THE ASSOCIATION BETWEEN BODY COMPOSITION, MITOCHONDRIAL FUNCTION AND FATIGABILITY AND PHYSICAL FUNCTION IN OLDER ADULTS

by

Adam J. Santanasto

BS, Health Information Management, University of Pittsburgh, 2008

MPH, Epidemiology, University of Pittsburgh, 2010

Submitted to the Graduate Faculty of

the Department of Epidemiology

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

Adam J. Santanasto, MPH

It was defended on

April 12, 2013

and approved by

Dissertation Advisor:
Anne B. Newman, MD, MPH
Chair, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

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

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

Elsa S. Strotmeyer, PhD
Assistant Professor, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Bret H. Goodpaster, PhD
Associate Professor, Department of Medicine
School of Medicine
University of Pittsburgh

Anne B. Newman, MD, MPH

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ABSTRACT

The 39 million Americans over the age of 65 accounted for 13% of the United States population in 2008. The absolute and relative number of older adults (age ≥65 years) is starting to rise rapidly as the baby boomers begin to turn 65. The prevalence of mobility disability in older (30%) adults is high and is a large public health concern as disability is associated with lower quality of life, higher health care costs, and mortality. There are many reasons for age-related disability, however; the role of changes to skeletal muscle remains unclear. Fatigue is also an independent risk factor for physical disability and is common among older adults. This dissertation aimed to provide novel insight into the association between skeletal muscle energetics, changes in regional body composition and physical function and fatigability in older adults. First, decreases in visceral and intermuscular adipose tissue as well as an increase in muscle density, a marker of intramyocellular fat, following a weight-loss and physical activity intervention, were shown to be related to improved physical performance. Next, mitochondrial function, measured by phosphocreatine recovery (mM ATP/s) in the quadriceps following an exercise-bout using ^{31}P magnetic resonance spectroscopy, was examined in relation to walking performance (time to walk 400m) and perceived performance fatigability (perceived exertion following a 0.67ms treadmill-walk). Mitochondrial function was related to walking performance in higher functioning older adults and older adults who were functionally impaired but able to ambulate 400m without discomfort. Mitochondrial function was also significantly lower in those

with high compared to low fatigability. This research provides novel evidence that function can be improved by targeting specific fat depots and mitochondrial function may impact overall function and fatigability. These findings could have large public health implications, as the etiology of age-related disability in regard to skeletal muscle is unclear. The prevalence of disability among older adults is quite high and is associated with increased health care costs and mortality. Clinicians, public health professionals and researchers can use this information to design interventions, treatments and future research studies focused on skeletal muscle to improve function in older adults.

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ACKNOWLEDGMENTS

I would first like to thank my committee. All five of these mentors have been a great source of knowledge and support concerning each of my research projects. I have learned a great deal under their tutelage and am looking forward to working with each of them in some capacity as I am staying at Pitt for more extensive post-doctoral training. I would especially like to thank my primary PhD advisor, Dr. Anne B. Newman. Although Dr. Newman is definitely the busiest person I know, being the chair of the large department of epidemiology and PI, co PI and consultant on numerous studies and research projects, she has always had sufficient time for me and has been a very attentive advisor. Her wisdom, both in the field of epidemiology and in general, is astonishing and I am grateful for the opportunity to have been her trainee. I would also like to thank Dr. Nancy W. Glynn, who is on my PhD committee, but also served as my MPH advisor and GSR supervisor. Under her mentorship, I learned a great deal and gained invaluable research experience in my formative years as an epidemiologist. In fact, Dr. Glynn introduced me to the field of aging epidemiology by responding to an inquiry I made to the department of epidemiology as an undergraduate student - seeking to conduct my senior project in the field of epidemiology. Dr. Glynn has been a constant source of support over the past 5 years and I would not have been able to accomplish this goal without her. I would also like to thank my parents. Their support over the past 5 years has been phenomenal and I could not have accomplished this goal without them. They have been very supportive and have always

encouraged me to seek out my goals. I am forever indebted to them for their love and support. Finally, I would like to thank my girlfriend of over 5 years, Adrienne. She has been by my side throughout this entire journey and was very supportive of my decision and commitment to attain my PhD. It would have been easy to have left during my 5 years as a graduate student. Instead she stuck by me and has supported every decision along the way. She believed in me even at moments when I doubted myself and my decision to seek a PhD. I definitely would not have been able to accomplish this goal without her unwavering support. I would also like to thank our 3-year old English bulldog, Lucy. She is a very big part of our lives, a constant source of joy and I would be remiss if I didn't mention her.

1.0 INTRODUCTION

The 39 million Americans over the age of 65 accounted for 13% of the United States population in 2008[1]. The absolute and relative number of older adults (age ≥65 years) is starting to rise rapidly as the baby boomers (born 1946-1964) begin to turn 65. By 2030, one in every five Americans (72 million) will be aged 65 and older[1]. Additionally, the United States Census Bureau estimates that the number of US citizens age 85 and older will increase 3-fold from 5.7 million in 2008, to 19 million by 2050[1]. The "squaring of the age pyramid", as some have called it, is expected to precipitate enormous public health problems that could have devastating effects on our country's healthcare system. The increasing risk for physical disability and declines in physical function that coincide with increasing age is one of the primary reasons why our rapidly aging population is of great public health concern[2-4]. It has been estimated that community-dwelling older adults who are functional disabled or transition to being functionally disabled spend $10,000 more on healthcare over a 2-year span compared to those who remain functionally independent[5]. Additionally, it is estimated that physically disability attributable to sarcopenia, the loss of muscle mass with age, alone in the United States in 2000 was between $11.8 billion and $26.2 billion[6]. Therefore, it is imperative to understand what the etiology of and risk factors for age-related physical disability in order to prevent its onset.

