is essential to apply an alternative test of cardiorespiratory fitness which is feasible to adults aged 80 years and older to examine the association with brain structure. It is also important to examine whether the associations would be influenced by health-related factors. Careful characterization of the brain imaging correlates of cardiorespiratory fitness in very old adults can help understand the biological mechanisms underlying the effect of fitness on the brain. This knowledge can also have implications to optimize prevention and treatment strategies aimed at improving brain health.

To address this gap in knowledge, **paper # 3** quantified the cross-sectional association of cardiorespiratory fitness measured by the Long-Distance Corridor Walk with brain microstructure in adults aged 80 years and older. Paper #3 also tested whether these associations would be attenuated by physical activity and chronic diseases known to be related to cardiorespiratory fitness and brain health.

3.0 GOAL AND SPECIFIC AIMS

The overall goal of the three studies is to provide scientific recommendations of precise prescriptions of physical activity on preventing brain abnormalities among community-dwelling older adults. Three specific aims are outlined: 1) quantify the dose-response relationship between physical activity, cardiorespiratory fitness, and brain structure in very old adults; 2) characterize brain regions/tracts that are most strongly related with physical activity; and 3) explore the moderating effects of health-related conditions and physical performance on the associations of physical activity, cardiorespiratory fitness on brain structure.

4.0 PAPER 1: EVERY STEP COUNTS, EVERY MINUTE MATTERS – OBJECTIVELY MEASURED PHYSICAL ACTIVITY AND WHITE MATTER TRATS IN ADULTS OVER 80 YEARS OF AGE

To be submitted for publication

Qu Tian[1], Nancy W. Glynn[†1], Kirk I. Erickson[†2], Howard J. Aizenstein[3], Eleanor M. Simonsick[4], Kristine Yaffe[5], Tamara B. Harris[6], Stephen B. Kritchevsky[7], Robert M. Boudreau[1], Anne B. Newman[1], Oscar L. Lopez[8], Judith Saxton[9], Caterina Rosano[1] for the Health ABC study

†Both contribute equally being the second authors

To be submitted for publication

[1]Department of Epidemiology, Center for Aging and Population Health, Graduate School of Public Health, University of Pittsburgh, 130 N. Bellefield Ave., Pittsburgh, PA 15213, USA. [2]Department of Psychology, University of Pittsburgh, 3107 Sennott Square, 210 S. Bouquet St., Pittsburgh, PA 15260, USA. [3]Departments of Psychiatry, Bioengineering, and Clinical and Translational Science, University of Pittsburgh, 3811 O'Hara Street, Pittsburgh, PA 15213, USA. [4]Intramural Research Program, National Institute on Aging, 3001 S. Hanover Street Baltimore, MD 21225, USA. [5]Department of Psychiatry, Neurology and Epidemiology, University of California, San Francisco, 4150 Clement Street, San Francisco, CA 94121, USA. [6]Intramural Research Program, National Institute on Aging, Laboratory of Epidemiology, Demography, and Biometry, Gateway Building 3C309, 7201 Wisconsin Avenue, Bethesda, MD 20892, USA. [7]Sticht Center on Aging, Section on Gerontology and Geriatric, Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA. [8]Department of Neurology, University of Pittsburgh, Pittsburgh, PA, USA. [9]Departments of Neurology and Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

4.1 ABSTRACT

Background. Loss of white matter integrity in frontal and medial temporal lobes is related to decline in memory and information processing speed. Physical activity (PA) has beneficial effects on memory and information processing, but it is unknown whether greater total PA and which components of PA are associated with greater white matter integrity in relevant networks. Furthermore, whether these associations exist in older adults with mild cognitive impairment (MCI) or dementia remains poorly understood. This study examined whether greater total PA was associated with fewer white matter hyperintensities (WMHs) and greater fractional anisotropy (FA) in memory- and information processing-related networks in adults over 80 with and without MCI or dementia. **Methods.** Diffusion-weighted and FLAIR sequences were obtained in 96 participants (mean age=86.1 years, 52.1% female, 44.8% black) with concurrent SenseWear Armband measures of active energy expenditure (AEE, kcal/day), number of steps per day, duration (minutes/day), and intensity of PA (metabolic equivalents, METs/day). WMHs and FA were computed for uncinate and superior longitudinal fasciculi related to memory and information processing, respectively. **Results.** For each standard deviation increase in AEE (304 kcal/day), steps taken (1321 per day or .56 miles/day), and duration of PA (42 minutes of PA/day), there was a 1% increase in FA in the superior longitudinal fasciculus (standardized β=.278, .255, and .242, respectively). For each standard deviation increase in steps taken, there was also a 1% increase in FA in uncinate fasciculus (standardized β=.218). Associations were minimally attenuated after adjustment for systolic blood pressure, diabetes, stroke, gait velocity, or joint pain. After stratification by cognitive status, associations with FA in the superior longitudinal fasciculus remained significant in cognitively intact adults (N=41), but not in adults

with MCI or dementia (N=55). Overall, associations between PA measures and WMHs in either tract were not significant. **Conclusions.** Objectively measured PA in adults over 80 years of age reveals that more steps taken and more minutes of being physically active are associated with elevated white matter integrity in tracts important for information processing, especially in adults who are not yet experiencing cognitive impairment. Further research is needed in longitudinal studies to confirm these associations and establish the causal association between PA and brain health.

4.2 INTRODUCTION

Loss of white matter integrity in frontal and medial temporal areas is common in older adults, and it is related to difficulties in memory and information processing [1, 2]. Demyelination of white matter may initiate the disruption of the axonal connectivity integrity. This, in turn, may accelerate neuronal degeneration of gray matter and further compromise brain function [3, 4]. Microstructural measures of white matter integrity can indicate early stages of impairment in memory and information processing [5]. Compelling evidence shows that being physically active is associated with enhanced memory and information processing (for review, see Erickson et al. 2012 [41]), but few neuroimaging studies have examined the association between physical activity (PA) and white matter integrity in older adults. The studies that have been conducted have shown promising associations such that higher PA has been associated with greater white matter volume [6, 7], greater white matter integrity, and fewer white matter lesions in adults in their seventies [7].

However, several important questions remain unanswered. First, the spatial distribution of the association between PA and white matter integrity has not been quantified in adults over 80 years of age, when the risk for developing cognitive impairment is high. This is critical for understanding the mechanisms underlying the neuroprotective effects of PA. Second, moderating effects of health-related conditions have not been examined in older adults. Health-related conditions, such as chronic diseases and physical functional limitations, may compromise the ability to safely engage in PA and may affect the integrity of the brain. Third, little is known about which components of PA (duration, intensity, or both), or whether a certain combination of PA components (e.g. lower intensity and longer duration), would be most strongly associated with brain structural integrity in older adults. This is mainly due to the major barrier of self-report measures which are unable to distinguish PA duration from intensity. Lastly, little is known about the relationships between PA and white matter integrity in adults with and without mild cognitive impairment (MCI) or dementia. Prior neuroimaging studies have reported robust associations between PA and brain volume in cognitively intact older adults [6, 8-10], but a few studies focusing on cardiorespiratory fitness, a physiologic marker of PA, have shown that higher fitness levels are associated with greater gray matter volume in adults with early stage Alzheimer's disease, not in cognitively intact older adults [11, 12]. Associations between PA and brain health may be attenuated in adults with MCI or dementia due to advance disease which could affect brain integrity. Understanding the associations between PA and white matter integrity in older adults with and without MCI or dementia may shed light on appropriate targeting strategies to preserve white matter integrity through individualized, tailored PA recommendations.

This study examined the cross-sectional association of objectively measured PA using a SenseWear Armband with white matter hyperintensities (WMHs) and fractional anisotropy (FA) in uncinate and superior longitudinal fasciculi supporting memory and information processing, respectively, in a well-characterized cohort of adults over 80 years of age. It was hypothesized that greater total PA, including higher active energy expenditure (AEE) and more steps taken, and longer duration and higher intensity of PA, would be associated with lower WMHs and higher FA in uncinate and superior longitudinal fasciculi. It was also hypothesized that these associations would be modified by health-related conditions and the associations would be attenuated in adults with MCI or dementia.

4.3 METHODS

4.3.1 Study population

Participants were recruited from the Health, Aging and Body Composition Study cohort, an ongoing longitudinal study that began in March 1997 to assess the relationship between changes in body composition and health outcomes in 3,075 community-dwelling older adults (52% female, 42% black) aged 70 to 79 years [13]. Among the 819 participants who were alive and seen in the clinic or had a home visit from the Pittsburgh site in 2006-2008, 325 received brain Magnetic Resonance Imaging (MRI) with Diffusion Tensor Imaging (DTI) at 3Tesla [14]. Of these 325, 163 received a follow-up MRI in 2010-2012 using the same MRI protocol. Of these, 103 wore the SenseWear Armband and 96 had usable PA data with at least 3 days of on-body

time [15] (Figure 4). The average percent of on-body time was 93.7% (SD=10.7). The average interval between the SWA and the MRI was 7.7 months (SD=6.3). Sex, race, and education were obtained at the Health ABC Study entry and age was obtained at the time of the SWA measurement.

4.3.2 MRI protocol and markers

MRI scans were obtained at the MR Research Center of the University of Pittsburgh with a 3Tesla Siemens TIM TRIO scanner equipped for echo-planer imaging using the protocol previously described [14]. T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) images and T2-weighted fluid-attenuated inversion recovery (FLAIR) images were acquired in the axial plane and the voxel size was 1mm*1mm. DTI were acquired using single-short spin-echo sequence in 12 diffusion directions and the voxel size was 2mm*2mm. There were no pathological findings for any participants included in this analysis as verified by a neuroradiologist.

The volume of WMHs was obtained from FLAIR images and was quantified with a fuzzy connected algorithm [16]. The volume of WMHs was computed by summing the voxels of WMHs and then normalized by total brain volume. DTI preprocessing was done using the FMRIB's Diffusion Toolbox to correct eddy current-induced distortions [17]. The fractional anisotropy (FA) map was computed and registered to the FMRIB58_FA template [17] using the FMRIB's non-linear image registration tool [42]. Mean FA was calculated for normal appearing WM. Localizations of white matter tracts were identified using a previously developed method of Automated Labeling Pathway [16, 18, 19].

White matter tracts to be analyzed were selected *a priori*, including uncinate and superior longitudinal fasciculi because of their associations with memory and information processing, respectively [20, 21]. The volume of WMHs in tracts of interest from left and right hemispheres were summed and then dichotomized using a median split due to heavily skewed distributions. FA in tracts of interest was quantified in common space and averaged across left and right hemispheres in a standardized unit.

4.3.3 Physical activity

Physical activity (PA) was measured by a SenseWear Armband (SWA, BodyMedia Inc., Pittsburgh, Pennsylvania). The SWA collects a variety of physiological data through portable multiple sensors (a three-axis accelerometer, heat flux sensor, skin temperature sensor, near-body ambient temperature sensor, and galvanic skin response sensor). Among various objective measures of PA, the SWA can detect various types of activities, using pattern detection algorithms based on physiological signals. It measures energy expenditure and provides good accuracy and reliability both at rest and during activities at low, moderate and high intensities [22-24].The unique combination of multiple sensors of SWA and its high accuracy, moderate price, and ease of assessment overcome limitations of other objective measures [25].

Participants were instructed to wear the SWA on the left upper arm for seven consecutive days and were allowed to remove the armband for up to 1 hour each day for a rest. Data with at least 3 days of on-body time was necessary to be included for analyses. PA was defined as activities greater than 3 METs. The SWA provided active energy expenditure (AEE, kcal), number of steps, duration (minutes of PA), and intensity (metabolic equivalents, METs). Daily

averages were computed by dividing AEE, steps, minutes of PA, and METs by the number of days the device was worn. Steps were also converted to miles using the average stride length measured by the GaitMat II [26].

4.3.4 Health-related conditions

Health-related conditions included brain and physical function, systolic blood pressure, prevalent disease, and joint pain, which have been related to PA participation or brain health. Cognitive function was measured by the modified mini-mental status examination (3MSE, range 0-100). Physical function was measured as gait velocity at a usual pace over a 4-m-long walkway using the GaitMat II^{TM} system (EQ Inc., Chalfont, Pennsylvania). Gait velocity was computed as the distance between the first switch closure of the first and last steps divided by the time between the earliest closures of the first and last steps. For those who did not have the GaitMat measure, gait velocity was computed in meters/second while walking at a usual pace over 3, 4 or 6 meters [27]. Gait velocity is a valid and reliable assessment of physical function among older adults in both clinical and aging research settings [28, 29]. Slower gait velocity is strongly associated with greater co-morbidity and higher mortality risk [30-32].

Cognitive status was characterized as cognitively intact or evidence of MCI or dementia, ascertained by an adjudication committee. The protocol of adjudication has been previously used in the Cardiovascular Health Study [33]. The classification of MCI or dementia was based on the number of impaired tests from the neuropsychological battery, severity of the impairment, scores on the Modified Mini-Mental State Examination, Digit Symbol Substitution Test, and Center for Epidemiologic Studies Depression Scale, and the evaluation of the instrumental activities of

daily living or the activities of daily living. Prevalent diabetes and stroke were measured using prevalent disease algorithms according to self-reported diagnoses by physicians and record of medication use at the time of the MRI in 2010-2012. Joint pain was measured as self-reported pain lasting for at least one month in or around either knee.

4.3.5 Statistical analysis

Associations between PA measures and MRI neuroimaging markers of interest were examined using multivariate regression models. Because the SWA accounted for age, sex, height, and weight in estimating PA, models were not adjusted for these factors. Models were adjusted for race, education, and health-related conditions, including systolic blood pressure, diabetes, stroke, joint pain, and gait velocity entered one at a time into separate models.

The strength of the association between PA and MRI outcomes was tested using forward stepwise analyses by entering health-related conditions in the model all at once (e.g., systolic blood pressure, diabetes, stroke, joint pain, and gait velocity). A p-value ≤ 0.10 was used as the criteria for entry into the model and a p-value ≥ 0.05 for removal from the model. The average interval between the SWA and the MRI, on-body percentage of time wearing the SWA, and days wearing the SWA, were also included as covariates to account for individual differences in a sensitivity analysis.

The moderating effects of health-related conditions on the strength of associations between PA and neuroimaging outcomes were tested using hierarchical multiple regression models: (1) the moderator (if continuous) was centered on the parent scale, (2) the interaction term of PA and the moderator was created, and (3) hierarchical multiple regression models were

conducted by first entering the measure of PA and the moderator and then adding the interaction term. A significant model change after adding the interaction term and significance level of the interaction term at $p<.05$ indicated a significant moderating effect.

Secondary analyses examined associations between PA and neuroimaging markers stratified by cognitive status and adjusted for race, education, and health-related conditions.

4.4 RESULTS

The 96 participants included in this study had a mean age of 86.1 (range: 83-92 years old) and were more likely to be female (52.1%) and white (55.2%) (Table 4). Those who received the brain MRI but did not have usable SWA data (N=67) were older ($p<.001$) and more likely to be female ($p=.034$) with a higher prevalence of stroke ($p=.008$) as compared to these 96 participants (Table 4).

More steps taken, but not AEE, minutes of PA, or METs, were associated with higher FA in the uncinate fasciculi ($p=.039$). Higher AEE, more steps taken, and more minutes of PA, but not METs, were associated with higher FA values in the superior longitudinal fasciculus ($p=.008$, .015, and .022, respectively). Associations between PA and WMHs in uncinate and superior longitudinal fasciculi were not significant (all $p>.05$). Demographics and health-related conditions were not significantly associated with FA or WMH in uncinate and superior longitudinal fasciculi (all $p>.05$).

For each standard deviation increase in AEE (304 kcal/day), steps taken (1321 per day, or .56 miles), and duration of PA (42 minutes of PA/day), there was a 1% increase in FA in the

superior longitudinal fasciculus (Table 5, standardized β=.278, .255, and .242, respectively). For each standard deviation increase in steps taken, there was also a 1% increase in FA in the uncinate fasciculus (Table 5, standardized β=.218). These associations remained significant after adjustment for race, education, systolic blood pressure, diabetes, stroke, gait velocity, and joint pain (Table 5). Adjustment for joint pain attenuated the association between minutes of PA and FA in the superior longitudinal fasciculus (Δβ>10%), because participants with joint pain had fewer minutes of PA than those without (mean difference=19, SE=8, p=.016). Adjustment for race strengthened the association between steps and FA in the uncinate fasciculus, because whites had more steps taken than blacks (md=.633, SE=264, p=.019). Adjustment for gait velocity strengthened the association between steps and FA in the uncinate fasciculus (Δβ>10%), because gait velocity was highly correlated with steps (r=.520, p<.001).

Moderating effects of health-related conditions on the strength of the association between steps and FA in the uncinate fasciculus and the association between AEE, steps, and minutes of PA and FA in the superior longitudinal fasciculus were not statistically significant (p for interaction terms > .05). In the forward stepwise analysis with all health-related conditions entered in the model, none of the variables were retained in the models of PA predicting FA in the superior longitudinal fasciculus. Adjustment for the average interval between the SWA assessment and MRI, on-body percentage of worn time, or days worn did not attenuate these associations (all Δβ<10%).

After stratification by cognitive status, Associations between AEE, steps, and minutes of PA with FA in the superior longitudinal fasciculus were significant in cognitively intact adults, but not in those with MCI or dementia (Table 6). In cognitively intact adults, associations between AEE and steps, but not minutes of PA, with FA in the superior longitudinal fasciculus

remained significant after adjustment for race, education, systolic blood pressure, diabetes, stroke, gait velocity, or joint pain (all $\Delta\beta<10\%$). The association between steps and FA in the uncinate fasciculus was not significant in either group.