The neuromuscular system contains most of the necessary machinery for physical function. Therefore it is a logical area of study when attempting to understand the etiology of

physical disability in older adults. This dissertation will focus on age-related changes to the neuromuscular system, specifically changes to skeletal muscle tissue and regional body composition depots and their effect on physical function and mobility in older adults. This dissertation will also explore the cellular basis for skeletal muscle performance by examining mitochondrial function, which is responsible for producing over 90% of the energy needed for locomotion[7], and how it relates to physical function and mobility.

Additionally, fatigue in older adults has been shown to be associated with physical function and disability, independent of disease status[8]. Therefore, fatigue is an independent risk factor for physical decline and is present in the disablement pathway. Additionally, a large proportion of it cannot be attributed to underlying diseases[9,10]. Fatigue is primarily considered an energy disorder, thus it has been hypothesized that age-related decreases in mitochondrial function may contribute to higher levels of fatigability in older adults[11,12]. This dissertation will also examine the relationship between in vivo mitochondrial function and fatigability. Fatigability is a whole-body measure of fatigue normalized to a set activity of a specific intensity and duration and has been hypothesized to provide better insight, compared to global fatigue, into the degree an individual is limited functionally due to fatigue[12]. The term whole-body in this context is emphasizing that fatigability as it is used in this dissertation is an overall sensation of fatigue at the "whole-body level" as opposed to skeletal muscle fatigability.

The research described in this dissertation will provide novel insight into the association between skeletal muscle energetics, changes in regional body composition and physical function and fatigability in old older adults. This dissertation will contribute information that will assist public health and medical professions in preventing age-related declines in physical function and disability.

1.1 THE DISABLEMENT PATHWAY: A CONCEPTUAL FRAMEWORK

It is practical to discuss age-related physical disability in the context of the Disablement Process model proposed by Verbrugge and Jette[13], who elaborated on a framework developed and proposed by Saad Nagi[14]. The Nagi model was adopted by an Institute of Medicine panel formed to reflect on the prevention of disability from a policy perspective[15]. As a result, it is sometimes referred to as the Institute of Medicine scheme. The Disablement Process model was constructed with prevention in mind and in a manner to appease both the research and medical communities. This model also recognizes the fact that disability is both a relative sociological concept, as well as a medical condition. Briefly, the main pathway consists of four concepts. These concepts are pathology, impairments, functional limitations and disability. The work presented in this dissertation deals with all four of these domains in a significant capacity. Therefore, for the purpose of the dissertation it is worthwhile to take some time to define these four concepts in the manor proposed by Verbrugge and Jette and first proposed by Nagi.

The first domain and furthest upstream in the pathway is pathology. Pathology refers to biochemical and physiologic disorders. Verbrugge and Jette state that these disorders are detectable and medically labeled as disease, injury or congenital/developmental conditions. For the purposes of this dissertation age-related physiologic and biochemical changes[16] that are not necessarily diseases, but "normal" aging phenomena belong in this domain of the disablement pathway. Verbrugge and Jette do state that these changes may not be directly measurable in usual medical care. This applies the two of the main topics of this dissertation; age induced changes in skeletal muscle and age-related related changes in mitochondrial function. In the more traditional sense Verbrugge and Jette give the examples of osteoarthritis, lung cancer, cataracts and Alzheimer's disease as being pathology. The pathologies that will be discussed in this

dissertation are decreasing muscle mass, muscle quality and reduced capacity for mitochondrial energy production.

The second domain is impairment. Impairments are defined as dysfunctions and structural abnormalities in specific body systems, such as musculoskeletal or cardiovascular impairments. Impairments are caused by pathology and can be thought of as signs or symptoms of pathology. Examples of impairments mentioned in this dissertation are decreased muscle strength and muscle power, the result of skeletal muscle pathology. Decreased muscle strength and muscle power are markers of neuromuscular impairment or dysfunction.

The third domain is functional limitation and is defined as restrictions in basic or fundamental physical or mental actions. These limitations depend largely on the individuals' lifestyle and can include things like walking, lifting objects, climbing stairs, visual acuity and hearing. The domains of impairment and functional limitation can also be thought of as "preclinical disability", a concept theorized by Lilienfeld & Lilienfeld[17] and elaborated on by Fried[18]. Preclinical disability is characterized by the development of functional limitations that have yet to manifest themselves clinically or interfere with a person's perceived ability to function "normally". It has been shown that persons who have difficulty with certain physical tasks (i.e. lifting 10lbs, walking up 10 steps, walking ¼ mile) are at an increased risk for further functional decline and disability[19,20]. In epidemiology we attempt to measure these functional impairments with performance measures such as the 6-minute walk test [21], the long distance corridor walk[22], the Short Physical Performance Battery[23], the timed up and go test[24] and simply gait-speed[25,26]. Additionally, fatigability will be examined in this dissertation and can be thought of as a functional impairment. Fatigability impairs one's ability to tolerate a certain intensity of physical activity or tasks of certain intensities. This may cause one to limit one's activity level

which can then leaded to decreases in fitness and ability level. Fatigue has been shown to be related to declining physical function and disability independent of disease status[9,10].