4.5 DISCUSSION

In this cohort of adults aged 80 years and older, an increase of 304 kcal per day in active energy expenditure, 1321 steps per day (or 0.56 miles per day), or 42 minutes of any physical activity per day is associated with a 1% increase in fractional anisotropy in the superior longitudinal fasciculus. This study extends previous investigations by using an objective measure of physical activity and high resolution diffusion tensor imaging to examine the microstructure of white matter in specific networks.

To the best of our knowledge, this study is the first to apply the objective measure of physical activity to examine the association between physical activity and white matter integrity in an older population (> 80 years) with extensive measures of health-related characteristics. The advantage of the SenseWear Armband is that it is believed to provide accurate and multifaceted measures of physical activity in a group of adults over 80 years of age who mostly engage in low intensity activities. Using objective measures to assess PA in older adults is critical for several reasons. First, older adults mostly engage in low intensity activities due to physical functional limitations and health-related conditions that may not be accurately identified by self-report measures, but could be captured by objective methods. Second, self-report measures require recall accuracy which is challenging for older persons with declining memory function. Findings

from this study indicate that among the physical activity components examined, steps taken and duration of activity at 3 or more METs seem to be most strongly associated with white matter integrity in the superior longitudinal fasciculus.

Participants included in this study were unique in that they were living with physical functional limitations and chronic diseases into their eighties [34-36]. According to recent data from "America on the Move", individuals with at least 5486 steps per day are likely to meet the current physical activity recommendations [37]. In this sample, the average number of daily steps was 1755, which is far from the recommended level. But even in such a cohort of old sedentary adults, we observed a positive association between physical activity, especially steps taken and physical activity duration, with white matter integrity in tracts important for information processing. Further, these positive associations remained largely unchanged after controlling for health-related conditions. It is possible that a moderating effect of health-related conditions on the association of physical activity predicting fractional anisotropy was unable to be detected with sample size in this study. Indeed, physical activity may protect against neurodegeneration by reducing peripheral risk factors, such as hypertension, diabetes, and obesity [38].

We found the associations between steps taken, physical activity duration, and fractional anisotropy were significant only in cognitively intact adults. Prior neuroimaging studies showed a robust association between physical activity and brain volume in cognitively intact older adults [6, 8-10], while a few other studies showed an opposite pattern that the associations were significant in adults with early Alzheimer's disease, but not in cognitively intact older adults [11, 12]. With a relatively small sample, our study may have insufficient power to detect associations

between physical activity and white matter integrity in adults with MCI or dementia, especially since those with MCI or dementia had uniformly very low levels of physical activity.

Contrary to expectations, the association between physical activity and white matter hyperintensities was not significant. These findings are inconsistent with previous studies reporting that higher physical activity either in midlife or late life was associated with fewer white matter lesions [7, 10]. These null findings may be attributed to a low prevalence of white matter hyperintensities in this sample. The lack of associations between physical activity components and gray matter integrity in memory- and information processing-related regions may indicate that physical activity does not have an impact on gray matter integrity in older age (Table 15).

We did not find an association between physical activity intensity and white matter integrity or white matter hyperintensities. No neuroimaging evidence is available to support the association of PA intensity with brain structural integrity. Only a few studies examining the association of physical activity duration and intensity with cognitive function yield mixed findings. One study found that greater decline in physical activity intensity, not in duration, was associated with greater cognitive decline in middle-aged adults [39], while another study showed that greater decline in both intensity and duration was associated with greater cognitive decline in adults in their mid-70s [40]. These inconsistent findings may be due to the use of self-report measures of physical activity, which poorly discriminate between intensity and duration. Hence, this null finding may indicate that physical activity intensity may not be as important for brain white matter health as the duration of physical activity in this old population. It is also possible that the effect of physical activity intensity on white matter integrity was not able to be detected in this study because of a truncated range of physical activity intensity for our participants. That

is, participants in our study were mostly sedentary and engaged in only low intensity activities (daily average intensity range: 0.72 to 1.75 METs/day). Future studies could be conducted to examine the association between physical activity intensity and white matter integrity in a group of older adults with a wider range of physical activity intensity, although advanced age itself may be the limiting factor for engaging in higher intensity activities.

Several limitations of this study require us to interpret the results with caution. First, we may not have been sufficiently powered to detect associations between physical activity intensity and white matter integrity and the potential moderating effects of physical function and health-related conditions. Future studies in larger samples are needed and should include participants with a wider range of physical activity intensities, if possible. Second, the direction of the effects is uncertain due to the cross-sectional nature of the design. Third, participants in our study tend to be healthier than the general population due to their voluntary participation and eligibility for the brain MRI.

In conclusion, more steps and more minutes of physical activity are associated with greater microstructural integrity in white matter tracts important for information processing, especially for cognitively intact older adults. Findings from this study may be useful in formulating physical activity recommendations for improving white matter health in adults aged 80 years of age. Future studies are needed to identify the minimal dose of physical activity necessary to preserve white matter integrity through older age. The contribution of physical activity intensity to the microstructural integrity and the moderating effects of physical function and health-related conditions are worthy exploring in larger samples with a wider range of activity intensities.

4.6 ACKNOWLEDGEMENTS

This research was supported by National Institute on Aging (NIA) Contracts N01-AG-6-2101, N01-AG-6-2103, N01-AG-6-2106, K23-AG028966-01; NIA grants R01-AG028050, R01-AG029232, P30-AG024827, and NINR grant R01-NR012459, and supported by 8th Annual Department of Epidemiology Small Grant program, University of Pittsburgh. This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging.

4.7 REFERENCES

1. Debette, S. and H.S. Markus, *The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis.* BMJ, 2010. **341**: p. c3666.

2. Le Bihan, D., et al., *Diffusion tensor imaging: concepts and applications.* J Magn Reson Imaging, 2001. **13**(4): p. 534-46.

3. Pantoni, L. and J.H. Garcia, *Pathogenesis of leukoaraiosis: a review.* Stroke, 1997. **28**(3): p. 652-9.

4. Nedergaard, M., B. Ransom, and S.A. Goldman, *New roles for astrocytes: redefining the functional architecture of the brain.* Trends Neurosci, 2003. **26**(10): p. 523-30.

5. Sasson, E., et al., *White matter correlates of cognitive domains in normal aging with diffusion tensor imaging.* Front Neurosci, 2013. **7**: p. 32.

6. Ho, A.J., et al., *The effects of physical activity, education, and body mass index on the aging brain.* Hum Brain Mapp, 2011. **32**(9): p. 1371-82.

7. Gow, A.J., et al., *Neuroprotective lifestyles and the aging brain: Activity, atrophy, and white matter integrity.* Neurology, 2012. **79**(17): p. 1802-1808.

8. Bugg, J.M. and D. Head, *Exercise moderates age-related atrophy of the medial temporal lobe.* Neurobiol Aging, 2011. **32**(3): p. 506-14.

9. Erickson, K.I., et al., *Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study.* Neurology, 2010. **75**(16): p. 1415-22.

10. Rovio, S., et al., *The effect of midlife physical activity on structural brain changes in the elderly.* Neurobiol Aging, 2010. **31**(11): p. 1927-36.

11. Honea, R.A., et al., *Cardiorespiratory fitness and preserved medial temporal lobe volume in Alzheimer disease.* Alzheimer Dis Assoc Disord, 2009. **23**(3): p. 188-97.

12. Burns, J.M., et al., *Cardiorespiratory fitness and brain atrophy in early Alzheimer disease.* Neurology, 2008. **71**(3): p. 210-6.

13. Simonsick, E.M., et al., *Measuring higher level physical function in well-functioning older adults: expanding familiar approaches in the Health ABC study.* J Gerontol A Biol Sci Med Sci, 2001. **56**(10): p. M644-9.

14. Venkatraman, V.K., et al., *Lower Digit Symbol Substitution Score in the Oldest Old is Related to Magnetization Transfer and Diffusion Tensor Imaging of the White Matter.* Front Aging Neurosci, 2011. **3**: p. 11.

15. Hart, T.L., et al., *How many days of monitoring predict physical activity and sedentary behaviour in older adults?* Int J Behav Nutr Phys Act, 2011. **8**: p. 62.

16. Wu, M., et al., *A fully automated method for quantifying and localizing white matter hyperintensities on MR images.* Psychiatry Res, 2006. **148**(2-3): p. 133-42.

17. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL.* Neuroimage, 2004. **23 Suppl 1**: p. S208-19.

18. Aizenstein, H.J., et al., *Prefrontal and striatal activation during sequence learning in geriatric depression.* Biol Psychiatry, 2005. **58**(4): p. 290-6.

19. Wu, M., et al., *Quantitative comparison of AIR, SPM, and the fully deformable model for atlas-based segmentation of functional and structural MR images.* Hum Brain Mapp, 2006. **27**(9): p. 747-54.

20. Hasan, K.M., et al., *Development and aging of the healthy human brain uncinate fasciculus across the lifespan using diffusion tensor tractography.* Brain Res, 2009. **1276**: p. 67-76.

21. Turken, A., et al., *Cognitive processing speed and the structure of white matter pathways: convergent evidence from normal variation and lesion studies.* Neuroimage, 2008. **42**(2): p. 1032-44.

22. Fruin, M.L. and J.W. Rankin, *Validity of a multi-sensor armband in estimating rest and exercise energy expenditure.* Med Sci Sports Exerc, 2004. **36**(6): p. 1063-9.

23. Drenowatz, C. and J.C. Eisenmann, *Validation of the SenseWear Armband at high intensity exercise.* Eur J Appl Physiol, 2011. **111**(5): p. 883-7.

24. Johannsen, D.L., et al., *Accuracy of armband monitors for measuring daily energy expenditure in healthy adults.* Med Sci Sports Exerc, 2010. **42**(11): p. 2134-40.

25. Andre, D. and D.L. Wolf, *Recent advances in free-living physical activity monitoring: a review.* J Diabetes Sci Technol, 2007. **1**(5): p. 760-7.

26. Rosano, C., et al., *Special article: gait measures indicate underlying focal gray matter atrophy in the brain of older adults.* J Gerontol A Biol Sci Med Sci, 2008. **63**(12): p. 1380-8.

27. Klepin, H.D., et al., *Physical performance and subsequent disability and survival in older adults with malignancy: results from the health, aging and body composition study.* J Am Geriatr Soc, 2010. **58**(1): p. 76-82.

28. Guralnik, J.M., et al., *Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery.* J Gerontol A Biol Sci Med Sci, 2000. **55**(4): p. M221-31.

29. Studenski, S., et al., *Physical performance measures in the clinical setting.* J Am Geriatr Soc, 2003. **51**(3): p. 314-22.

30. Montero-Odasso, M., et al., *Gait velocity as a single predictor of adverse events in healthy seniors aged 75 years and older.* J Gerontol A Biol Sci Med Sci, 2005. **60**(10): p. 1304-9.

31. Studenski, S., et al., *Gait speed and survival in older adults.* JAMA, 2011. **305**(1): p. 50-8.

32. Brach, J.S., et al., *Gait variability in community-dwelling older adults.* J Am Geriatr Soc, 2001. **49**(12): p. 1646-50.

33. Lopez, O.L., et al., *Evaluation of dementia in the cardiovascular health cognition study.* Neuroepidemiology, 2003. **22**(1): p. 1-12.

34. Pate, R.R., J.R. O'Neill, and F. Lobelo, *The evolving definition of "sedentary".* Exerc Sport Sci Rev, 2008. **36**(4): p. 173-8.

35. Abellan van Kan, G., et al., *Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an International Academy on Nutrition and Aging (IANA) Task Force.* J Nutr Health Aging, 2009. **13**(10): p. 881-9.

36. Cesari, M., et al., *Prognostic value of usual gait speed in well-functioning older people--results from the Health, Aging and Body Composition Study.* J Am Geriatr Soc, 2005. **53**(10): p. 1675-80.

37. Bassett, D.R., Jr., et al., *Pedometer-measured physical activity and health behaviors in U.S. adults.* Med Sci Sports Exerc, 2010. **42**(10): p. 1819-25.

38. Cotman, C.W., N.C. Berchtold, and L.A. Christie, *Exercise builds brain health: key roles of growth factor cascades and inflammation.* Trends Neurosci, 2007. **30**(9): p. 464-72.

39. Angevaren, M., et al., *Physical activity and 5-year cognitive decline in the Doetinchem cohort study.* Ann Epidemiol, 2010. **20**(6): p. 473-9.

40. van Gelder, B.M., et al., *Physical activity in relation to cognitive decline in elderly men: the FINE Study.* Neurology, 2004. **63**(12): p. 2316-21.

41. Erickson et al., *Physical activity and brain plasticity in late adulthood: a conceptual review.* Ageing Research, 2012. **4**(e6): p.34-47.

4.8 TABLES

Table 4: Demographics, physical activity measures, neuroimaging markers of interest, and health-related conditions of the analytic sample (n=96) compared to Healthy Brain Project (HBP) cohort who had MRI but didn't have usable SWA measures (n=67).

	Total (n=96)	HBP cohort with MRI but without usable SWA (n=67)	p-value
Demographics			
Age, years, mean±SD	86.1±2.3	87.9±3.1	<.001
Sex, female, N (%)	50 (52.1)	46 (68.7)	.034
Race, black, N (%)	43 (44.8)	27 (40.3)	.569
Education > high school, N (%)	48 (50.0)	34 (50.7)	.748
Physical Activity, mean±SD			
AEE, kcal/day	197±304	-	-
Number of steps per day	1755±1321	-	-
Duration, minutes/day	38±42	-	-
Intensity, METs/day	1.1±0.2	-	-
Neuroimaging markers			
White matter hyperintensities (WMHs)^, mm^3, median (iqr)			
Uncinate fasciculus	.00032 (.00195)	.00041 (.00169)	.495
Superior longitudinal fasciculus	.00024 (.00540)	.00017 (.00708)	.733
Fractional anisotropy, mean±SD			
Uncinate fasciculus	.3310±.0154	.3264±.0181	.095
Superior longitudinal fasciculus	.3534±.0146	.3504±.0146	.214
Health-related conditions, mean±SD or N (%) as noted			
3MSE score (range 0-100)	93.6±6.4	91.7±7.9	.113
Gait velocity, meters/second	.80±.20	.80±.21	.855
Systolic blood pressure, mm Hg	128.9±19.2	124.5±14.1	.121
MCI/Dementia	55 (57.3)	39 (58.2)	.244
Diabetes	22 (23.2)	14 (21.9)	.850
Stroke	4 (4.2)	11 (16.4)	.008
Joint pain	27 (28.4)	15 (23.4)	.485

Note: ^The volume of WMHs was dichotomized by a median split due to heavily skewed distributions. MCI=mild cognitive impairment. 3MSE=modified mini-mental state examination. AEE=active energy expenditure. METs=metabolic equivalents. P-values were computed from independent t-test or chi-square test as appropriate.

Table 5: Results of linear regression models of associations between physical activity measures and MRI neuroimaging markers significant at p<.05, standardized units (n=96).

	unadjusted	Adjusted for race	Adjusted for education	Adjusted for Systolic blood pressure	Adjusted for diabetes	Adjusted for stroke	Adjusted for gait velocity	Adjusted for joint pain
				FA in the superior longitudinal fasciculus, standardized β (95% CI), p-value				
Active energy expenditure[2] (kcal/day)	.278 (.073, .483) .008	.281 (.071, .490) .009	.266 (.062, .470) .011	.284 (.074, .493) .009	.280 (.073, .487) .009	.277 (.070, .484) .009	.259 (.050, .468) .016	.253 (.045, .461) .018
Number of steps per day[3]	.255 (.051, .460) .015	.261 (.049, .474) .016	.232 (.024, .441) .029	.255 (.047, .462) .017	.259 (.049, .468) .016.	.255 (.049, .461) .016	.244 (.001, .486) .049	.250 (.046, .454) .017
Duration[4] (minutes of PA/day)	.242 (.035, .449) .022	.241 (.031, .452) .025	.226 (.018, .433) .033	.243 (.033, .453) .024	.245 (.035, .455) .023	.241 (.032, .450) .025	.223 (.013, .433) .038	.213 (.003, .424) .047
				FA in the uncinate fasciculus, standardized β (95% CI), p-value				
Number of steps per day[1]	.218 (.011, .425) .039	.266 (.057, .476) .013	.225 (.013, .437) .038	.221 (.011, .430) .039	.227 (.016, .439) .035	.217 (.010, .424) .040	.280 (.037, .524) .025	.213 (.007, .419) .043

Notes: The standard deviation of FA in the uncinate and superior longitudinal fasciculi is .0154 and .0147, respectively.

[1] For each SD increase in steps (1321 per day), there is an increase of .0033 (=.214*.0154) in FA in the uncinate fasciculus, which corresponds to 1% of the average FA in the uncinate fasciculus of this analytic sample.

[2] For each SD increase in active energy expenditure (304 kcal/day), there is an increase of .00352 (=.272*.0147) in FA in the SLF, which corresponds to 1% of the average FA in the SLF of this sample.

[3] For each SD increase in steps (1321 per day), there is an increase of .00363 (=.247*.0147) in FA in the SLF, which corresponds to 1% of the average FA in the SLF of this sample.

[4] For each SD increase in duration (42 minutes of physical activity/day), there is an increase of .00345 (=.235*.0147) in FA in the SLF, which corresponds to 1% of the average FA in the SLF of this sample.

Table 6: Unadjusted linear regression models of associations between physical activity measures and MRI neuroimaging markers significant at p<.05 by cognitive status, standardized units.