In epidemiologic research you will often see the term mobility disability. This term is distinct from instrumental activities of daily living (IADL), activities of daily living (ADL), or more simply, physical disability which will be discussed in the next paragraph. Mobility disability is a functional limitation and is therefore further up the pathway than physical disability. Mobility disability can be measured by both self-report and performance testing. Self-report mobility disability is usually defined as being unable to or having difficulty walking 400m-500m (about ¼ mile) and/or climbing 1 flight of stairs[22,27-31]. This definition varies across study and even within different analyses from the same study. For example, the Cardiovascular Health Study, EPESE and the Woman's Health and Aging II studies characterized mobility disability as the self-reported inability to walk ½ a mile (8 blocks) and/or climb up and down 1 flights of stairs[32-34] and the InCHIANTI study defines mobility disability as the self-reported inability to walk 1km in one analysis[35] and ½ a km in another[27]. There are also performance based measures of mobility disability in older adult such as the inability to walk 400m without sitting down and/or under 15 minutes [36-39], 4m or 6m usual gait-speed < 1.0m/s[40] and fast-paced gait-speed <1.2m/s[27]. Mobility disability is an important epidemiological outcome for studies involving older adults because of its strong relationship with further functional decline, disability[19,20], cardiovascular disease and mortality[22,41].

When referring to physical disability, researchers are usually referring to impairment in the ability of a person to independently perform tasks that fall into one of two categories. The first category is called instrumental activities of daily living (IADLs), which include: using the telephone, shopping, preparing meals, housekeeping, laundry, transportation (public or personal),

managing medications and managing finances[42]. The second category is called activities of daily living (ADLs), which include: bathing, dressing, toileting, transfer (getting in and out of bed/chair), continence, and feeding[43]. IADLs are further upstream the disablement pathway than ADLs and are meant to capture activities necessary for maintaining independent status in the context of one's cultural setting whereas ADLs are activities that are necessary for survival[13]. To help discern the subtle difference, one can also think of ADLs as self-care tasks and IADLs as household management activities.

As mentioned previously, age-related physical disability is an enormous public health issue, an issue that is growing due to the rapid aging of the population. With increased age, comes an increased risk for physical disability; with physical disability comes an increased risk for further disability, institutionalization and ultimately death[2,3,44]. The next section will quantify this growing public health issue by providing an overview of the epidemiology of disability in older adults.

1.1.1 Epidemiology of Disability in Older Adults

When attempting to quantify or study disability in older adults, it is very important to pay attention to the specific definition of disability, as it varies across population and epidemiological studies. For example the National Long term Care Study (NLTCS) defines disability as the inability to perform an IADL or ADL task due to health or aging[45] whereas the Established Populations for Epidemiologic Studies of the Elderly (EPESE) project defines disability as self- or proxy-report of needing help with or being unable to perform one or more IADL tasks[46] and NHANES defines disability as having any difficultly performing these tasks.

Disability in older adults is an immense public health issue. According to NHANES, 20% of those aged 60-69 have ADL disability, 23% have IADL disability, 30% have mobility disability and 48% experience functional limitations[47]. This issue is not unique to the United States. According to the INCHIANTI study in Tuscany, Italy 5.5% of those age 65 and older had ADL disability and 22.2% had IADL disability[48]. Additionally, a study by Woo et al. conducted in Hong Kong revealed that 23.0, 29.4 and 35.9% of women and 9.5, 13.2 and 20.9% of men age 65-69,70-74 and 75+ respectively have IADL disability[49]. These data show that the prevalence of physical and mobility disability are quite high. Again, this is alarming because of the aging nature of the population and the increased utilization of health care services and health care costs associated with being disabled or dependent[5].

It is important to note that women report more IADL or ADL disability than men (47% vs. 35%)[1]. There are a few reasons for why this sex disparity exists. First of all, this disparity seems to be real and not an artifact of inherent bias in self-reported disability. It was thought that women might be more likely to "admit" to difficulty performing certain tasks than men or men might report not doing or being able to do certain household activities such as preparing meals because they've never performed these tasks. However, previous research shows good agreement as well as no sex differences in terms of accuracy when comparing self-reported and observed performance on similar tasks[50]. Previous work suggests that the disparity also does not seem to simply be a result of woman developing disability more often than men; but rather a combination of the fact that woman tend to live longer with disability than men[44,51,52] and the fact that diseases precipitating disability in men and woman differ[53]. For example, women tend to suffer from more comorbidities that are risk factors for physical disability, such as arthritis, osteoporosis and obesity, than men and present more physical disability at every level of

comorbidity when compared to men[53]. Moreover, men are 50% more likely to experience sudden cardiac death, which often occurs without physical disability, than women; this also likely contributes to the prevalence discrepancy between men and women[54,55]. In the same vein, men tend suffer from more life-threatening diseases than women such as heart disease and congestive heart failure. Thus, men are more likely to die and women are more likely to live with disability[44]. It has also been suggested that due to inherent genetic differences among sexes, men have great physical advantages than women. Therefore, it takes less pathology and impairments for woman to cross the threshold for mobility and physical disability. For example, in men, a larger proportion of total body mass is muscle or lean mass compared to women[53]. Interestingly, despite the apparent differences between men and women concerning the prevalence of disability, studies have shown that there is no difference regarding incident rates of disability between the sexes[56]. It is hypothesized that this is due to women living longer with disability than men and the fact that men may experience shorter bouts of disability, which are not captured in cross-sectional ascertainment at long intervals in large epidemiologic studies[56].