	Cognitively intact (n=41)	Mild cognitively impaired or demented (N=55)
	FA in the superior longitudinal fasciculus standardized β (95% CI), p-value	
Active energy expenditure (kcal/day)	.449 (.127, .770) .007	.126 (-.136, .389) .339
Number of steps per day	.393 (.055, .732) .024	.142 (-.113, .398) .269
Duration (minutes of PA/day)	.315 (.002, .627) .049	.155 (-.133, .444) .285
	FA in the uncinate fasciculus standardized β (95% CI), p-value	
Number of steps per day	.297 (-.015, .609) .062	.174 (-.103, .452) .213

4.9 FIGURES

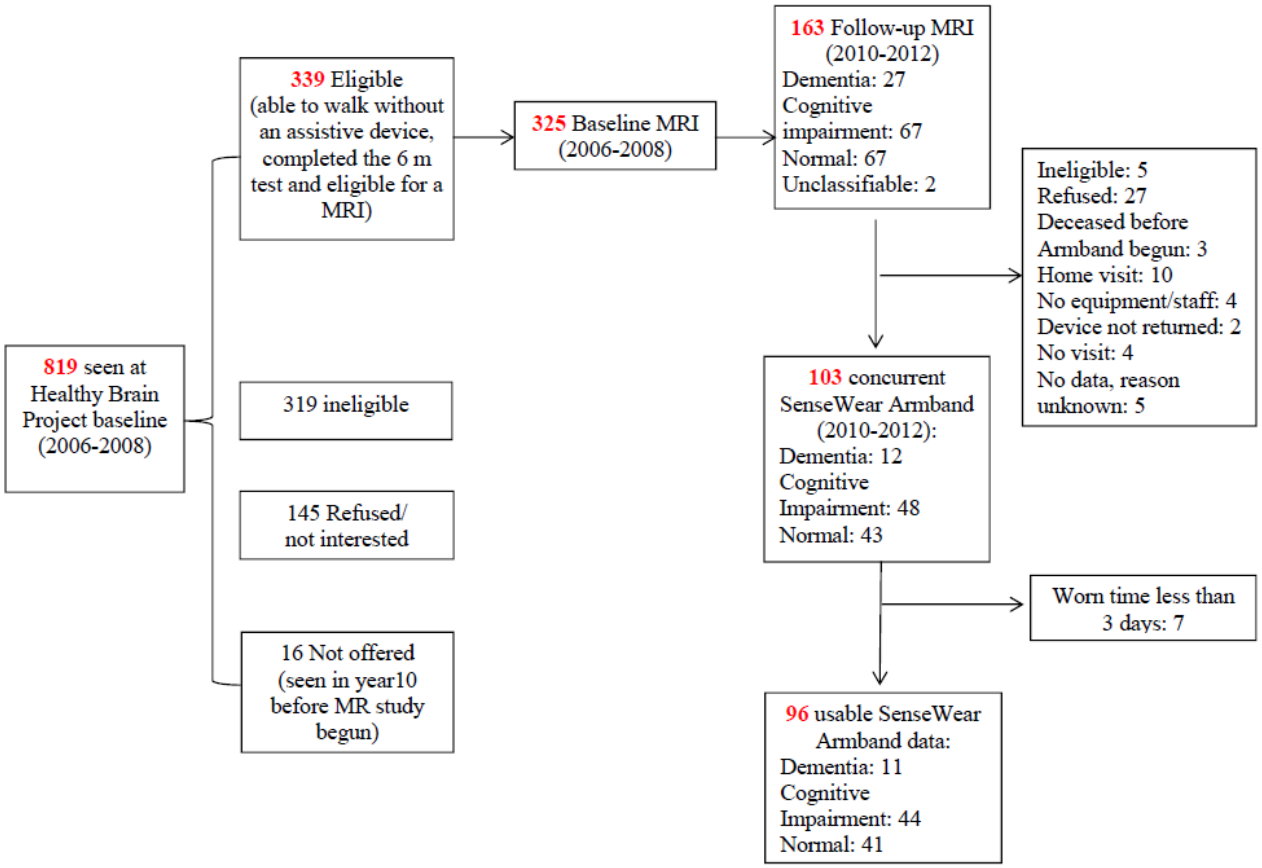

Figure 4: Flow chart of the Healthy Brain Project cohort.

5.0 PAPER 2: PHYSICAL ACTIVITY PREDICTS MICROSTRUCTURAL INTEGRITY IN MEMORY-RELATED NETWORKS IN VERY OLD ADULTS

Qu Tian[1], Kirk I. Erickson[2], Eleanor M. Simonsick[3], Howard J. Aizenstein[4], Nancy W. Glynn[1], Robert M. Boudreau[1], Anne B. Newman[1], Stephen B. Kritchevsky[5], Kristine Yaffe[6], Tamara Harris[7], Caterina Rosano[1] for the Health ABC study

Submitted to the Journal of Gerontology: Medical Sciences for publication

[1]Department of Epidemiology, Center for Aging and Population Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; [2]Department of Psychology, University of Pittsburgh, Pittsburgh, PA; [3]Intramural Research Program, National Institute on Aging, Baltimore, MD; [4]Departments of Psychiatry, Bioengineering, and Clinical and Translational Science, University of Pittsburgh, Pittsburgh, PA; [5]Sticht Center on Aging, Section on Gerontology and Geriatric, Medicine, Wake Forest School of Medicine, Winston Salem, NC; [6]Department of Psychiatry, Neurology and Epidemiology, University of California, San Francisco, CA; [7]Intramural Research Program, National Institute on Aging, Laboratory of Epidemiology, Demography, and Biometry, Maryland, MD.

5.1 ABSTRACT

Background. Although the beneficial effects of physical activity (PA) on memory and executive function are well established in older adults, little is known about the relationship between PA and brain microstructure and the contributions of physical functional limitations and chronic diseases. This study examined whether higher PA would be longitudinally associated with greater microstructural integrity in memory- and executive function-related networks and whether these associations would be independent of physical function and chronic diseases. **Methods.** Diffusion Tensor Imaging was obtained in 2006-2008 in 276 participants (mean age=83.0years, 58.7%female, 41.3%black) with PA (sedentary, lifestyle active, and exercise active) measured in 1997-1998. Gait speed, cognition, depressive symptoms, cardiovascular and pulmonary diseases, hypertension, stroke, and diabetes were measured at both time points. Mean diffusivity (MD) and fractional anisotropy were computed from normal appearing gray and white matter in fronto-parietal and subcortical networks. Moderating effects of physical function and chronic diseases were tested using hierarchical regression models. **Results.** Compared to the sedentary, the exercise active group had lower MD in the medial temporal lobe and the cingulate cortex (β, p: -.405, .023 and -.497, .006, respectively), independent of age, sex, and race. Associations remained independent of other variables, although they were attenuated after adjustment for diabetes. Associations between PA and other neuroimaging markers were not significant. **Conclusions.** Being exercise active predicts greater memory-related microstructural integrity in older adults. Future studies in older adults with diabetes are warranted to examine the neuroprotective effect of PA in these networks.

5.2 INTRODUCTION

Fronto-parietal and subcortical networks are highly susceptible to changes in blood oxygenation levels [1, 2], because of their watershed vascularization. Loss of structural integrity in these networks, specifically in dorsolateral prefrontal and hippocampal areas, is related to difficulties in memory and executive function and an increased risk of developing dementia [3, 4]. Because of the cardiorespiratory nature of physical activity (PA) [5], these networks may selectively benefit from the exposure to higher PA.

It is well accepted that higher PA is associated with improved brain health (see review [6]) and these effects are stronger for memory and executive function than for motor control (see meta-analysis [7]). Neuroimaging studies indicate positive effects of PA on brain macrostructure and function [8-16]. However, most of previous studies were cross-sectional designs and relied on low resolution imaging and volumetric measurements of the brain. Data on the association between PA and brain microstructure are sparse, with one observational study indicating a positive association between PA and white matter (WM) integrity [13] and one intervention study reporting increases in fitness from walking were associated with increases in WM integrity [17]. Understanding the longitudinal association with brain microstructure in older adults may have important implications in designing interventions to preserve structural integrity by improving PA. Furthermore, it is essential to differentiate the effects of PA on gray matter (GM) and WM to understand the mechanisms of how PA influences the structural integrity in the brain.

It is also critical to examine the contributions of physical functional limitations and chronic diseases which are common in older adults. The relationships between PA, physical function, and chronic diseases become increasingly complex in late life. For example, PA may

be beneficial for physical function and cardiovascular health, but in turn poorer physical function and cardiovascular diseases may limit PA participation [18]. Both PA and these potential modifiers are related to brain integrity [13].

This study quantified the associations between PA and microstructural integrity of GM and WM in a well-characterized cohort of very old adults, by accounting for health-related conditions. It was hypothesized that higher PA was associated with greater microstructural integrity localized in frontal and medial temporal lobes important for memory and executive function as compared to other regions, for example regions related to motor control. It was also hypothesized that these associations was attenuated by physical function and chronic diseases.

5.3 METHODS

5.3.1 Study population

Participants were recruited from the Health, Aging and Body Composition Study which began in March 1997 to assess the relationship between changes in body composition and health outcomes in a cohort of 3,075 community-dwelling older adults (52%women, 42%Black) aged 70 to 79 years [19]. 325 participants at the Pittsburgh site received a brain Magnetic Resonance Imaging (MRI) scan. Of 325, 276 had Diffusion Tensor Imaging in 2006-2008 and PA measured in 1997-1998. The study protocol was approved by the University of Pittsburgh and all participants provided informed consent.

5.3.2 MRI protocol and markers

MRI scans were obtained at the MR Research Center of the University of Pittsburgh with 3Tesla Siemens TIM TRIO scanners equipped for echo-planer imaging using the protocol previously described [20]. Magnetization-prepared rapid gradient echo images and Fluid attenuated inversion recovery images were acquired in the axial plane to obtain volumes of GM, WM, and WMHs, respectively [20]. Diffusion Tensor Images (DTI) were acquired using single-short spin-echo sequence with 12 directions and pre-processed using the FMRIB's Diffusion Toolbox [21] to correct eddy current-induced distortions The voxel size was 2 mm*2mm. There were no pathological findings from MR images for this study as verified by a neuroradiologist.

MRI outcomes were mean diffusivity (MD) from normal appearing GM and fractional anisotropy (FA) from normal appearing WM in standardized units. Using Automated Labeling Pathway [22, 23], localizations of regions and tracts were identified using Automated Anatomical Labeling Atlas [24] and Johns Hopkins University White Matter Atlas [25], respectively. Regions and tracts of interest were selected as *a prior*, including medial temporal lobe, cingulate cortex, dorsolateral prefrontal cortex, posterior parietal cortex, and uncinate and superior longitudinal fasciculi (Figure 5). Other regions were examined as a comparison to the hypothesized regions and tracts, including striatum, primary sensorimotor cortex, and supplementary motor cortex. MD in regions of interest from left and right hemispheres was computed as the mean weighted by GM volume. FA in tracts of interest from left and right hemispheres was computed as the mean.

Parenchyma atrophy was calculated by subtracting volumes of GM and WM from intracranial volume. The volume of WM hyperintensities (WMHs) normalized by total brain volume was dichotomized at the median due to a skewed distribution.

5.3.3 Physical activity

Physical activity (PA) was measured in 1997-98 using a standardized questionnaire [26]. Participants were categorized into sedentary, lifestyle active, and exercise active groups as previously defined (Table 16) [27, 28]. Those with lifestyle activities < 2,719 kcal/week and exercise activities < 1,000 kcal/week were defined as sedentary (n=39, 14.1%). Those with lifestyle activities >2,719 kcal/week and exercise activities <1,000 kcal/week were defined as lifestyle active (n=148, 53.6%) and those with exercise activities >1,000 kcal/week, regardless the amount of lifestyle activities, were defined as exercise active (n=89, 32.2%). In additional sensitivity analyses, the sedentary group was defined by the amount of lifestyle activities only (< 2,719 kcal/week), because being sedentary most of the day and exercising regularly can also be considered as sedentary [29]. Those with exercise activities > 1,000 kcal/week and lifestyle activities > 2,719 kcal/week were defined as exercise active.

Self-reported time spent walking in minutes per week was measured annually. Change was computed by subtracting walk time at study entry from walk time at time of MRI.

5.3.4 Other measures of interest

Gait speed at usual pace over 3, 4 or 6 meters was measured at study entry, years 4 and 6, and time of MRI. Gait speed is a valid and reliable assessment of physical function for older adults [30]. Slower gait speed is strongly associated with severity of health conditions and higher mortality risk [31, 32]. Change was computed by subtracting speed at study entry from speed at time of MRI. Annual percent change was computed by dividing relative change over 9 years by 9.

The digit symbol substitution test (DSST) was measured at study entry, years 5, 7, 8, and 9, and time of MRI. The modified mini-mental status examination (3MSE) was measured at study entry, years 3, 5, 7, and 9, and time of MRI. The Center for Epidemiologic Studies Depression Scale (CES-D) was measured at study entry, years 3-9, and time of MRI. Changes were computed by subtracting scores at study entry from scores at time of MRI. Annual percent changes were computed by dividing relative changes over 9 years by 9. The annual percent change was 0 when participants had 0 at study entry and time of MRI. For those with 0 values at study entry but non-zero values at time of MRI, 0 values at study entry were added to 1 in order to compute annual percent changes.

Chronic diseases, including prevalent cardiovascular disease (CVD), pulmonary disease, hypertension, and diabetes were obtained at study entry and time of MRI using prevalent disease algorithms according to self-reported diagnoses by physicians and records of medication use. Prevalent CVD was defined by self-report prevalent coronary heart disease or cerebrovascular disease. Prevalent pulmonary disease was defined by self-report or medication use. Prevalent diabetes and hypertension were defined by self-report and confirmed by medication use. Incident

CVD and stroke were ascertained using annual self-report questionnaires from the study entry to time of MRI.

In addition to age, sex, and race obtained at study entry, other characteristics were also measured, including education, body mass index, smoking status, alcohol consumption, pulmonary function, and prevalent knee osteoarthritis defined as consistent knee pain for at least 1 month in the past 12 months. Pulmonary function was assessed by the ratio of forced expiratory volume in the first second and forced vital capacity.

5.3.5 Statistical analysis

Univariate associations of PA with MD, FA, parenchyma atrophy, and WMHs were tested using ANOVA or χ^2 tests as appropriate. Associations with neuroimaging markers with $p<0.10$ were tested in multivariate regression models with PA as dummy coded vectors using the sedentary group as referent. Models were adjusted for age, sex, and race, which were related to PA or the outcomes, and further adjusted for physical function and chronic diseases at study entry. Models were also adjusted for education for its known associations with PA and brain health.

The hypothesized moderators, physical function and chronic diseases, were tested using hierarchical multivariate regression models: (1) the moderator (if continuous) was centered on the parent scale, (2) the interaction term between PA and the moderator was created, and (3) models were conducted by entering PA and the moderator and then adding the interaction. A significant model change after adding the interaction and the interaction with $p<.05$ indicated a significant moderating effect.

The strength of the associations between PA and neuroimaging markers was tested using forward stepwise analysis with all population characteristics in the model. A $p \leq 0.10$ was used as the criteria for entry into the model and a $p \geq 0.05$ for removal from the model.

5.4 RESULTS

Compared to the parent cohort, the 276 participants in this study were less likely to be sedentary [28]. The sedentary group had lower body mass index than the lifestyle active group and they had less time spent walking at study entry than the exercise active group. Physical and brain function and chronic diseases at study entry or education did not differ significantly between PA groups (Table 7).

Neuroimaging markers from total brain did not differ significantly between groups (Table 8). By contrast, PA was significantly associated with MD in medial temporal lobe and cingulate cortex (Table 9, Model 1). Associations followed a dose-response relationship (linear trend p=.012 and .009, respectively). Markers in other regions and tracts did not differ significantly between groups, including striatum, primary sensorimotor cortex, supplementary motor area, and uncinate and superior longitudinal fasciculi (Tables 17 and 18).

In multivariate regression models of PA predicting MD in medial temporal lobe, the exercise active group had lower MD than the sedentary group, independent of age, sex, and race (Table 9, Model 2). The association was attenuated after adjustment for diabetes (Table 9, Model 3 vs. 2; $\Delta\beta$=11.4%) and hypertension (Table 9, Model 4 vs. 2; $\Delta\beta$=4.0%), but remained significant. The interactions between PA and diabetes or hypertension were not significant

(p>.05). Adjustment for gait speed, other chronic diseases, or education did not substantially attenuate the association (Δβ<10% for all). In stepwise analyses, the association remained significant (β=-.407, p=.024) with age, sex, and hypertension retained in the model.

In multivariate regression models of PA predicting MD in cingulate cortex, the exercise active group had lower MD than the sedentary group, independent of age, sex, and race (Table 10, Model 2). The association was attenuated after adjustment for diabetes (Table 10, Model 3 vs. 2; Δβ=23.3%), but remained significant. The interaction of PA and diabetes was not significant (p=.677). Adjustment for gait speed, other chronic diseases, or education did not substantially attenuate the association (Δβ<10% for all). In stepwise analyses, the association remained significant (β=-.381, p=.037) with age, sex, race, diabetes, and DSST score retained in the model.

When the sedentary group was defined by the amount of lifestyle activities only, associations with MD in medial temporal lobe and cingulate cortex remained similar (β, p-value: -.314, .059 and -.506, .006 respectively).

5.5 DISCUSSION

In this cohort of adults aged 70 to 79, a relatively large proportion of participants engaged in lifestyle and exercise activities. Our findings suggest that being exercise active, such as walking for exercise, exercising or doing recreational activities, for at least 1,000 kcal every week, may be optimal for microstructural integrity in gray matter among older adults with a range of physical and brain function and chronic diseases.

This study extended prior investigations on the dose-response relationship between PA and brain structure in older adults [10, 13]. First, the application of high resolution diffusion tensor imaging allowed the identification of very focal associations at the microstructural level. Although previous studies identified the hippocampus as a region related to PA [9, 10], these studies relied on low resolution imaging and volumetric measurements of the brain. Examining specific networks of the microstructure can help understand the mechanisms underlying the neuroprotective effects of PA in old age. Second, this study included a comprehensive characterization of health-related conditions at multiple time points which could affect PA and were important contributors to brain integrity. Our findings indicate that diabetes may have the moderating effect on the association of PA with microstructural integrity. It has been proposed that PA can protect against cognitive dysfunction and brain neurodegeneration by reducing cardiovascular disease conditions, such as hypertension, diabetes, and obesity [33]. However, a significant interaction was not detected in this study, possibly due to the small sample. The moderating effect of diabetes needs to be further explored. This knowledge may be helpful to formulate individualized prescriptions of PA for promoting brain health in older adults with and without diabetes.

Contrary to our expectations, we did not find an association of PA with white matter integrity. These null findings appeared inconsistent with previous report. Recent cross-sectional studies indicated that higher PA and fitness were associated with greater white matter integrity [13, 34], and one intervention study reported increases in fitness from walking was associated with elevated white matter integrity in young older adults [17]. It is possible that being exercise active may not impact on white matter integrity a decade later, because the evolution of white matter degeneration is stronger for very old adults than for young older adults and it may

override the short-term but potentially beneficial effects of PA. It is also possible that the association was not detected due to the small sample.

One of the limitations is using PA as a categorical variable, which is less sensitive to small variations in PA-related behaviors than using the continuous variable. We chose this coding because it has been previously validated in the parent cohort [27, 28]. Furthermore, self-reported PA may be less sensitive in measuring low intensity activities in which older adults are mostly engaging. Objective measures, for example the SenseWear Armband, are more valid and accurate in detecting low intensity activities than self-report measures. Future neuroimaging studies are warranted applying objective measures to quantify small amounts and small variations of PA. Another methodological limitation is that the presence of crossing fibers may affect the estimate of fractional anisotropy in white matter [35]. Because it primarily limits the ability to conduct tractography, we chose the predefined anatomic regions of interest approach, which is less susceptible to this limitation than tractography.

Because brain MRI was not obtained at study entry, a possible reverse causality between PA and brain integrity cannot be ruled out. Those with a more favorable neuroimaging profile at study entry would have had higher PA which may explain the observed associations. However, adjustment for brain function at study entry, a surrogate marker of brain integrity, did not modify these associations. Another limitation is that PA was not obtained at time of MRI. Therefore, the contribution of PA transition to the observed associations cannot be assessed. Those in the sedentary group may have become lifestyle or exercise active and those in lifestyle or exercise active groups may have reduced exercise over time. Those in the sedentary group may have become lifestyle or exercise active and those in lifestyle or exercise active groups may have reduced exercise over time. However, such extreme transitions appeared unlikely in this cohort,

as indicated by the similar declines in time spent walking over 9 years among three groups (p=.092). Time spent walking declined in each group (p<.001). Results were similar when using all available time points of time spent walking. Although less comprehensive than the PA measure examined here, time spent walking was positively associated with PA type (p=.008) and it has been used as a measure of PA in other studies [10, 11]. Even if such crossovers had occurred, this study would have underestimated the effect size of the relationship between PA and brain health. Lastly, this cohort may not well represent the general population of older adults due to the voluntary participation and MRI eligibility. However, the analytic sample and those seen in 2006-2008 without a brain MRI shared similar baseline characteristics.

As the number of very old adults rises, so does the incidence of cognitive impairment and dementia. A major public health priority is to identify strategies to prevent or delay the progression of brain abnormalities using effective interventions for older people. This study suggests that being exercise active may help preserve brain microstructural integrity in memory-related networks among community-dwelling older adults. Future studies are warranted to explore the moderating effect of diabetes on the neuroprotective effect of PA.

5.6 ACKNOWLEDGEMENTS

This research was supported by National Institute on Aging (NIA) Contracts N01-AG-6-2101, N01-AG-6-2103, N01-AG-6-2106, K23-AG028966-01; NIA grants R01-AG028050, R01-AG029232, P30-AG024827, and NINR grant R01-NR012459. This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging.

5.7 REFERENCES

1. Buckner, R.L., *Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate.* Neuron, 2004. **44**(1): p. 195-208.

2. Rovio, S., et al., *Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease.* Lancet Neurol, 2005. **4**(11): p. 705-11.

3. Pantano, P., et al., *Regional cerebral blood flow and oxygen consumption in human aging.* Stroke, 1984. **15**(4): p. 635-41.

4. Lu, H., et al., *Alterations in cerebral metabolic rate and blood supply across the adult lifespan.* Cereb Cortex, 2011. **21**(6): p. 1426-34.

5. Black, J.E., et al., *Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats.* Proc Natl Acad Sci U S A, 1990. **87**(14): p. 5568-72.

6. Kramer, A.F., K.I. Erickson, and S.J. Colcombe, *Exercise, cognition, and the aging brain.* J Appl Physiol, 2006. **101**(4): p. 1237-42.

7. Colcombe, S. and A.F. Kramer, *Fitness effects on the cognitive function of older adults: a meta-analytic study.* Psychol Sci, 2003. **14**(2): p. 125-30.

8. Benedict, C., et al., *Association between physical activity and brain health in older adults.* Neurobiol Aging, 2013. **34**(1): p. 83-90.

9. Bugg, J.M. and D. Head, *Exercise moderates age-related atrophy of the medial temporal lobe.* Neurobiol Aging, 2011. **32**(3): p. 506-14.

10. Erickson, K.I., et al., *Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study.* Neurology, 2010. **75**(16): p. 1415-22.

11. Ho, A.J., et al., *The effects of physical activity, education, and body mass index on the aging brain.* Hum Brain Mapp, 2011. **32**(9): p. 1371-82.

12. Rovio, S., et al., *The effect of midlife physical activity on structural brain changes in the elderly.* Neurobiol Aging, 2010. **31**(11): p. 1927-36.

13. Gow, A.J., et al., *Neuroprotective lifestyles and the aging brain: Activity, atrophy, and white matter integrity.* Neurology, 2012. **79**(17): p. 1802-1808.

14. Colcombe, S.J., et al., *Aerobic exercise training increases brain volume in aging humans.* J Gerontol A Biol Sci Med Sci, 2006. **61**(11): p. 1166-70.

15. Erickson, K.I., et al., *Exercise training increases size of hippocampus and improves memory.* Proc Natl Acad Sci U S A, 2011. **108**(7): p. 3017-22.

16. Rosano, C., et al., *Psychomotor speed and functional brain MRI 2 years after completing a physical activity treatment.* J Gerontol A Biol Sci Med Sci, 2010. **65**(6): p. 639-47.

17. Voss, M.W., et al., *The influence of aerobic fitness on cerebral white matter integrity and cognitive function in older adults: Results of a one-year exercise intervention.* Hum Brain Mapp, 2012.

18. Pahor, M., et al., *Effects of a physical activity intervention on measures of physical performance: Results of the lifestyle interventions and independence for Elders Pilot (LIFE-P) study.* J Gerontol A Biol Sci Med Sci, 2006. **61**(11): p. 1157-65.

19. Simonsick, E.M., et al., *Measuring higher level physical function in well-functioning older adults: expanding familiar approaches in the Health ABC study.* J Gerontol A Biol Sci Med Sci, 2001. **56**(10): p. M644-9.

20. Rosano, C., et al., *Neuroimaging differences between older adults with maintained versus declining cognition over a 10-year period.* Neuroimage, 2012. **62**(1): p. 307-13.

21. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL.* Neuroimage, 2004. **23 Suppl 1**: p. S208-19.

22. Wu, M., et al., *A fully automated method for quantifying and localizing white matter hyperintensities on MR images.* Psychiatry Res, 2006. **148**(2-3): p. 133-42.

23. Aizenstein, H.J., et al., *Prefrontal and striatal activation during sequence learning in geriatric depression.* Biol Psychiatry, 2005. **58**(4): p. 290-6.

24. Tzourio-Mazoyer, N., et al., *Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain.* Neuroimage, 2002. **15**(1): p. 273-89.

25. Wakana, S., et al., *Fiber tract-based atlas of human white matter anatomy.* Radiology, 2004. **230**(1): p. 77-87.

26. Taylor, H.L., et al., *A questionnaire for the assessment of leisure time physical activities.* J Chronic Dis, 1978. **31**(12): p. 741-55.

27. Brach, J.S., et al., *The association between physical function and lifestyle activity and exercise in the health, aging and body composition study.* J Am Geriatr Soc, 2004. **52**(4): p. 502-9.

28. Peterson, M.J., et al., *Physical activity as a preventative factor for frailty: the health, aging, and body composition study.* J Gerontol A Biol Sci Med Sci, 2009. **64**(1): p. 61-8.

29. Craft, L.L., et al., *Evidence that women meeting physical activity guidelines do not sit less: an observational inclinometry study.* Int J Behav Nutr Phys Act, 2012. **9**: p. 122.

30. Guralnik, J.M., et al., *Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery.* J Gerontol A Biol Sci Med Sci, 2000. **55**(4): p. M221-31.

31. Montero-Odasso, M., et al., *Gait velocity as a single predictor of adverse events in healthy seniors aged 75 years and older.* J Gerontol A Biol Sci Med Sci, 2005. **60**(10): p. 1304-9.

32. Studenski, S., et al., *Gait speed and survival in older adults.* JAMA, 2011. **305**(1): p. 50-8.

33. Cotman, C.W., N.C. Berchtold, and L.A. Christie, *Exercise builds brain health: key roles of growth factor cascades and inflammation.* Trends Neurosci, 2007. **30**(9): p. 464-72.

34. Marks, B.L., et al., *Role of aerobic fitness and aging on cerebral white matter integrity.* Ann N Y Acad Sci, 2007. **1097**: p. 171-4.

35. Budde, M.D. and J. Annese, *Quantification of anisotropy and fiber orientation in human brain histological sections.* Front Integr Neurosci, 2013. **7**: p. 3.

5.8 TABLES

Table 7: Characteristics and health-related conditions over 9 years by PA.

	Total (N=276)	Sedentary (n=39, 14.1%)	Lifestyle active (n=148, 53.6%)	Exercise active (n=89, 32.2%)	p-value
Demographics					
Age, yrs	72.9±2.7	73.1±2.9	72.9±2.8	72.7±2.6	.792
Female sex	162 (58.7)	22 (56.4)	94 (63.5)	46 (51.7)	.192
Black race	114 (41.3)	12 (30.8)	67 (45.3)	35 (39.3)	.236
Physical function					
Gait speed (m/sec)	1.28±.24	1.23±.21	1.27±.25	1.33±.24	.065
Change in gait speed (m/sec)	-0.27±0.26	-0.30±0.28	-0.27±0.26	-0.26±0.24	.646
Annual % change in gait speed	-2.23±2.06	-2.63±2.52	-2.25±2.03	-2.02±1.87	.299
Chronic disease conditions					
Prevalent cardiovascular disease	40 (14.8)	7 (18.4)	20 (14.0)	13 (14.6)	.790
Prevalent pulmonary disease	28 (10.2)	6 (15.4)	14 (9.5)	8 (9.0)	.513
Prevalent hypertension	107 (38.9)	14 (35.9)	64 (43.5)	29 (32.6)	.226
Prevalent diabetes	30 (10.9)	8 (20.5)	14 (9.5)	8 (9.0)	.112
Incident cardiovascular disease	33 (12.6)	2 (5.4)	18 (12.9)	13 (15.3)	.315
Incident stroke	9 (3.3)	0 (0.0)	7 (4.9)	2 (2.3)	.268
Cognitive function					
DSST score (0-90)	42.4±12.7	41.5±13.3	41.8±12.8	43.8±12.4	.437
Change in DSST score	-5.9±10.8	-7.3±10.5	-6.3±10.9	-4.6±10.7	.334
Annual % change in DSST	-.99±6.94	-2.05±3.47	-0.77±9.04	-0.89±2.95	.586
3MSE score (0-100)	92.2±6.3	91.3±6.5	92.7±5.9	91.8±6.8	.310
Change in 3MSE score	0.6±5.8	1.1±6.5	-0.1±5.8	1.5±5.5	.116
Annual % change in 3MSE	0.10±0.73	0.16±0.80	0.01±0.71	0.21±0.72	.113
Depressive symptoms					
CES-D score (0-60)	4.1±4.4	4.6±5.0	4.3±4.4	3.4±4.0	.203
Change in CES-D score	2.9±6.3	4.4±6.0	2.6±6.0	2.7±6.8	.255
Annual % change in CES-D	21.31±45.72	26.32±44.89	19.78±39.21	21.63±55.58	.729
Other characteristics related to PA					
Education > high school	140 (50.9)	14 (35.9)	76 (51.4)	50 (56.8)	.200
Body mass index, kg/m^2	27.4±4.6	25.5±3.6	28.1±4.7	26.9±4.5	.004
Current smokers	13 (4.7)	2 (5.1)	9 (6.1)	2 (2.2)	.649
Alcohol consumption >7 drinks/week	23 (8.3)	3 (7.7)	15 (10.1)	5 (5.6)	.428
Pulmonary function	77.0±7.4	77.8±9.1	77.2±7.7	76.5±6.3	.603
Prevalent knee osteoarthritis	18 (6.5)	1 (2.6)	12 (8.1)	2 (5.6)	.421
Time spent walking (min/week)					
Time spent walking	60 (225)	20 (135)	40 (143)	190 (385)	.008
Change in walk time	-104.3±380.0	-13.0±117.1	-91.5±347.2	-167.6±488.7	.092

Note: Values are mean±SD, N (%) or median (iqr). DSST=digit symbol substitution test. 3MSE=modified mini-mental state examination. CES-D=Center for Epidemiologic Studies Depression Scale. P-values were obtained from ANOVA tests for continuous variables and χ^2 tests for dichotomous and categorical variables.

Table 8: Neuroimaging markers from total brain and univariate associations with physical activity (N=276).

	Fractional Anisotropy	Mean diffusivity^	Parenchyma atrophy, cm^3	White matter hyperintensities*
Total (N=276)	.3581±.0140	1.3057±.1102	908.4±137.0	138 (50.0)
Sedentary (N=39, 14.1%)	.3552±.0176	1.3326±.1337	892.3±153.3	22 (56.4)
Lifestyle active (N=148, 53.6%)	.3581±.0132	1.3062±.1105	910.0±129.3	78 (52.7)
Exercise Active (N=89, 32.2%)	.3594±.0136	1.2930±.0968	912.6±143.2	38 (42.7)
p-value	.298	.174	.726	.226

Table 9: Regression models of physical activity predicting mean diffusivity in medial temporal lobe (N=276).

	Model 1: unadjusted	Model 2: adjusted for age, sex, and race	Model 3: Model 2 + Diabetes	Model 4: Model 2 + Hypertension
		β (95% CI), p		
Sedentary	Reference	Reference	Reference	Reference
Lifestyle active	-.192 (-.544, .160) .283	-.131 (-.458, .196) .433	-.086 (-.414, .242) .606	-.148 (-.471, .175) .368
Exercise active	-.431 (-.806, -.055) .025	-.405 (-.753, -.057) .023	-.359 (-.708, -.010) .044	-.389 (-.733, -.046) .027
Diabetes	-	-	.362 (.005, .718) .047	-
Hypertension	-	-	-	.347 (.122, .572) .003

Table 10: Regression models of physical activity predicting mean diffusivity in cingulate cortex (N=276).

	Model 1: unadjusted	Model 2: adjusted for age, sex, and race	Model 3: Model 2 + Diabetes
Sedentary	Reference	Reference	Reference
Lifestyle active	-.395 (-.745, -.044) .028	-.328 (-.660, .004) .053	-.253 (-.592, .086) .131
Exercise active	-.536 (-.910, -.162) .005	-.497 (-.851, -.144) .006	-.421 (-.781, -.061) .019
Diabetes	-	-	.602 (.234, .970) .001

5.9 FIGURES

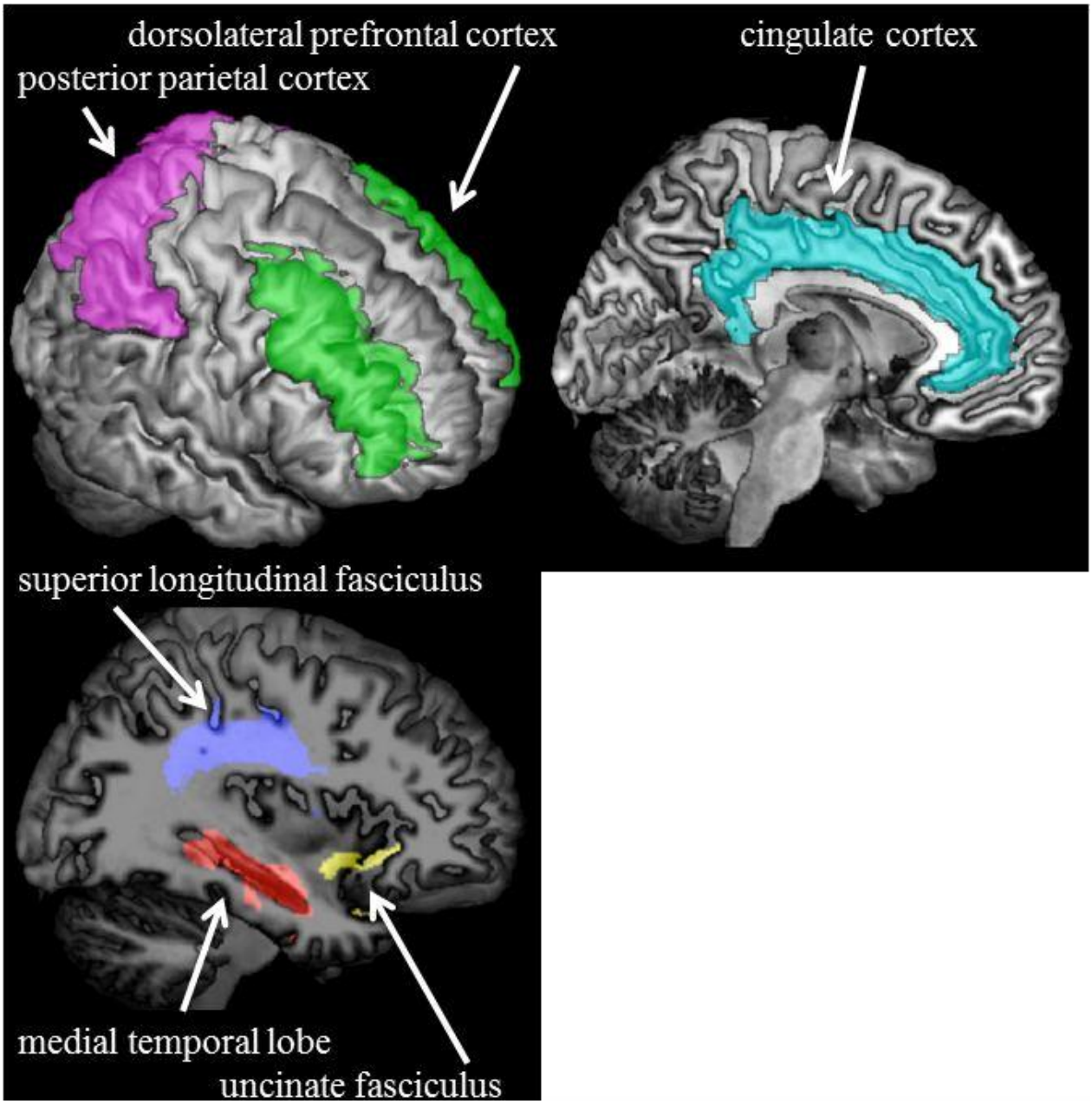

Figure 5: Regions and tracts of interest.