In addition to the sex disparity in the prevalence of age-related physical disability, racial disparities exist as well with non-Hispanic blacks having higher prevalence rates of disability compared to whites[46,57-59]. The incidence of disability between African-Americans and whites is also different with African-Americans being 1.61 times more likely to develop disability according to a study using data from the Health and Retirement Study[60]. A study using data from the EPESE study showed that African-Americans were 2.3 times more likely to develop physical disability over a 7-9 year follow-up period[58]. These differences are partly attributable to differences in socio-economic status and education level, but significant disparities still exist when these factors are taken into account[58,60]. It should also be noted that these discrepancies are

greater among women than men[46,58]. It is thought that in addition to socioeconomic status, the reason that African-Americans are at greater risk for disability may originate earlier in life where African-Americans are at greater risk for developing certain chronic conditions that are risk factors for disability including hypertension, heart disease and diabetes [58,61-64].

Risk factors for mobility, ADL and IADL disability other than age, race and sex include lower levels of physical activity[65,66], mobility disability or previous disability[19,20,67] hypertension[65,66], obesity[65,68-70], fractures[32,65], arthritis[65], stroke[32], prevalent cancer[32], number of chronic conditions[2,32], smoking[71], diabetes[72-75] and others[44,65,76]. Deteriorating neuromuscular function, in the form of lower muscle and strength, power is well documented to be associated with physical disability[77-79]. Declining muscle function is part of the disablement pathway leading to ADL and IADL disability and can be thought of as both a precursor to and risk factors for disability. It is a precursor to disability in the sense that deteriorating muscle is present in the disablement pathway leading to disability. It can also be thought of as risk factors for disability because those with lower muscle performance are at higher risk for developing disability and interventions exist to improve muscle function which may also then confer improvements to overall physical function and prevent disability.

It is important to note that the non-neuromuscular risk factors for ADL and IADL disability mentioned above can also affect muscle mass and muscle performance, and that not all of the changes to the neuromuscular system are due to aging per se. Changes to the neuromuscular system with age are due both in part to illness, which can cause weight-loss, inactivity and inflammation which in-turn negatively affect muscle mass and performance, and intrinsic changes that occur with aging.

Muscle performance in the form of strength has been shown in the Health Aging and Body Composition Study (Health ABC) to be an independent risk factor for mobility disability[30]. Also in Health ABC, high and low relative knee extensor strength in both men and woman was shown to be related to fast-paced gait-speed over 20 meters[80]. These results have been replicated in Invecchiare in Chianti (InCHIANTI) study. In this study, over a 3-year follow-up period, low muscle power was associated with a 9- and 3-fold greater risk of developing mobility disability in men and women, respectively. In this same study, low knee extensor strength was associated with gait-speed declines of 0.24 and 0.06m/s over the 3-year period in men and women respectively. Additionally, hand-grip strength and declining hand-grip strength, as measures of muscle performance, have also been shown to be strong predicators of physical disability in older adults[81,82]

However, the role of changes in the neuromuscular system and their effect on disability are still unclear. It was largely believed that the loss of muscle mass was largely responsible for declining muscle strength and neuromuscular performance. However, longitudinal evidence shows that declining muscle mass only explains about 5% of the variance in declining muscle strength[83]. This dissertation will attempt to fill certain gaps in this knowledge base. The neuromuscular system is a logical point of study when attempting to understand the etiology of and prevent physical impairments and disability in older adults. The role of the neuromuscular system in the disablement pathway is depicted in supplemental Figure 1. Age-related changes in the neuromuscular system and proposed mechanisms for their decline are described in the subsequent sections.