6.0 PAPER 3: CARDIORESPIRATORY FITNESS AND DIFFUSION TENSOR IMAGING MEASURES OF MEMORY-RELATED NETWORKS IN VERY OLD ADULTS

Qu Tian[1], Eleanor M. Simonsick[2], Kirk I. Erickson[3], Howard J. Aizenstein[4], Nancy W. Glynn[1], Robert M. Boudreau[1], Anne B. Newman[1], Stephen B. Kritchevsky[5], Kristine Yaffe[6], Tamara Harris[7], Caterina Rosano[1] for the Health ABC study

To be submitted for publication

[1]Department of Epidemiology, Center for Aging and Population Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; [2]Intramural Research Program, National Institute on Aging, Baltimore, MD; [3]Department of Psychology, University of Pittsburgh, Pittsburgh, PA; [4]Departments of Psychiatry, Bioengineering, and Clinical and Translational Science, University of Pittsburgh, Pittsburgh, PA; [5]Sticht Center on Aging, Section on Gerontology and Geriatric, Medicine, Wake Forest University School of Medicine, Winston-Salem, NC; [6]Department of Psychiatry, Neurology and Epidemiology, University of California, San Francisco, San Francisco, CA; [7]Intramural Research Program, National Institute on Aging, Laboratory of Epidemiology, Demography, and Biometry, Bethesda, MD.

6.1 ABSTRACT

Background: Higher cardiorespiratory fitness (CRF) is associated with better memory and executive function. However, the associations of CRF with brain microstructure have not been characterized in adults older than 80 years of age with chronic diseases and uniformly low levels of physical activity (PA). This cross-sectional study tested the hypothesis that higher CRF would be associated with greater microstructural integrity in adults aged 80 and older free of disability. **Methods:** Diffusion tensor imaging was obtained at 3Tesla in 164 participants (mean age=83.0 years, 57.1% female, 40.3% black) with CRF measured as time to complete a 400m walk as quickly as possible, which provided a valid estimate of peak VO_2. Associations of CRF with fractional anisotropy (FA) from normal appearing white matter in the cingulum and uncinate and superior longitudinal fasciculi and mean diffusivity (MD) from normal appearing gray matter in the dorsolateral prefrontal cortex and medial temporal lobe were tested using sex-adjusted linear regression models. Moderating effects of chronic diseases and PA were tested using hierarchical regression models. **Results:** Average completion time of the 400m walk was 5.7 minutes. One minute faster to complete the 400m walk was associated with a 1% greater FA in the cingulum, a 2.4% lower MD in the entorhinal cortex, and a 3.8% lower MD in the hippocampus (sex-adjusted standardized β, p-value: -.170, .005; .220, .006; and .165, .035; respectively). After adjustment for age, only the association with MD in the entorhinal cortex remained significant. The association with MD in the entorhinal cortex was also attenuated but remained significant after adjustment for hypertension (Δβ=10.9%). The interaction of 400m time and hypertension was not significant. Adjustment for other chronic diseases or PA did not substantially attenuate any of these associations. Associations with other neuroimaging markers were not significant.

Conclusion: Higher CRF measured using the 400m walk is associated with greater microstructural integrity in the medial temporal lobe, especially in the entorhinal cortex, in adults older than 80 years of age. Future studies should examine the contribution of hypertension to the neuroprotective effects of CRF in this area and their implications for reducing cognitive decline.

6.2 INTRODUCTION

Cumulative evidence shows that higher cardiorespiratory fitness (CRF) is associated with improved memory and executive function in older adults (for meta-analysis, see [1]; for review, see [2, 3]). However, the impact of CRF on brain structure is less clear. Some studies have shown that higher CRF is associated with reduced brain atrophy localized in the prefrontal and medial temporal lobes [4-7]. These regions support memory and executive function [8] and are also highly susceptible to changes in blood oxygenation levels [9]. By contrast, other studies found no significant associations between CRF and brain atrophy in cognitively normal older adults, but rather the effects of CRF were significant in older adults with early dementia [10, 11]. Most of prior neuroimaging studies have applied macroscopic measurements of the brain in younger old.

The association between CRF and microstructural integrity of gray and white matter is even less clear. Although pioneer studies using diffusion tensor imaging have shown a positive association between CRF and white matter microstructure [12-15], it is not known whether the associations are localized in regions and tracts related to memory and executive function. Thus, a careful characterization of the relationship between CRF and brain microstructure in both gray

and white matter is needed to further the understanding on how CRF influences the structural integrity in the brain. It is also not known whether chronic diseases and physical activity (PA) which can affect both CRF and brain health, would moderate the relationship between CRF and the structural integrity in older adults, especially in adults older than 80 years of age. The decrease in CRF with advancing age may be primarily determined by limited PA participation, a potential consequence of chronic disease conditions common in older persons.

Among adults older than 80 years of age, the safest and most valid approach of assessing CRF remains a source of debate. Most prior studies focusing on well-functioning adults in their mid-sixties applied the graded maximal exercise test [4, 6, 12, 13], which can pose safety risks for adults older than 80 years of age [16, 17]. Compared to the graded maximal exercise test, the self-paced 400-m long-distance corridor walk test has high safety and validity in measuring CRF and provides a good estimate of VO_2max in adults aged 70 and older [18].

This study quantified the association between CRF, measured as time to complete a 400m walk as quickly as possible [18], and neuroimaging markers of brain microstructure in a cohort of adults aged 80 years and older free of disability, while accounting for chronic disease conditions and PA. It was hypothesized that higher CRF would be associated with greater brain integrity in the prefrontal cortex and medial temporal lobe. It was also hypothesized that these associations would be moderated by prevalent diseases and PA.

6.3 METHODS

6.3.1 Study population

Participants were recruited from the Health, Aging and Body Composition study cohort, an ongoing longitudinal study that began in March 1997 in Pittsburgh, PA and Memphis, TN to assess the relationship between changes in body composition and health outcomes in 3,075 community-dwelling older adults (52% women, 42% Black) aged 70 to 79 [19]. Among 1,527 participants seen at the Pittsburgh site at study entry (1997-8), 819 were alive and seen in the clinic or at home in 2006-8. Of the 819, 193 did not meet the MRI eligibility, 145 refused, 52 did not come for the clinic or had home visit, and 16 were not offered MRI before the study began. 325 had Magnetic Resonance Imaging (MRI) of the brain. Of this group, 226 had Diffusion Tensor Imaging (DTI) at 3Tesla and were asked to perform the Long-Distance Corridor Walk (LDCW) test. Twenty-three (10.2%) were excluded from the test due to medical contraindications, including electrocardiogram abnormalities, mobility-related exclusions or recent chest pain, shortness of breath, or fainting and thirty-nine (17.3%) were unable to complete the test. One hundred and sixty-four (72.5%) completed the test and had 400m time measured to the nearest second (Figure 6). The study protocol was approved by the University of Pittsburgh and all participants provided informed consent.

6.3.2 MRI protocol and markers

MRI scans were obtained at the MR Research Center of the University of Pittsburgh with 3Tesla Siemens TIM TRIO scanners equipped for echo-planer imaging and with Diffusion Tensor using single-short spin-echo sequence and a protocol previously described [20]. There were no pathological findings from the MR images for any study participant in this study as verified by a neuroradiologist.

MRI outcomes were microstructural indices of mean diffusivity (MD) from normal appearing gray matter (GM) and fractional anisotropy (FA) from normal appearing WM. Diffusion tensor imaging (DTI) preprocessing was done using the FMRIB's Diffusion Toolbox to correct eddy current-induced distortions [21]. The tensor was computed [22] and diagonalized to determine the eigenvalues from which the FA and MD maps were computed [23].

Regions and tracts were identified using the previously published Automated Labeling Pathway [24-26]. The anatomic localizations of GM regions and WM tracts were identified using the Automated Anatomical Labeling Atlas [27] and the Johns Hopkins University White Matter Atlas [28], respectively. Regions and tracts of interest were selected as *a prior* (Figure 7), including the medial temporal lobe (hippocampus, parahippocampus, entorhinal cortex), the dorsolateral prefrontal cortex, uncinate and superior longitudinal fasciculi, and the cingulum [29]. MD in GM regions of interest from left and right hemispheres was computed as the mean weighted by GM volume. FA in WM tracts from left and right hemispheres was computed as the mean.

6.3.3 Cardiorespiratory fitness

Cardiorespiratory fitness was measured as time to complete the Long-Distance Corridor Walk (LDCW) test as quickly as possible. The LDCW consists of a 2-minute walk followed immediately by a 400m walk with the instruction to "walk as quickly as possible". The detailed procedures for the LDCW are described elsewhere [30]. Time to complete 400m has been shown to be a valid estimate of peak VO_2 among older adults [18]. In this study, 400m time in seconds was computed in a standardized unit.

6.3.4 Other measures of interest

Chronic conditions, including prevalent cardiovascular disease (CVD), hypertension, myocardial infarction, stroke, and diabetes, were all obtained concurrently with the LDCW test and brain MRI in 2006-08 using prevalent disease algorithms according to self-reported diagnoses by physicians and record of medication use. Prevalent CVD was defined by self-reported prevalent coronary heart disease or cerebrovascular disease. Prevalent diabetes and hypertension were defined by self-report and confirmed by medication use.

Cognitive function was measured by the digit symbol substitution test (DSST) and the modified mini-mental status examination (3MSE) and depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D). Physical function was measured as gait speed using the GaitMat II^{TM} system (EQ Inc., Chalfont, Pennsylvania). Participants were asked to walk at their usual pace on the 4-m-long GaitMat II^{TM} walkway. For those who did not have the GaitMat measure, gait speed was computed in meters/second while

walking at a usual pace over 3, 4 or 6 meters [31, 32]. Gait speed is a valid and reliable assessment of physical function among older adults in both the clinical settings and in aging research [33, 34]. Slower gait speed is strongly associated with severity of health conditions and higher mortality risk [35, 36].

In addition to age, sex, and race obtained at study entry, other characteristics related to CRF were measured at the time of the MRI, including education, body mass index (BMI, kg/m^2), smoking status, alcohol consumption, and systolic blood pressure (mm Hg). Systolic blood pressure was obtained as the average from two measurements. Pulmonary function was assessed by the ratio of forced expiratory volume in the first second and forced viral capacity (FEV1/FVC). Physical activity (PA) was measured as walking and climbing stairs in the past week by self-report at the time of the MRI [37]. PA was computed as a continuous variable in kcal/kg/week.

6.3.5 Statistical analysis

Associations of 400m time with characteristics and neuroimaging markers were tested using a partial correlation analysis adjusted for sex (Tables 11 and 12). Associations between 400m time and neuroimaging markers significant at $P<.05$ were further adjusted for age, chronic diseases, or PA in multivariate linear regression models (Table 13).

Moderating effects of chronic diseases and PA on the strengths of associations between 400m time and neuroimaging markers were tested using hierarchical multiple regression models: (1) the moderator (if continuous) was centered on the parent scale, (2) the interaction term between 400m time and the moderator was created, and (3) hierarchical multiple regression

models were conducted by first entering 400m time and the moderator and then adding the interaction term. A significant model change after adding the interaction term and also the significance of the interaction term at p<.05 indicated a statistically significant moderating effect.

6.4 RESULTS

The 164 participants in this study who had a brain MRI and completed the LDCW in 2006-08 were 51.8% female, 40.3% black (Table 11) and were similar to those who completed the LDCW in the parent cohort at study entry (48.8% female, 37.7% black) [38]. Compared to those who received a brain MRI but did not complete the LDCW test, the 164 completers were more likely to be men (p=.016) and had higher DSST score (p=.019), faster gait speed (p<.001), and lower BMI (p=.016). The completers tended to have lower prevalence of hypertension and diabetes, although group differences were not statistically significant (p=.058 and .060, respectively).

Faster 400m time was associated with younger age, white race, higher DSST and 3MSE scores, lower CES-D score, faster gait speed, and lower BMI (sex-adjusted p<.05 for all). Associations of 400m time with chronic diseases and PA were in the expected directions and were statistically significant for prevalent hypertension (p=.031) and PA (p=.002) (Table 11). Compared to women, men had faster 400m time, were less likely to be black, had hypertension and stroke, were more likely to be current or former smokers (p<.001), reported higher alcohol consumption, and had lower pulmonary function as indicated by FEV1/FVC (Table 11).

Higher FA in the cingulum was associated with younger age (p=.030), male sex (p=.001), prevalent hypertension (p=.045) and stroke (p=.006), higher education (p=.039), and less alcohol consumption (p=.012). Lower MD in the medial temporal lobe was associated with younger age (p<.001), female sex (p=.017), higher DSST (p=.007) and 3MSE scores (p=.035), faster gait speed (p=.016), and less alcohol consumption (p=.002) (Table 19).

Faster 400m time was significantly associated with higher FA in the cingulum at p<0.05, but not with FA in uncinate and superior longitudinal fasciculi (Table 12). Faster 400m time was significantly associated with lower MD in the medial temporal lobe at p<0.05, but not with MD in the dorsolateral prefrontal cortex (Table 12). Among the subregions of the medial temporal lobe, associations were significant at p<0.05 for the entorhinal cortex and the hippocampus.

In sex-adjusted models (Table 13), one standard deviation decrease in 400m time (64 seconds) was associated with an increase of .00388 (=.170*.0228) in FA in the cingulum, which was equivalent to 1% of the mean FA in the cingulum of this sample (mean=.3966, Table 12). Similarly, each standard deviation decrease in 400m time was associated with a decrease of .00004 (=.165*.00024) in MD in the hippocampus, which was 2.4% of the mean MD in the hippocampus of this sample (mean=.00165, Table 12) and a decrease of .000055 (=.220*.00025) in MD in the entorhinal cortex, which was 3.8% of the average MD in the entorhinal cortex of this sample (mean=.00144, Table 12).

The sex-adjusted associations of 400m time with FA in the cingulum and MD in the hippocampus and the entorhinal cortex were attenuated after adjustment for age; the change in the regression coefficient of 400m time was of 20.0%, 58.8% and 23.2%, respectively (Table 13). Only the association with MD in the entorhinal cortex remained significant at p<0.05 after adjustment for age. The interactions between 400m time and age on these associations were not

significant (p>.05 for all). The sex-adjusted association with MD in the entorhinal cortex was attenuated after adjustment for hypertension (Δβ=10.9%). The interaction between 400m time and hypertension on the association with MD in the entorhinal cortex was not significant (p>.05). Trends of the associations with MD in the entorhinal cortex were similar for those with and without hypertension. Adjustment for cardiovascular disease, stroke, myocardial infarction, diabetes, or PA, did not substantially attenuate the associations with FA in the cingulum or MD in the hippocampus and in the entorhinal cortex (Table 13).

6.5 DISCUSSION

In this cohort of adults aged 80 years and older free of disability, higher cardiorespiratory fitness was associated with higher microstructural integrity in the white matter of the cingulum and the gray matter of the medial temporal lobe with stronger associations in the gray matter of the entorhinal cortex. Associations were independent of physical activity and chronic disease conditions.

The application of high-resolution diffusion tensor imaging allowed investigation of the spatial distribution of microstructural integrity related to cardiorespiratory fitness in this sample of very old adults and it extends previous work in young old adults (mid-sixties) [13, 14]. The application of the long-distance corridor walk test allowed testing the association between cardiorespiratory fitness and brain health in a sample of very old persons. The sample had a mean age of 83 years and only 14.9% walked at a pace of at least 1.34 meters/second, the typical

starting pace for treadmill protocols [38]. Thus, the majority of these participants would not have been able to perform a traditional treadmill test to quantify fitness.

Associations were localized in the medial temporal lobe, which are vulnerable to lower perfusion mainly because of their watershed vascularization [9]. Cardiorespiratory fitness may preserve integrity in these networks by improving oxygen transport and utilization in the cerebral vascular system and increase oxidative capacity in the brain [39, 40]. The finding that hypertension attenuated the association with integrity in the entorhinal cortex is consistent with this pathway linking CRF and higher integrity. However, a significant interaction term of cardiorespiratory fitness and hypertension was not detected and the patterns of the association were similar after stratification by hypertension (Figure 8). Whether changes in blood pressure underlie the relationship between cardiorespiratory fitness and brain microstructural integrity needs further exploration.

Because the long-distance corridor walk performance is associated with chronic disease conditions, such as cardiovascular disease, stroke, and diabetes [30, 38, 41], we hypothesized that these disease conditions would explain the associations between cardiorespiratory fitness and neuroimaging markers of interest. However, adjustment for cardiovascular disease, myocardial infarction, stroke, and diabetes, did not substantially attenuate the association of CRF with neuroimaging outcomes. It is possible that adults who have survived to this old age free from clinically overt disabilities, even though have been exposed to these chronic conditions, are exceptionally robust and have developed resilience to such chronic conditions.

We found that associations were also independent of physical activity. This could have been related to the type of the physical activity measurement, which was based on self-reported information of a few specific activities (i.e. walking and climbing stairs) and may not have

captured the whole spectrum of free-living activities, especially for those activities with light-to-low intensity. Future studies applying objective measures of physical activity can clarify the contribution of physical activity to the association between cardiorespiratory fitness and brain structure.

Although the observed associations were attenuated after adjustment for age, the interactions between 400m time and age were not significant. Because the age range of this analytic sample was narrow (79-90 years old), the effect of age on these associations may be due to the collinearity between 400m time and age, rather than a true age-related moderation. Others also reported that the association of cardiorespiratory fitness with microstructural integrity was attenuated after adjustment for age and sex [13].

Because of the cross-sectional design, this study cannot assess a causal relationship between cardiorespiratory fitness and brain structural integrity and reverse causality cannot be ruled out. For example, those with greater brain integrity may have a higher healthy life-style consciousness and thus choose to engage in fitness promoting activities. However higher physical activity was not associated with brain integrity in this study. A second limitation is that the long-distance corridor walk test cannot estimate the fitness level for those ineligible to participate and those who stopped. Lastly, our study participants are likely to be healthier than the general population due to their ability to complete the long distance corridor test and eligibility for brain MRI. While our findings may be applicable only to very old adults free of disability, they are not likely to be driven by illness or frailty status.

6.6 ACKNOWLEDGEMENTS

This research was supported by National Institute on Aging (NIA) Contracts N01-AG-6-2101, N01-AG-6-2103, N01-AG-6-2106, K23-AG028966-01; NIA grants R01-AG028050, R01-AG029232, P30-AG024827, and NINR grant R01-NR012459. This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging.

6.7 REFERENCES

1. Colcombe, S. and A.F. Kramer, *Fitness effects on the cognitive function of older adults: a meta-analytic study.* Psychol Sci, 2003. **14**(2): p. 125-30.

2. Kramer, A.F., et al., *Fitness, aging and neurocognitive function.* Neurobiol Aging, 2005. **26 Suppl 1**: p. 124-7.

3. Kramer, A.F., K.I. Erickson, and S.J. Colcombe, *Exercise, cognition, and the aging brain.* J Appl Physiol, 2006. **101**(4): p. 1237-42.

4. Colcombe, S.J., et al., *Aerobic fitness reduces brain tissue loss in aging humans.* J Gerontol A Biol Sci Med Sci, 2003. **58**(2): p. 176-80.

5. Colcombe, S.J., et al., *Aerobic exercise training increases brain volume in aging humans.* J Gerontol A Biol Sci Med Sci, 2006. **61**(11): p. 1166-70.

6. Erickson, K.I., et al., *Aerobic fitness is associated with hippocampal volume in elderly humans.* Hippocampus, 2009. **19**(10): p. 1030-9.

7. Erickson, K.I., et al., *Exercise training increases size of hippocampus and improves memory.* Proc Natl Acad Sci U S A, 2011. **108**(7): p. 3017-22.

8. Park, D.C., et al., *Cerebral aging: integration of brain and behavioral models of cognitive function.* Dialogues Clin Neurosci, 2001. **3**(3): p. 151-65.

9. Bladin, C.F., B.R. Chambers, and G.A. Donnan, *Confusing stroke terminology: watershed or borderzone infarction?* Stroke, 1993. **24**(3): p. 477-8.

10. Burns, J.M., et al., *Cardiorespiratory fitness and brain atrophy in early Alzheimer disease.* Neurology, 2008. **71**(3): p. 210-6.

11. Honea, R.A., et al., *Cardiorespiratory fitness and preserved medial temporal lobe volume in Alzheimer disease.* Alzheimer Dis Assoc Disord, 2009. **23**(3): p. 188-97.

12. Johnson, N.F., et al., *Cardiorespiratory fitness is positively correlated with cerebral white matter integrity in healthy seniors.* Neuroimage, 2012. **59**(2): p. 1514-23.

13. Marks, B.L., et al., *Aerobic fitness and obesity: relationship to cerebral white matter integrity in the brain of active and sedentary older adults.* Br J Sports Med, 2011. **45**(15): p. 1208-15.

14. Marks, B.L., et al., *Role of aerobic fitness and aging on cerebral white matter integrity.* Ann N Y Acad Sci, 2007. **1097**: p. 171-4.

15. Voss, M.W., et al., *The influence of aerobic fitness on cerebral white matter integrity and cognitive function in older adults: Results of a one-year exercise intervention.* Hum Brain Mapp, 2012.

16. Hollenberg, M., et al., *Treadmill exercise testing in an epidemiologic study of elderly subjects.* J Gerontol A Biol Sci Med Sci, 1998. **53**(4): p. B259-67.

17. Wilson, P.W., et al., *Assessment methods for physical activity and physical fitness in population studies: report of a NHLBI workshop.* Am Heart J, 1986. **111**(6): p. 1177-92.

18. Simonsick, E.M., E. Fan, and J.L. Fleg, *Estimating cardiorespiratory fitness in well-functioning older adults: treadmill validation of the long distance corridor walk.* J Am Geriatr Soc, 2006. **54**(1): p. 127-32.

19. Simonsick, E.M., et al., *Measuring higher level physical function in well-functioning older adults: expanding familiar approaches in the Health ABC study.* J Gerontol A Biol Sci Med Sci, 2001. **56**(10): p. M644-9.

20. Rosano, C., et al., *Neuroimaging differences between older adults with maintained versus declining cognition over a 10-year period.* Neuroimage, 2012. **62**(1): p. 307-13.

21. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL.* Neuroimage, 2004. **23 Suppl 1**: p. S208-19.

22. Basser, P.J., J. Mattiello, and D. LeBihan, *Estimation of the effective self-diffusion tensor from the NMR spin echo.* J Magn Reson B, 1994. **103**(3): p. 247-54.

23. Pierpaoli, C., et al., *Diffusion tensor MR imaging of the human brain.* Radiology, 1996. **201**(3): p. 637-48.

24. Aizenstein, H.J., et al., *Prefrontal and striatal activation during sequence learning in geriatric depression.* Biol Psychiatry, 2005. **58**(4): p. 290-6.

25. Wu, M., et al., *Quantitative comparison of AIR, SPM, and the fully deformable model for atlas-based segmentation of functional and structural MR images.* Hum Brain Mapp, 2006. **27**(9): p. 747-54.

26. Wu, M., et al., *A fully automated method for quantifying and localizing white matter hyperintensities on MR images.* Psychiatry Res, 2006. **148**(2-3): p. 133-42.

27. Tzourio-Mazoyer, N., et al., *Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain.* Neuroimage, 2002. **15**(1): p. 273-89.

28. Wakana, S., et al., *Fiber tract-based atlas of human white matter anatomy.* Radiology, 2004. **230**(1): p. 77-87.

29. Rosano, C., et al., *A regions-of-interest volumetric analysis of mobility limitations in community-dwelling older adults.* J Gerontol A Biol Sci Med Sci, 2007. **62**(9): p. 1048-55.

30. Newman, A.B., et al., *Walking performance and cardiovascular response: associations with age and morbidity--the Health, Aging and Body Composition Study.* J Gerontol A Biol Sci Med Sci, 2003. **58**(8): p. 715-20.

31. Klepin, H.D., et al., *Physical performance and subsequent disability and survival in older adults with malignancy: results from the health, aging and body composition study.* J Am Geriatr Soc, 2010. **58**(1): p. 76-82.

32. Inzitari, M., et al., *Gait speed predicts decline in attention and psychomotor speed in older adults: the health aging and body composition study.* Neuroepidemiology, 2007. **29**(3-4): p. 156-62.

33. Guralnik, J.M., et al., *Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery.* J Gerontol A Biol Sci Med Sci, 2000. **55**(4): p. M221-31.

34. Studenski, S., et al., *Physical performance measures in the clinical setting.* J Am Geriatr Soc, 2003. **51**(3): p. 314-22.

35. Montero-Odasso, M., et al., *Gait velocity as a single predictor of adverse events in healthy seniors aged 75 years and older.* J Gerontol A Biol Sci Med Sci, 2005. **60**(10): p. 1304-9.

36. Studenski, S., et al., *Gait speed and survival in older adults.* JAMA, 2011. **305**(1): p. 50-8.

37. Taylor, H.L., et al., *A questionnaire for the assessment of leisure time physical activities.* J Chronic Dis, 1978. **31**(12): p. 741-55.

38. Newman, A.B., et al., *Association of long-distance corridor walk performance with mortality, cardiovascular disease, mobility limitation, and disability.* JAMA, 2006. **295**(17): p. 2018-26.

39. Etnier, J.L. and D.M. Landers, *Brain function and exercise. Current perspectives.* Sports Med, 1995. **19**(2): p. 81-5.

40. Ainslie, P.N., et al., *Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing.* J Physiol, 2008. **586**(16): p. 4005-10.

41. Enright, P.L., et al., *The 6-min walk test: a quick measure of functional status in elderly adults.* Chest, 2003. **123**(2): p. 387-98.

6.8 TABLES

Table 11: Characteristics of the analytic sample by sex (N=164).

	Completed (n=164)	Men (n=79, 48.2%)	Women (n=85, 51.8%)	p-value
Demographics				
Age, years	82.9±2.6**	82.8±2.8	82.9±2.5	.772
Female	85 (51.8)	-	-	-
Black race	63 (38.4)**	22 (27.8)	41 (48.2)	.007
Cardiorespiratory fitness				
400m time, seconds	344.4±63.7	329.3±61.8	358.4±62.6	.003
Physical Activity				
Walking and climbing stairs, kcal/kg/week	6.2±10.5*	6.0±10.7	6.4±10.3	.810
Chronic disease conditions[1]				
Cardiovascular disease	42 (25.6)	23 (29.1)	19 (22.4)	.322
Hypertension	102 (62.2)*	41 (51.9)	62 (72.9)	.005
Myocardial infarction	20 (12.2)	13 (16.5)	7 (8.2)	.108
Stroke	12 (7.3)	1 (1.3)	11 (12.9)	.004
Diabetes	37 (22.6)	23 (29.1)	14 (16.5)	.053
Cognitive function				
DSST score (range 0-90)	38.5±13.2**	38.2±12.7	38.8±13.8	.768
3MSE score (range 0-100)	93.9±5.8*	94.4±5.4	93.4±6.2	.313
Depressive symptoms				
CES-D score (range 0-60)	6.2±5.4*	5.9±4.7	6.4±6.0	.572
Physical function				
Gait speed, meters/second	1.01±.23**	.98±.18	.94±.17	.104
Other characteristics related to CRF				
Postsecondary Education	89 (54.6)	49 (62.8)	40 (47.1)	.092
Current or former smokers	75 (45.7)	51 (64.5)	24 (28.2)	<.001
Alcohol consumption ever >=5drinks/day	9 (5.8)	9 (11.4)	0 (0)	.001
Body mass index, kg/m^2	26.8±4.1**	27.3±3.8	26.4±4.3	.192
Systolic blood pressure, mm Hg	133.6±19.0	132.5±18.4	134.7±19.6	.483
FEV1/FVC	70.8±9.3	69.1±9.0	72.4±9.3	.026

Abbreviations: DSST=digit symbol substitution test. 3MSE=modified mini-mental state examination. CES-D=Center for Epidemiologic Studies Depression Scale. FEV1/FVC=forced expiratory volume in first second/forced vital capacity, unitless. Note: Values are mean±SD or N(%) as noted. [1] Disease prevalence at the time of MRI and LDCW measurements. **sex-adjusted correlations with 400m time p<.001,*p<.05. Partial correlation coefficient ranged from .18 to .32 and from -.55 to -.23. P-values for sex differences were computed from independent t-test or χ^2 test as appropriate.

Table 12: Fractional anisotropy (FA, higher=greater integrity) in tracts of interest and mean diffusivity (MD, lower=greater integrity) in regions of interest and partial correlations with 400m time in seconds (faster=higher fitness) after controlling for sex.

Regions and tracts of interest	Mean±SD	Partial correlations, r	p-value
Microstructural integrity of white matter			
FA in the cingulum	.3966±.0228	-.185	.018
FA in the uncinate fasciculus	.3247±.0264	.008	.918
FA in the superior longitudinal fasciculus	.3547±.0200	-.116	.142
Microstructural integrity of gray matter			
MD in the dorsolateral prefrontal cortex	.00108±.00011	-.058	.466
MD in the medial temporal lobe	.00157±.00021	.180	.022
in the hippocampus	.00165±.00024	.166	.035
in the parahippocampus	.00154±.00020	.147	.062
in the entorhinal cortex	.00144±.00025	.215	.006

Table 13: Sex-adjusted multivariate regression models of 400m time (faster=higher fitness) predicting fractional anisotropy (FA, higher=greater integrity) in the cingulum and mean diffusivity (MD, lower=greater integrity) in the hippocampus and the entorhinal cortex, standardized units (N=164).

Covariates	FA in the cingulum	MD in the hippocampus	MD in the entorhinal cortex
	β (95% CI), p		
sex	-.170 (-.310, -.029) .018	.165 (.012, .317) .035	.220 (.064, .376) .006
sex + age	-.136 (-.282, .010) .067*	.068 (-.083, .219) .376*	.169 (.008, .329) .040*
Sex+ cardiovascular disease	-.171 (-.311, -.031) .017	.163 (.010, .315) .036	.222 (.066, .378) .006
sex + hypertension	-.156 (-.300, -.012) .034	.153 (-.003, .310) .055	.196 (.037, .355) .016*
sex + myocardial infarction	-.170 (-.310, -.029) .019	.165 (.012, .318) .035	.220 (.064, .376) .006
sex + stroke	-.166 (-.305, -.027) .020	.164 (.011, .317) .036	.217 (.062, .373) .006
sex + diabetes	-.168 (-.310, -.026) .020	.166 (.012, .321) .035	.216 (.058, .373) .008
sex + physical activity	-.157 (-.302, -.012) .034	.163 (.005, .321) .043	.213 (.052, .374) .010

Notes: Covariates included age, chronic disease conditions and physical activity, which were known to be associated with 400m time. *Changes in regression coefficients compared to Model 1 > 10%.

6.9 FIGURES

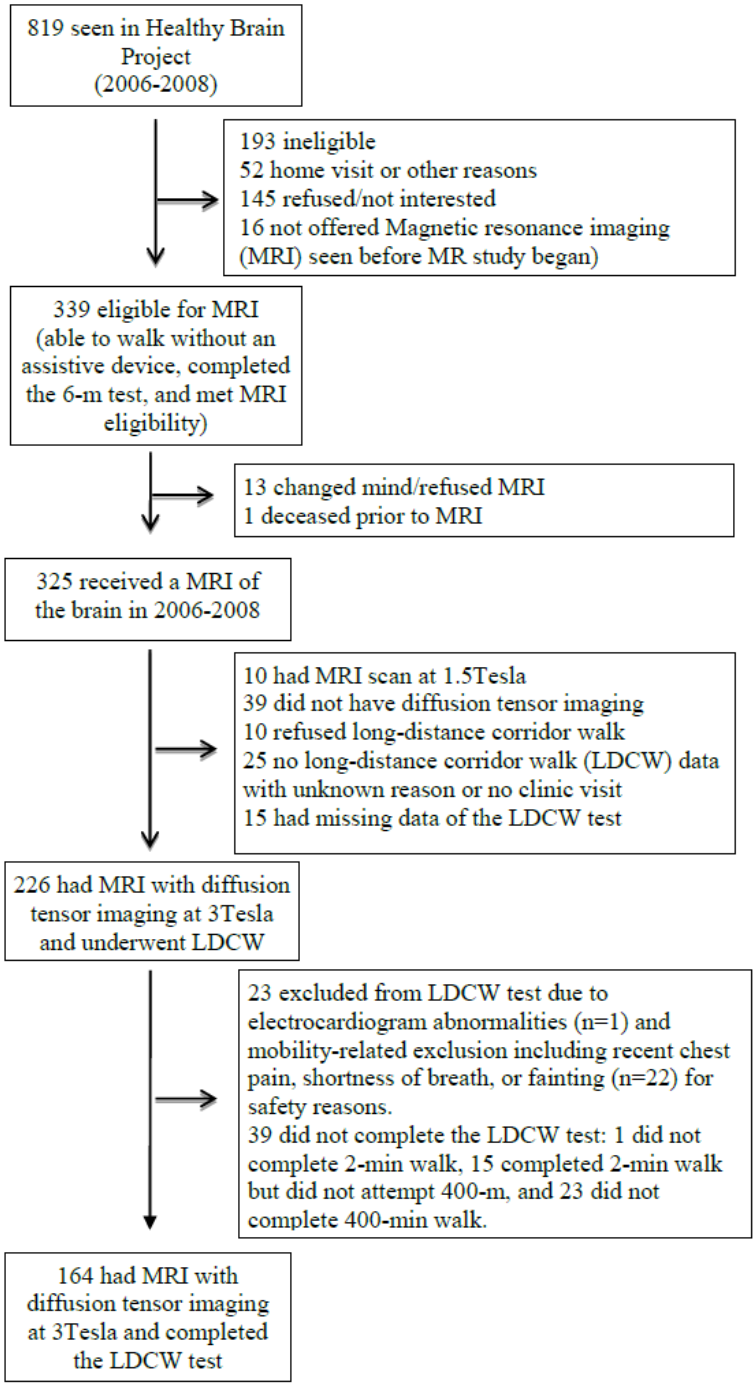

Figure 6: Flow chart of the analytic sample drawn from the Health, Aging and Body Composition study cohort.