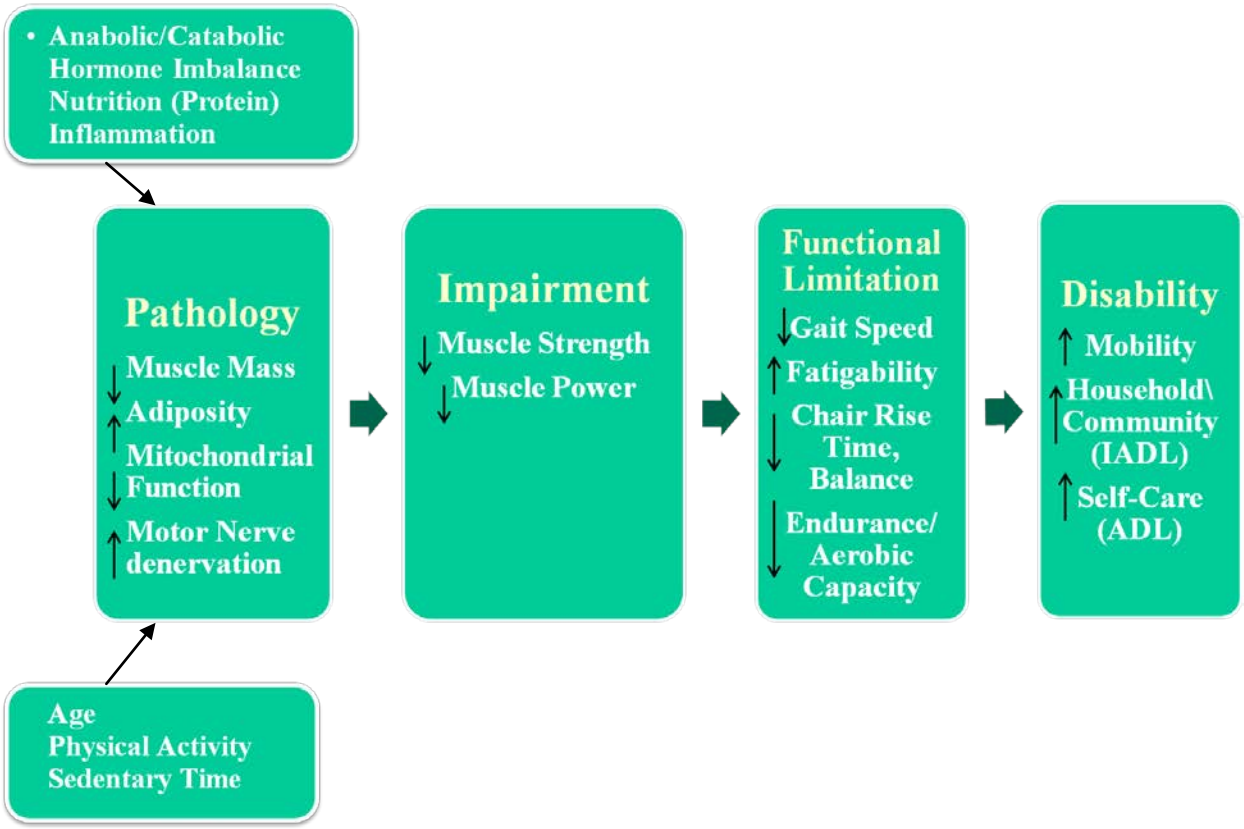


Figure 1. The Role of the Nueromuscular System in the Disablement Pathway

1.2 CHANGES TO SKELETAL MUSCLE WITH AGE

Muscle cross-sectional area (CSA) and muscle mass can be measures in several different ways. Before presenting change in muscle mass and CSA, briefly presenting these methods and the pros and cons of each is important. Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) are considered to be the gold standard method for measuring muscle CSA[84]. They both produce high resolution images which can be used to assess both lean and fat mass depots including muscle density which is an indirect marker of intramyocellular adipose tissue[85]. MRI may be more advantages than CT because subjects are not exposed to radiation. However, high cost of using these technologies is a barrier to widespread use and in larger studies. Dual energy X-ray absorptiometry (DXA) is one of the most widely used methods for measuring muscle and fat mass due to its low cost, good accuracy and the fact that it does not require highly trained personnel to operate. DXA can also be used to estimate regional body composition as well as bone mass. It should be noted that DXA can over estimate lean mass in older adults because it does not differentiate between water and bone-free lean mass[86]. However DXA is a valid and reliable tool[87]. Bioelectrical impedance is also a relatively common, accessible and easy way to measure muscle mass, however it has limited accuracy and validity[88,89]. Ultrasound is another relatively lost cost method that can be used to measure muscle CSA of individual muscle groups[90]. However, this method requires highly trained technicians and cannot provide whole body muscle mass estimates[84]. Urinary creatinine excretion is used as a measure of total muscle mass, because creatinine in the body is restricted almost exclusively to skeletal muscle. This method has several limitations including, delay in availability of results, complicated procedure, participants must ingest a meat-free diet the day before collection and creatinine excretion may vary from day to day[84]. Peripheral quantitative computerized tomography (pQCT)

is becoming a more widely used technique because of its portability. However this method provides less accurate estimates of muscle when compared to MRI or CT as it was initially developed to measure bone[84]. This method is attractive though because it is portable, less expensive than MRI and CT and can provide reliable cross-sectional area estimates of specific fat depots present in skeletal muscle such as intermuscular and subcutaneous adipose tissue as well as muscle cross-sectional area and muscle density, which is a surrogate measure of fat infiltration into muscle fibers[84,91-93].

Perhaps the most fundamental and well documented structural change in skeletal muscle with age is decreasing muscle size or mass. This reduction has been shown both cross-sectionally, comparing young adults to older adults, and longitudinally using various measurement techniques[83,94-116]. Reductions have been observed in muscle groups of both the upper and lower extremities[95,105,107,116,117]. Muscles cross-sectional area (CSA), as measured by MRI, of the lower extremities has been shown to decrease more than those of the upper body[105]. This suggests that there are non-systemic causes of muscle loss with age. For example, the reason for larger decreases in lower compared to upper body muscle mass may be due to age-related reductions in physical activity levels. Reducing activity levels would affect the lower extremity muscle groups more than the upper because ambulation is powered by the lower extremities. However, changes across muscle groups are highly correlated, which also implies systematic reasons for muscle loss (which will be discussed later), so one muscle group is often used to represent changes in muscle mass or CSA of the whole body. This review will mainly discuss changes to the lower extremities, in particular the quadriceps, because lower extremity muscle performance is more vital to performing IADL and ADL tasks and for mobility. The following will present observed changes in muscle mass with age and proposed mechanisms

driving the atrophy. There are several other very important qualitative and physiologic changes that take place in the aging skeletal neuromuscular system. These changes and their implications will also be presented and discussed.