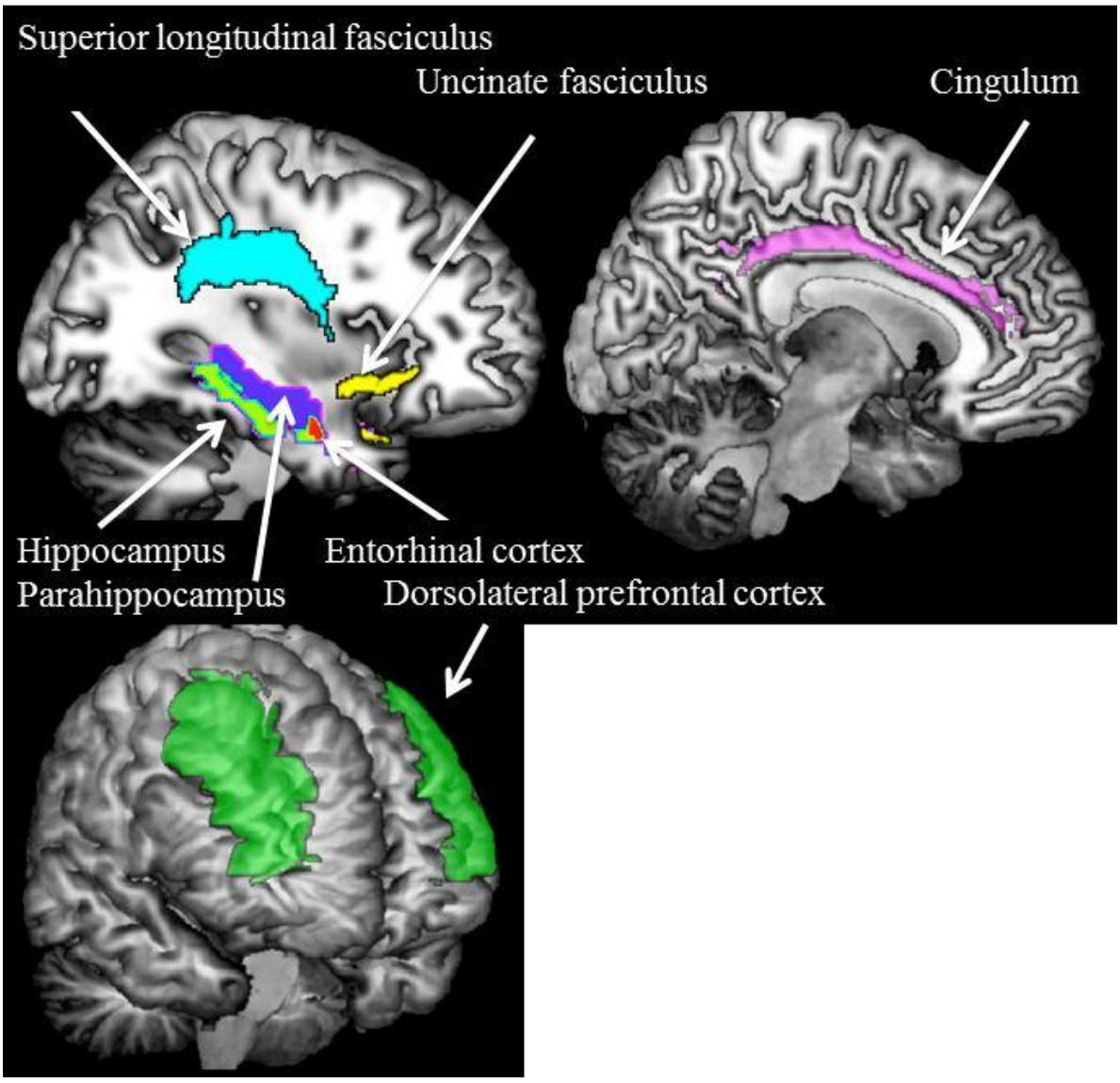

Figure 7: Regions and tracts of interest.

7.0 DISCUSSION AND FUTURE DIRECTIONS

The overall goal of my dissertation work was to provide scientific recommendations of physical activity on preserving brain structural integrity in community-dwelling older adults. Papers #1-3 quantified the associations of physical activity and cardiorespiratory fitness (e.g. a physiological marker of physical activity) with brain structure while accounting for physical function and chronic disease conditions known to be related to both physical activity participation and brain health. In a nutshell, I found that these associations were significant only for the micro- but not the macrostructure of the brain and they were mostly localized in the medial temporal lobe, independent of health-related conditions (Table 14). Specifically, being exercise active and higher levels of cardiorespiratory fitness were associated with greater microstructural integrity in subcortical networks localized to the medial temporal lobe (paper #2 and paper #3) in adults aged 80 years and older. Prevalence of hypertension and diabetes attenuate but not moderate the observed associations. In a small subset of well-characterized older adults aged 80 years and older, I also discovered that greater amounts of physical activity by the objective measure, especially longer duration, were associated with greater microstructural integrity in the superior longitudinal fasciculus connecting the frontal lobe and the lateral portion of the temporal lobe (paper #1).

The application of the SenseWear Armband provided valuable data on physical activity dose, duration, and intensity. To the best of my knowledge, paper#1 is the first study examining the association of physical activity using the objective measure with brain structure. Several strengths from paper #1 are worth highlighting. First, it provided neuroimaging evidence supporting the beneficial effects of physical activity on brain function in very old age. It identified that overall dose and longer duration, but not intensity of physical activity, were strongly associated with brain structural integrity in the superior longitudinal fasciculus connecting the frontal lobes to the lateral portions of the medial temporal lobe. Third, the careful characterization of physical function and health-related conditions allowed testing the potential moderating effects on the observed associations. Last but not least, this study examined a group of very old adults who are at high risk of developing brain abnormalities and dementia, which has not been previously studied. Thus, findings have important implications in designing intervention strategies in preventing or delaying the progression of brain abnormalities in very old age. Because the study sample is mostly sedentary with a limited range of intensity levels, the effect of intensity may not be detected. The causation is also unclear due to the cross-sectional design of the study.

Paper #2 primarily focused on the moderating effects of physical function and chronic disease conditions on the association between physical activity and brain structure. Although a few existing studies controlled for disease conditions, none of prior studies have accounted for physical function or tested the moderating effects of physical function and chronic diseases. Findings from paper #2 shows prevalent diabetes and hypertension at the time of physical activity measurement attenuated the observed associations, especially the association with microstructural integrity in the medial temporal lobe. The hypothesis that physical activity may

benefit brain health by reducing peripheral risk factors, such as hypertension and insulin resistance, seems to be supported. A significant moderating effect of hypertension or diabetes was not detected possibly due to the small sample size and the low prevalence conditions.

Paper #3 examined the association of cardiorespiratory fitness with brain microstructure and tested the contribution of physical activity and the effects of chronic diseases. This paper extended previous work by applying the long-distance corridor work in a group of very old adults and examining microstructural integrity in both gray and white matter using high resolution tensor imaging. Similarly, prevalence of hypertension attenuated the association with microstructural integrity in the entorhinal cortex, one sub-region of the medial temporal lobe. The moderating effect was not detected possibly due to the small sample. The causation cannot be determined due to the cross-sectional design of the study.

Future studies in large samples are needed to examine the potential moderating effects of hypertension and diabetes on the association of physical activity, cardiorespiratory fitness predicting brain structure. Studies in a sample with a wide range of the intensity level are needed to identify the effect of physical activity intensity on brain structure.

Table 14: A summary of papers #1-3 examining the associations of physical activity and cardiorespiratory fitness with brain structure in older adults.

Papers	Study Design	Age	N	Exposure	Outcome measures	Covariates	Main findings	Moderating effects
Paper#1	Cross-sectional	Mean age=86	93	PA by the objective measure of the SenseWear Armband	White matter hyperintensities and fractional anisotropy (FA) in uncinate and superior longitudinal fasciculi	Age, sex, race, physical and brain function, chronic diseases, and PA-related characteristics	Higher PA (dose and duration, not intensity) -higher FA in the superior longitudinal fasciculus	None
Paper#2	Longitudinal	Mean age=72.9 at the time of PA measurement	276	PA (sedentary, lifestyle active, and exercise active) by a self-report questionnaire	Mean diffusivity (MD) in the medial temporal lobe, the cingulate cortex, the dorsolateral prefrontal cortex, and posterior parietal cortex; FA in the uncinate and superior longitudinal fasciculus.	Age, sex, race, physical and brain function, chronic diseases, and PA-related characteristics	Higher PA -lower MD in the cingulate cortex and the medial temporal lobe	None; Both associations were attenuated by diabetes. The association with MD in the medial temporal lobe was also attenuated by hypertension.
Paper#3	Cross-sectional	Mean age=82.9	164	Cardiorespiratory fitness (CRF) by the 400m walk	MD in the medial temporal lobe and dorsolateral prefrontal cortex and FA in the uncinate fasciculus and cingulum	Age, sex, race, physical and brain function, chronic diseases, and PA-related characteristics	Higher CRF -higher FA in the cingulum -lower MD in the medial temporal lobe (especially in the entorhinal cortex)	None; All associations were attenuated by age. The association with MD in the entorhinal cortex was attenuated by hypertension.

8.0 PUBLIC HEALTH SIGNIFICANCE

Findings from these studies lay a foundation for my future work to formulate precise prescriptions of free-living physical activity that may be beneficial for brain abnormalities among very old community-dwelling adults. Identifying brain regions using state-of-the-art MRI can serve as biomarkers of quantify the associations between physical activity and brain structural changes help elucidate the underlying mechanisms and contribute to identify those adults who may be more likely to benefit from physical activity. Investigating the relationships between cardiorespiratory fitness and brain health furthers the understanding of potential mechanisms underlying the beneficial effects of physical activity on brain health in very old age.

APPENDIX: SUPPLEMENTARY TABLES AND FIGURES

Table 15: Correlations of physical activity measures with mean diffusivity in gray matter regions of interest (lower mean diffusivity=lower integrity in gray matter).

	medial temporal lobe	cingulate cortex	dorsolateral prefrontal cortex	posterior parietal cortex
Active energy expenditure (kcal/day)	-.092	-.013	-.061	-.017
Number of steps per day	-.106	-.022	.059	.205
Duration (minutes of PA/day)	-.070	-.064	-.016	-.002
Intensity (METs/day)	-.248	-.050	-.028	.004

Notes: Correlations were computed using Pearson Correlation Coefficients. P values for all > 0.5.

Table 16: Definition of physical activity measured in 1997-1998.

	Lifestyle activities		Exercise activities
Sedentary	< 2719 kcal/week	and	< 1000 kcal/week
Lifestyle active	>2719 kcal/week	and	< 1000 kcal/week
Exercise active	≤ or > 2719 kcal/week	and	> 1000 kcal/week

Notes: Lifestyle activities included household chores (sum of outdoor chores, heavy chores, light housework, grocery shopping, and laundry), walking (sum of exercise walking and other walking) and climbing stairs, paid work, volunteer work and child/adult care. Exercise activities included exercise/recreation (sum of aerobic dancing, weight training, and high and moderate intensity activities regardless of effort) and walking for exercise.

Table 17: Mean diffusivity in regions of interest and univariate associations with physical activity.

	Regions of interest hypothesized to be related to physical activity				Other regions		
	Medial temporal lobe	Cingulate cortex	Dorsolateral prefrontal cortex	Posterior parietal cortex	Striatum	Primary sensorimotor cortex	Supplementary motor cortex
Sedentary, n=39 (14.1%)	1.6289±.2664	1.3579±.1729	1.0945±.1322	1.3163±.1942	1.8448±.2254	1.2322±.1478	1.2039±.1878
Lifestyle active, n=148 (53.6%)	1.5879±.2058	1.3040±.1374	1.0917±.1270	1.3098±.1504	1.7690±.1986	1.2117±.1123	1.1814±.1587
Exercise Active, n=89 (32.2%)	1.5370±.1949	1.2847±.1108	1.0902±.0982	1.3106±.1337	1.7746±.2074	1.2099±.1011	1.1738±.1210
ANOVA p-value	.054	.020	.982	.971	.106	.561	.587

Notes: Values of mean diffusivitiy are multiplied by 1000 and expressed as mean±SD.

Table 18: Fractional anisotropy in tracts of interest and univariate associations with physical activity.

	Uncinate fasciculus	Superior longitudinal fasciculus
Sedentary, n=39 (14.1%)	.3208±.0264	.3528±.0197
Lifestyle active, n=148 (53.6%)	.3239±.0273	.3539±.0217
Exercise Active, n=89 (32.2%)	.3247±.0243	.3563±.0184
ANOVA p-value	.743	.585

Note: Values of fractional anisotropy are mean±SD.

Table 19: Univariate associations of characteristics with fractional anisotropy (FA, higher=greater integrity) in the cingulum and mean diffusivity (MD, lower=greater integrity) in the medial temporal lobe (N=164).

	FA in the cingulum		MD in the medial temporal lobe	
	r	p-value	r	p-value
Demographics				
Age, yrs	-.17	.030	.29	<.001
Female	-.26	.001	-.19	.017
Black race	-.15	.055	-.10	.210
Physical Activity				
Walking and climbing stairs, kcal/kg/week	.09	.258	-.06	.484
Chronic disease conditions[1]				
Cardiovascular disease	-.05	.502	-.02	.842
Hypertension	-.16	.045	.11	.175
Myocardial infraction	.06	.485	-.02	.762
Stroke	-.21	.006	.03	.741
Diabetes	.01	.943	.08	.301
Cognitive function				
DSST score (range 0-90)	.10	.190	-.21	.007
3MSE score (range 0-100)	.14	.085	-.17	.035
Depressive symptoms				
CES-D score (range 0-60)	-.14	.068	.11	.177
Physical function				
Gait speed, meters/sec	-.00	.986	-.19	.016
Other characteristics related to CRF				
Postsecondary Education	.16	.039	-.01	.941
Current or former smokers	.07	.347	.04	.576
Alcohol consumption ever ≥ 5 drinks / day	-.20	.012	.25	.002
Body mass index, kg/m^2	.04	.607	-.10	.233
Systolic blood pressure, mm Hg	-.05	.518	-.04	.639
FEV1/FVC	-.14	.082	.05	.516

Abbreviations: DSST=digit symbol substitution test. 3MSE=modified mini-mental state examination. CES-D=Center for Epidemiologic Studies Depression Scale. CRF=cardiorespiratory fitness. FEV1/FVC=forced expiratory volume in first second/forced vital capacity, unitless. Notes: Correlations and p-values were obtained from Pearson correlation coefficients and Point-Biserial correlation coefficients as appropriate.[1] Disease prevalence at the time of MRI and LDCW measurements.

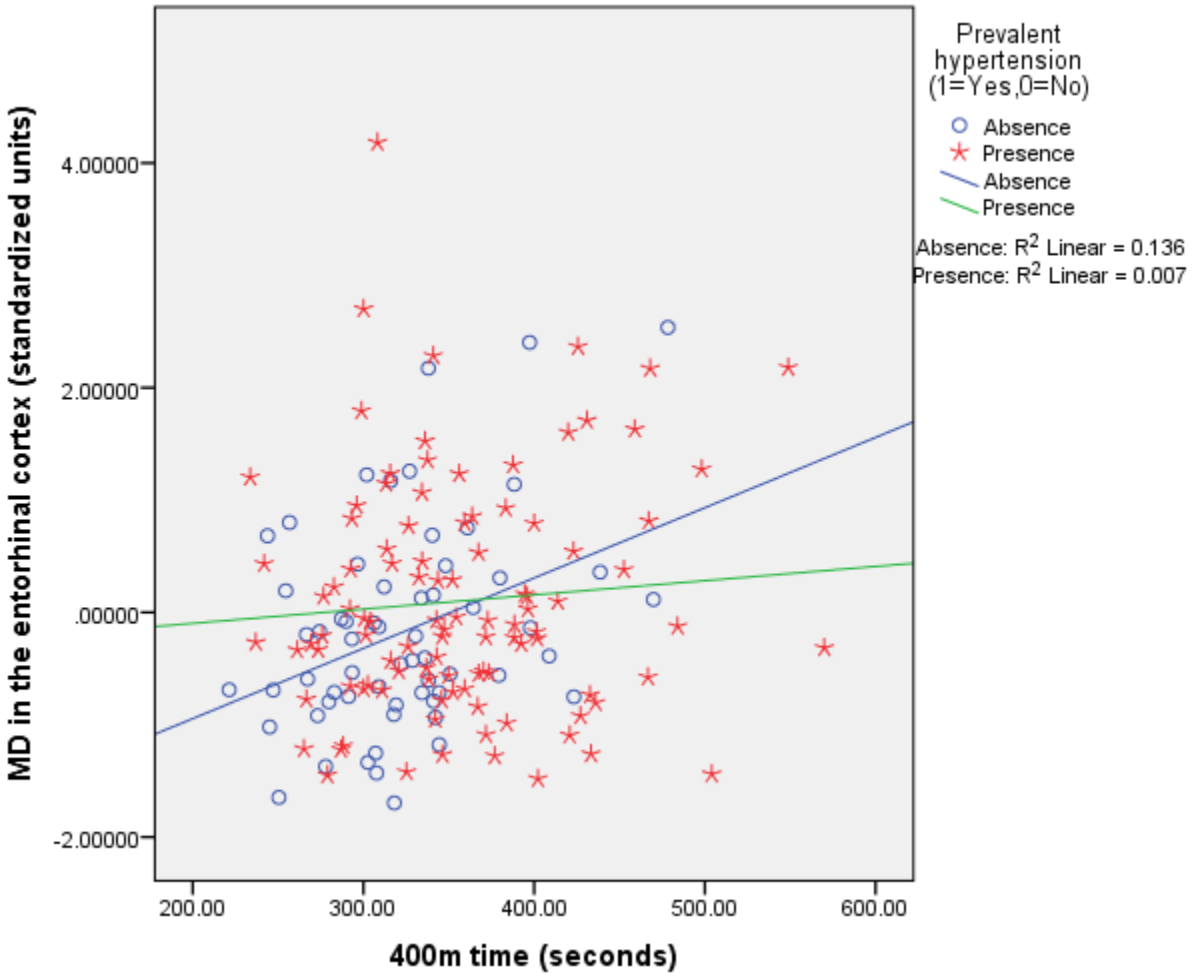

Figure 8: Scatter plots of the association between 400m time (faster=higher fitness) and mean diffusivity (MD, lower=greater integrity) in the entorhinal cortex stratified by hypertension.