1.2.1 Cross-sectional Evidence for Age-Related declines in Skeletal Muscle Mass or Area

A majority of the studies reporting age-related muscle atrophy have been cross-sectional, comparing the muscle size or mass of older adults to that of younger adults. These studies are shown in Table 1. These studies were conducted using a variety of techniques, including, ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), bioelectrical impedance, creatine excretion, dual energy X-ray absorptiometry (DXA) and direct measurement from post-mortem subjects. Annual declines in muscle CSA, as measured by ultrasound, MRI and CT, from cross-sectional studies have been shown to be ~0.5% per year and changes in muscle mass on the order of ~0.48% in men and ~0.51% per year mm in woman. These rates are rough estimates calculated from cross-sectional studies. Muscle CSA has been shown to peak around the age of 25 and decline thereafter, with accelerated declines after age 50[106]. Additionally, it has been shown by full body MRI that muscle CSA of the lower body tend to decrease more than in the upper body[105].

Young et al. conducted studies comparing the CSA of the quadriceps in older (age 70-79) compared to younger (20-29) men and woman using ultrasound[111,112]. They showed that quadriceps CSA was 33% smaller in older woman and 25% smaller in older men compared to their younger counterparts. Borkan et al. conducted a similar study comparing older (mean age 69.4) to younger (mean age 46.3) men using CT and found similar results[104]. This study showed a 12% reduction in CSA of the quadriceps in older compared to younger men, a similar annual

decline as the Young et al. study (Table 1.). These results were confirmed yet again in another study of younger and older men using CT[117]. Although most of the research in this area focuses on the lower extremities, it should be noted that reductions in CSA of the triceps and biceps of older men compared to younger men have also been reported using CT[107].

The studies described in the previous paragraph all had relatively small sample sizes (n= n=24-41), were done mostly in men and were limited to a few muscle groups. The participants in the older adult groups also tended to be in their 60s or 70s, thus the oldest and frailest of older adults are not represented. Larger cross-sectional studies have been conducted using different methods in much larger samples of both men and women. For example, Frontera et al. conducted a study of 200 45-78 year old men and woman, using creatinine excretion to measure total muscle mass[103]. This study showed 9.6% and 19.1% lower muscle mass in men and woman aged 65-78 compared to those aged 45-54. Similar differences have been shown using creatinine excretion to measure total muscle mass in studies of 184[118] and 959[119] younger men and women. These studies reported a 23.4% and 22.0% decrease in men and woman between the age of 30 and 70[118] and an average 50 year decrease of 33%[119]. Baumgartner et al. measured total appendicular skeletal muscle mass (ASMM) using DXA in 426 and 382 older men (mean age 73.3) and woman (mean age 73.7) in the New Mexico Health Survey as well as 107 and 122 young men (mean age 28.7) and women (mean age 29.7)[94]. This study showed an ASMM difference of 17.6% and 18.1% in older men and woman compared to their younger counterparts. A similar study using DXA to measure ASMM showed 14.8% and 10.8% differences in older compared to younger men and woman, after adjustment for height, body weight and age[95]. In summary, cross-sectional studies show that muscle mass decreases by about 40% between the

ages of 20 and 80 with greater decreases seen in lower extremity groups compared to upper extremity.

Table 1. Cross-sectional Studies examining Changes in Muscle Mass or Area with Age

Author	YN, ON	Technique	Measurement	Sex	Young Age	Older Age	Young Value	Old Value	Difference	% Difference	% Annual Difference
Baumgartner[94]	553, 883	DXA	AMM, kg	m	28.7±5.1	73.6±5.8	27.3	22.5	-4.8	-17.6	-0.39
				f	29.7±5.9	73.7±6.1	17.7	14.5	-3.2	-18.1	-0.41
Frontera[103]	24,34	hydrostatic weighting	whole-body muscle mass, kg	m	50.5±2.8	68.5±2.8	60.2	55.4	-4.8	-8.0	-0.44
				f	50.2±2.6	69.6±3.8	40.5	36.2	-4.3	-10.6	-0.55
Borkan[104]	21,20	CT	upper leg CSA, cm^2	m f	46.3±2.6	69.4±4.1	147.3	129.1	-18.2	-12.4	-0.53
		CT	upper arm CSA, cm^2				54.6	48.2	-6.4	-11.7	-0.51
		total body potassium	lean body mass, kg				56.2	49.6	-6.6	-11.7	-0.51
Jansen[105]	66,11	MRI	upper body SMM	m	18-29	70+	14.3	13.5	-0.8	-5.6	~-0.10
			lower body SMM	m	18-29	70+	18.5	13.8	-4.7	-25.4	~-0.45
	40,19		upper body SMM	f	18-29	70+	8.7	7.7	-1.0	-11.5	~-0.21
			lower body SMM	f	18-29	70+	12.5	9.7	-2.8	-22.4	~-0.40
Lexell[106]	9,9	Post-mortem	whole vastus lateralis, cm^2	m	32.0	73.0	75.0	56.0	-19.0	-25.3	-0.62
Young[111]	12,12	Ultrasound	quad CSA, cm^2	m	20-29	70-79	-	-	-	-25.0	-0.50
Young[112]	25,25	Ultrasound	quad CSA. cm^2	f	20-29	70-79	-	-	-	-33.0	-0.66
Overrend[117]	13,12	CT	quad CSA. cm^2	m	24.5±1.5	70.7	84.7	65.7	-19.0	-22.4	-0.49

Abbreviations: YN= Number of young participants; ON= number of older adult participants: DXA = Dual X-ray Absorptiometry; AMM= appendicular muscle mass; kg = kilogram; CT= computed tomography; CSA = cross-sectional area; m = male; f= female; SMM= skeletal muscle mass; cm= centimeters; quad= quadriceps

1.2.2 Longitudinal Evidence for Age-Related Declines in Skeletal Muscle Mass or Area

Although cross-sectional studies are easier, less expensive and produce data more quickly than longitudinal studies, they are subject to inherent biases and can only be used to roughly estimate "true" age-related changes in muscle mass. Longitudinal studies show greater declines in muscle CSA and muscle mass than were previously estimated cross-sectionally, likely due to the inherent selection bias associated with cross-sectional studies. That is, older adults who are healthier than the general older adult population may be more likely to volunteer for research studies, and therefore may have experienced less muscle atrophy than the average older adult. This would lead to an underestimation of muscle loss with age than if the same individuals were followed from middle to old age. However, longitudinal prospective cohort studies are not immune to a healthy participant bias. Older adults who remain in prospective cohort studies for the longest amount of time are also likely to be healthier than those who have either dropped out or died. That being said, longitudinal studies, compared to cross-sectional, are significantly better equipped to determine actual change over time. Longitudinal studies of muscle mass or muscle area across are shown in Table 2.

The two of the earliest examples of observational prospective studies measuring changes in muscle CSA or mass were conducted in a small number of participants who had originally took part in an exercise intervention[102,120]. In these studies, participants were only measured twice, once at baseline and once the follow-up visit. In a study by Greig et al., 4 men (aged 79 to 84) and 10 women (aged 79 to 89) showed an average of 6.4% decrease in the CSA of the quadriceps after 8 years[120]. Muscle CSA was measured by ultrasound at baseline and by CT at the 8-year follow-up visit. In a similar study by Fontera et al., 9 men presented 12-year 16.1%

and 14.9% decreases in the CSAs of the quadriceps femoris and flexors as measured by CT[102]. Smaller changes in the CSA of the quadriceps were observed in a similar 8-year follow-up study by Frontera et al., in which only 12 out of 24 participants had follow-up data. This study consisted of a subset of men and woman originally aged 45-70, who were an average of 60.4 years old after a mean follow-up time of 9.7 ± 1.1 years, total muscle mass measured by creatinine excretion, decreased 12.9 ± 15.5% per decade in men and in −5.3 ± 18.2% per decade women from CT[121].

The Health Aging and Body Composition Study (HABC) is a large prospective cohort study designed to measure changes in body composition using both DXA and CT and how they relate to functional measures in older adults. This study enrolled and is following 3,075 men (48.4%) and woman (51.6%) of whom 41.6% were black, who were aged 70-79 at baseline[99]. For these reasons, it is arguably the most well equipped study to document longitudinal changes in muscle mass and CSA. In a 3-year follow-up study of the Health ABC cohort by Goodpaster et al. annual leg mass declines from DXA of 1.01% in white men, 1.32% in black men, 0.86% in white women and .93% in black woman were observed[83]. These were significant gender differences within races and significant racial differences within genders. During a 5 year follow-up period in Health ABC, muscle CSA of the thigh was shown to decrease 0.98%/year in men and 0.64%/year in woman[122]. Finally, 1178 female and 1129 male Health ABC participants, annual percent changes in lower leg lean mass as measured by DXA of 0.71% and 0.85% were observed over a 7-year follow-up period[123]. The differences in the percent changes observed in the three Health ABC analyses is likely attributable to a healthy participant effect, as the analyses that were conducted over longer periods of time actually show less severe declines in muscle mass. It is unlikely that muscle mass decline is attenuated over time in old-age; instead, these

differences are likely due to the healthier participants, who presumably lose less muscle mass, surviving longer. In summary, these longitudinal data are invaluable and suggest that muscle mass declines are greater in men than woman and African Americans compared to whites, although the reasons are not clear. As previously mentioned African Americans have been shown to have a higher prevalence of chronic diseases, such as diabetes and higher levels of inflammation, which may in part explain the racial disparity in muscle loss. They also start with higher levels of muscle mass, and those who start with the most muscle mass have been shown to lose the most.

Table 2. Longitudinal Changes in Muscle Mass or Area in Older Adults

Study	N	Technique	Measurement	Sex	BL Age	FU-Time (Yrs)	BL Value	FU Value	Change	% Change	% Annual Change
Frontera[102]	7	CT	Total Thigh Muscle CSA, cm^2	M	65.4±4.2	12.2	135.7	115.8	-19.8	-12.5	-1.02
			Thigh Extensor CSA, cm^2				64.8	54.5	-10.3	-16.1	-1.32
			Thigh Flexors CSA, cm^2				34.2	29	-5.2	-14.9	-1.22
Hughes[124]	78	Hydro-densitometry	Total Body Muscle Mass, kg	F	60±7.4	9.4	41.9	41.8	-0.1	-0.24	-0.03
	53			M	61.1±8.1		58.8	57.7	-1.1	-1.87	-0.20
Goodpaster[83]		DXA	Leg Lean Mass, kg	BF	73.0±2.8	3	7.0	6.8	0.2	-2.78	-0.93
				WF	73.4±2.8		5.9	5.7	0.2	-2.59	-0.86
				BM	73.4±2.8		9.3	8.9	0.4	-3.97	-1.32
				WM	73.7±2.8		8.7	8.4	0.3	-3.03	-1.01
Delmonico[122]	865	CT	Total Thigh Muscle CSA, cm^2	F	73.2±2.9	5	92.9	89.7	-3.20	-3.44	-0.69
	813			M	73.6±2.8		133.2	126.4	-6.80	-5.11	-1.02
Koster[123]	1178	DXA	Leg Lean Mass, kg	F	73.9±2.8	7	12.4	11.8	-0.62	-5.00	-0.71
	1129			M	74.2±2.8		17.2	16.2	-1.02	-5.90	-0.85