BIBLIOGRAPHY

1. Morris, J.N., *Exercise in the prevention of coronary heart disease: today's best buy in public health.* Med Sci Sports Exerc, 1994. **26**(7): p. 807-14.

2. Hillman, C.H., K.I. Erickson, and A.F. Kramer, *Be smart, exercise your heart: exercise effects on brain and cognition.* Nat Rev Neurosci, 2008. **9**(1): p. 58-65.

3. Kramer, A.F., K.I. Erickson, and S.J. Colcombe, *Exercise, cognition, and the aging brain.* J Appl Physiol, 2006. **101**(4): p. 1237-42.

4. Erickson, K.I., A.M. Weinstein, and O.L. Lopez, *Physical activity, brain plasticity, and Alzheimer's disease.* Arch Med Res, 2012. **43**(8): p. 615-21.

5. Pahor, M., et al., *Effects of a physical activity intervention on measures of physical performance: Results of the lifestyle interventions and independence for Elders Pilot (LIFE-P) study.* J Gerontol A Biol Sci Med Sci, 2006. **61**(11): p. 1157-65.

6. Garber, C.E., et al., *American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise.* Med Sci Sports Exerc, 2011. **43**(7): p. 1334-59.

7. Ainsworth, B.E., et al., *Compendium of physical activities: an update of activity codes and MET intensities.* Med Sci Sports Exerc, 2000. **32**(9 Suppl): p. S498-504.

8. Warburton, D.E., C.W. Nicol, and S.S. Bredin, *Health benefits of physical activity: the evidence.* CMAJ, 2006. **174**(6): p. 801-9.

9. Bucksch, J. and W. Schlicht, *Health-enhancing physical activity and the preventionof chronic diseases--an epidemiological review.* Soz Praventivmed, 2006. **51**(5): p. 281-301.

10. Sallis, J.F. and B.E. Saelens, *Assessment of physical activity by self-report: status, limitations, and future directions.* Res Q Exerc Sport, 2000. **71**(2 Suppl): p. S1-14.

11. Troiano, R.P., et al., *Physical activity in the United States measured by accelerometer.* Med Sci Sports Exerc, 2008. **40**(1): p. 181-8.

12. Chandler, J.M. and E.C. Hadley, *Exercise to improve physiologic and functional performance in old age.* Clin Geriatr Med, 1996. **12**(4): p. 761-84.

13. Lord, S.R. and S. Castell, *Physical activity program for older persons: effect on balance, strength, neuromuscular control, and reaction time.* Arch Phys Med Rehabil, 1994. **75**(6): p. 648-52.

14. Williams, P.T., *Physical fitness and activity as separate heart disease risk factors: a meta-analysis.* Med Sci Sports Exerc, 2001. **33**(5): p. 754-61.

15. Bouchard, C. and T. Rankinen, *Individual differences in response to regular physical activity.* Med Sci Sports Exerc, 2001. **33**(6 Suppl): p. S446-51; discussion S452-3.

16. Eaton, C.B., et al., *Physical activity, physical fitness, and coronary heart disease risk factors.* Med Sci Sports Exerc, 1995. **27**(3): p. 340-6.

17. Tager, I.B., M. Hollenberg, and W.A. Satariano, *Association between self-reported leisure-time physical activity and measures of cardiorespiratory fitness in an elderly population.* Am J Epidemiol, 1998. **147**(10): p. 921-31.

18. Dishman, R.K., J.F. Sallis, and D.R. Orenstein, *The determinants of physical activity and exercise.* Public Health Rep, 1985. **100**(2): p. 158-71.

19. Lakoski, S.G., et al., *Impact of body mass index, physical activity, and other clinical factors on cardiorespiratory fitness (from the Cooper Center longitudinal study).* Am J Cardiol, 2011. **108**(1): p. 34-9.

20. Lakka, T.A., et al., *Relation of leisure-time physical activity and cardiorespiratory fitness to the risk of acute myocardial infarction.* N Engl J Med, 1994. **330**(22): p. 1549-54.

21. Myers, J., et al., *Fitness versus physical activity patterns in predicting mortality in men.* Am J Med, 2004. **117**(12): p. 912-8.

22. Rauramaa, R., et al., *Physical activity and health-related fitness in middle-aged men.* Med Sci Sports Exerc, 1995. **27**(5): p. 707-12.

23. Dvorak, R.V., et al., *Respiratory fitness, free living physical activity, and cardiovascular disease risk in older individuals: a doubly labeled water study.* J Clin Endocrinol Metab, 2000. **85**(3): p. 957-63.

24. Schmidt, M.D., et al., *A comparison of subjective and objective measures of physical activity and fitness in identifying associations with cardiometabolic risk factors.* Ann Epidemiol, 2008. **18**(5): p. 378-86.

25. Lee, D.C., et al., *Mortality trends in the general population: the importance of cardiorespiratory fitness.* J Psychopharmacol, 2010. **24**(4 Suppl): p. 27-35.

26. Caspersen, C.J., K.E. Powell, and G.M. Christenson, *Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research.* Public Health Rep, 1985. **100**(2): p. 126-31.

27. Schutz, Y., R.L. Weinsier, and G.R. Hunter, *Assessment of free-living physical activity in humans: an overview of currently available and proposed new measures.* Obes Res, 2001. **9**(6): p. 368-79.

28. Gledhill, N., D. Cox, and R. Jamnik, *Endurance athletes' stroke volume does not plateau: major advantage is diastolic function.* Med Sci Sports Exerc, 1994. **26**(9): p. 1116-21.

29. Myers, J., *Cardiology patient pages. Exercise and cardiovascular health.* Circulation, 2003. **107**(1): p. e2-5.

30. Rivera-Brown, A.M. and W.R. Frontera, *Principles of exercise physiology: responses to acute exercise and long-term adaptations to training.* PM R, 2012. **4**(11): p. 797-804.

31. Abernethy, P.J., R. Thayer, and A.W. Taylor, *Acute and chronic responses of skeletal muscle to endurance and sprint exercise. A review.* Sports Med, 1990. **10**(6): p. 365-89.

32. Hickson, R.C., *Skeletal muscle cytochrome c and myoglobin, endurance, and frequency of training.* J Appl Physiol, 1981. **51**(3): p. 746-9.

33. Kiens, B., et al., *Skeletal muscle substrate utilization during submaximal exercise in man: effect of endurance training.* J Physiol, 1993. **469**: p. 459-78.

34. Kaliman, P., et al., *Neurophysiological and epigenetic effects of physical exercise on the aging process.* Ageing Res Rev, 2011. **10**(4): p. 475-86.

35. Cotman, C.W. and N.C. Berchtold, *Exercise: a behavioral intervention to enhance brain health and plasticity.* Trends Neurosci, 2002. **25**(6): p. 295-301.

36. Vaynman, S., Z. Ying, and F. Gomez-Pinilla, *Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition.* Eur J Neurosci, 2004. **20**(10): p. 2580-90.

37. Rasmussen, P., et al., *Evidence for a release of brain-derived neurotrophic factor from the brain during exercise.* Exp Physiol, 2009. **94**(10): p. 1062-9.

38. Cotman, C.W., N.C. Berchtold, and L.A. Christie, *Exercise builds brain health: key roles of growth factor cascades and inflammation.* Trends Neurosci, 2007. **30**(9): p. 464-72.

39. Blomstrand, E., et al., *Administration of branched-chain amino acids during sustained exercise--effects on performance and on plasma concentration of some amino acids.* Eur J Appl Physiol Occup Physiol, 1991. **63**(2): p. 83-8.

40. Gomez-Pinilla, F., et al., *Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity.* J Neurophysiol, 2002. **88**(5): p. 2187-95.

41. Berchtold, N.C., J.P. Kesslak, and C.W. Cotman, *Hippocampal brain-derived neurotrophic factor gene regulation by exercise and the medial septum.* J Neurosci Res, 2002. **68**(5): p. 511-21.

42. Molteni, R., Z. Ying, and F. Gomez-Pinilla, *Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray.* Eur J Neurosci, 2002. **16**(6): p. 1107-16.

43. Boecker, H., et al., *The runner's high: opioidergic mechanisms in the human brain.* Cereb Cortex, 2008. **18**(11): p. 2523-31.

44. Carroll, S. and M. Dudfield, *What is the relationship between exercise and metabolic abnormalities? A review of the metabolic syndrome.* Sports Med, 2004. **34**(6): p. 371-418.

45. Weuve, J., et al., *Physical activity, including walking, and cognitive function in older women.* JAMA, 2004. **292**(12): p. 1454-61.

46. Buchman, A.S., R.S. Wilson, and D.A. Bennett, *Total daily activity is associated with cognition in older persons.* Am J Geriatr Psychiatry, 2008. **16**(8): p. 697-701.

47. Lytle, M.E., et al., *Exercise level and cognitive decline: the MoVIES project.* Alzheimer Dis Assoc Disord, 2004. **18**(2): p. 57-64.

48. Etgen, T., et al., *Physical activity and incident cognitive impairment in elderly persons: the INVADE study.* Arch Intern Med, 2010. **170**(2): p. 186-93.

49. Larson, E.B., et al., *Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older.* Ann Intern Med, 2006. **144**(2): p. 73-81.

50. Ravaglia, G., et al., *Physical activity and dementia risk in the elderly: findings from a prospective Italian study.* Neurology, 2008. **70**(19 Pt 2): p. 1786-94.

51. Rovio, S., et al., *Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease.* Lancet Neurol, 2005. **4**(11): p. 705-11.

52. Floel, A., et al., *Physical activity and memory functions: are neurotrophins and cerebral gray matter volume the missing link?* Neuroimage, 2010. **49**(3): p. 2756-63.

53. Ho, A.J., et al., *The effects of physical activity, education, and body mass index on the aging brain.* Hum Brain Mapp, 2011. **32**(9): p. 1371-82.

54. Gow, A.J., et al., *Neuroprotective lifestyles and the aging brain: Activity, atrophy, and white matter integrity.* Neurology, 2012. **79**(17): p. 1802-1808.

55. Erickson, K.I., et al., *Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study.* Neurology, 2010. **75**(16): p. 1415-22.

56. Angevaren, M., et al., *Physical activity and 5-year cognitive decline in the Doetinchem cohort study.* Ann Epidemiol, 2010. **20**(6): p. 473-9.

57. Hendrie, H.C., et al., *The NIH Cognitive and Emotional Health Project. Report of the Critical Evaluation Study Committee.* Alzheimers Dement, 2006. **2**(1): p. 12-32.

58. Colcombe, S. and A.F. Kramer, *Fitness effects on the cognitive function of older adults: a meta-analytic study.* Psychol Sci, 2003. **14**(2): p. 125-30.

59. Seab, J.P., et al., *Quantitative NMR measurements of hippocampal atrophy in Alzheimer's disease.* Magn Reson Med, 1988. **8**(2): p. 200-8.

60. Barnes, D.E., et al., *A longitudinal study of cardiorespiratory fitness and cognitive function in healthy older adults.* J Am Geriatr Soc, 2003. **51**(4): p. 459-65.

61. Baloh, R.W. and H.V. Vinters, *White matter lesions and disequilibrium in older people. II. Clinicopathologic correlation.* Arch Neurol, 1995. **52**(10): p. 975-81.

62. Nitkunan, A., et al., *Multimodal MRI in cerebral small vessel disease: its relationship with cognition and sensitivity to change over time.* Stroke, 2008. **39**(7): p. 1999-2005.

63. Ylikoski, A., et al., *White matter hyperintensities on MRI in the neurologically nondiseased elderly. Analysis of cohorts of consecutive subjects aged 55 to 85 years living at home.* Stroke, 1995. **26**(7): p. 1171-7.

64. Garde, E., et al., *Relation between age-related decline in intelligence and cerebral white-matter hyperintensities in healthy octogenarians: a longitudinal study.* Lancet, 2000. **356**(9230): p. 628-34.

65. Junque, C., et al., *Leuko-araiosis on magnetic resonance imaging and speed of mental processing.* Arch Neurol, 1990. **47**(2): p. 151-6.

66. Debette, S. and H.S. Markus, *The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis.* BMJ, 2010. **341**: p. c3666.

67. Kempton, M.J., et al., *Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder.* Arch Gen Psychiatry, 2008. **65**(9): p. 1017-32.

68. Videbech, P., *MRI findings in patients with affective disorder: a meta-analysis.* Acta Psychiatr Scand, 1997. **96**(3): p. 157-68.

69. Gallo, A., et al., *Diffusion-tensor magnetic resonance imaging detects normal-appearing white matter damage unrelated to short-term disease activity in patients at the earliest clinical stage of multiple sclerosis.* Arch Neurol, 2005. **62**(5): p. 803-8.

70. Le Bihan, D., et al., *Diffusion tensor imaging: concepts and applications.* J Magn Reson Imaging, 2001. **13**(4): p. 534-46.

71. Filippi, M., et al., *Diffusion tensor magnetic resonance imaging in multiple sclerosis.* Neurology, 2001. **56**(3): p. 304-11.

72. Assaf, Y. and O. Pasternak, *Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review.* J Mol Neurosci, 2008. **34**(1): p. 51-61.

73. Drenowatz, C. and J.C. Eisenmann, *Validation of the SenseWear Armband at high intensity exercise.* Eur J Appl Physiol, 2011. **111**(5): p. 883-7.

74. Johannsen, D.L., et al., *Accuracy of armband monitors for measuring daily energy expenditure in healthy adults.* Med Sci Sports Exerc, 2010. **42**(11): p. 2134-40.

75. Andre, D. and D.L. Wolf, *Recent advances in free-living physical activity monitoring: a review.* J Diabetes Sci Technol, 2007. **1**(5): p. 760-7.

76. Colcombe, S.J., et al., *Aerobic fitness reduces brain tissue loss in aging humans.* J Gerontol A Biol Sci Med Sci, 2003. **58**(2): p. 176-80.

77. Gordon, B.A., et al., *Neuroanatomical correlates of aging, cardiopulmonary fitness level, and education.* Psychophysiology, 2008. **45**(5): p. 825-38.

78. Erickson, K.I., et al., *Aerobic fitness is associated with hippocampal volume in elderly humans.* Hippocampus, 2009. **19**(10): p. 1030-9.

79. Johnson, N.F., et al., *Cardiorespiratory fitness is positively correlated with cerebral white matter integrity in healthy seniors.* Neuroimage, 2012. **59**(2): p. 1514-23.

80. Marks, B.L., et al., *Role of aerobic fitness and aging on cerebral white matter integrity.* Ann N Y Acad Sci, 2007. **1097**: p. 171-4.

81. Marks, B.L., et al., *Aerobic fitness and obesity: relationship to cerebral white matter integrity in the brain of active and sedentary older adults.* Br J Sports Med, 2011. **45**(15): p. 1208-15.

82. Voss, M.W., et al., *The influence of aerobic fitness on cerebral white matter integrity and cognitive function in older adults: Results of a one-year exercise intervention.* Hum Brain Mapp, 2012.

83. Reynolds, G. (2012, April 22). *How exercise lead to a better brain?* New York Times, p. MM46.

84. Alzheimer's Association, *2012 Alzheimer's Disease Facts and Figures*, Alzheimer's & Dementia,Volume 8, Issue 2.

85. Taylor, A.W. and Johnson, W., 2008. Physiology of Exercise and Healthy Aging.

86. Borg, G., 1998. *Borg's perceived exertion and pain scales*. Champaign: Human Kinetics.

87. Lee, I., *Putting the 2008 physical activity guidelines intro practice to prevent cardiovascular disease*, 2010. 4: p. 277-283.

88. U.S. Department of Health and Human Services. *2008 Physical Activity Guidelines for Americans.* Available at http://www.health.gov/paguidelines/. Accessed February 9, 2010 This document provides information for health professionals on evidence based guidance to help Americans improve their health through appropriate physical activity.

89. Wilmore JH, Costill DL. *Physiology of sport and exercise.* Champaign, IL: Human Kinetics, 1994.

90. United States. Public Health Service. Office of the Surgeon General, National Center for Chronic Disease Prevention, Health Promotion (US), President's Council on Physical Fitness, & Sports (US). (1996). Physical Activity and Health: A Report of the Surgeon. Jones & Bartlett Learning.

91. Rowell, L. B. (1993). Human cardiovascular control. Oxford University Press.

92. Thompson, W. R., Gordon, N. F., & Pescatello, L. S. (2009). ACSM's guidelines for exercise testing and prescription. Hubsta Ltd.

93. MacDougall, J. D. (1994). Blood pressure responses to resistive, static and dynamic exercise. Cardiovascular Response to Exercise. Mount Kisco, NY: Futura Publishing Co, Inc, 155-173.

94. McArdle, W. D., Katch, F. I., & Katch, V. L. (2010). Exercise physiology: nutrition, energy, and human performance. Lippincott Williams & Wilkins.

95. Marcus, M. L. (1983). The coronary circulation in health and disease (Vol. 983). New York: McGraw-Hill.