Abbreviations: N= Number participants; BL= baseline; FU= follow-up; Yrs= Years; CT= computed tomography; CSA = cross-sectional area; cm = centimeters; m= male; kg= kilograms; f= female; DXA= dual x-ray absorptiometry; bf = black female; wf= white females; bm= black male; wm= white male;

1.2.3 Physiologic and Structural Mechanisms underlying Changes in Muscle Mass with Age

This section will discuss the underlying age-related physiologic and structural changes in the neuromuscular system that have been postulated to cause reductions in muscle mass with age. These are, in part, hypothesized to be distinct from the general consensus that age-related declines in physical activity levels result in many of the age-related changes in skeletal muscle. It is important to note that these physiologic changes, driving the loss of muscle mass, (as well as other changes in skeletal muscle that will be discussed later) likely contribute to functional declines in skeletal muscle function independent of muscle mass. This is because it has been shown that the age-related declines in muscle strength are 2-5 times larger than changes in muscle strength[83,125]. Furthermore, several studies well equipped to do so, including Health ABC[83,99,122] and the Baltimore Longitudinal Study of Aging (BLSA)[116,126] have shown a decrease in muscle quality, defined as muscle strength per unit muscle mass with age. This suggests that qualitative neuromuscular changes, not just changes in mass, largely affect changes in neuromuscular function. This idea will be discussed in more detail throughout the remainder of this document.

Muscle mass and CSA are functions of both the mass or area and number of individual muscle fibers. The general consensus in the literature is that the loss of muscle mass with age is a combination of both a reduction in muscle fiber size and number. Cross-sectional research using both *in vivo* biopsy techniques and direct measurement in cadavers shows reductions in the number of both type I and type II muscle fibers[106,109,113]. The percent reduction in type I fiber number is markedly smaller than the decrease in the number of type II fibers, with some studies

even showing increases in type I fiber number[102,106,108,109,113,127]. In addition to the preferential loss of type II fibers with age, it has been shown that type II fibers also atrophy with age, whereas type I fibers seem to preserve their size[106,109]. However some studies show no reduction is type II fiber size[102,109]. Conversely, a compensatory fiber hypertrophy has also been observed in both humans and rats where remaining fibers become enlarged to compensate for the reductions in number[121,128,129].

Contradictory findings have also been reported in the literature. Two small longitudinal studies in older adults conducted by Frontera et al. showed no reduction in the number of type II fibers after 8 years and a surprising increase in the proportion of type II fibers after 12 years[102,121]. There have been very few longitudinal studies that examined changes in fiber type number and sizes, but they do not seem to corroborate this finding by Frontera et al.[128,130]. Longitudinal research in rats also shows a reduction in the number both type I and type II fibers, with greater reductions in type II[131,132]. Assessing number and size of fiber types is subject to large biases. Lexell et al., found that in a ordinary sized biopsy (~600 fibers), containing equal proportions of both fiber types, the 95% confidence interval for the estimate of the proportion of fiber type can vary by as much as 40-60%[113]. Additionally, the proportion of type I fiber type taken for different areas of the quadriceps can vary from 32 to 65% [113]. Anderson et al. have shown that aged muscle fibers may express two or three myosin isoforms (how fiber types are determined), thus histochemical analysis could misclassify fibers. In summary, the current literature seems to suggest losses in muscle mass result mainly from reductions in the number of both type I and type II muscle fibers, with reductions in individual muscle fiber size of the latter.

Reduction in muscle fiber number and size is thought to be due in large part to ongoing denervation and renervation process[127,133,134]. Muscle fibers are functionally grouped together

into motor units. They are called motor units, because all of the muscle fibers in a motor unit are innervated by a single motor neuron. The hypothesis that there is a progressive age-related denervation and renervation process was suggested in response to the observed grouping of type I fibers that occur with age[106,127,135]. It is thought that motor neurons enervating type II fibers begin to fail, causing a neighboring motor neuron to expand its territory by renervation the fibers that were originally enervated by the failing motor neuron. There is experimental evidence supporting this hypothesis. It has been shown that the number of excitable motor units declines with age, using the electrophysiological technique[136]. Furthermore, indirect evidence suggesting a greater number of muscle fibers per motor unit has been found using the macro-electromyographic (EMG) technique. This technique showed an increase in size of motor units of the vastus lateralis, the tibialis anterior, and the biceps brachii[137,138]. There is also controversial evidence showing that the number of muscle satellite or stem cells decreases with age[139,140]. As muscle cells are post mitotic, a decreasing number of these cells would result in a limited capacity to regenerate losses in muscle tissue[140].

In addition to being implicated in the etiology of sarcopenia, the loss of muscle mass with age, poor peripheral nerve function has also been associated with lower muscle performance and physical function in older adults[141,142]. In Health ABC, Strotmeyer et al. showed that the poorest motor nerve conduction amplitude quartile (<1.7 mV) was associated with 8% lower quadriceps strength compared to the highest quartile, after adjustment for covariates including muscle mass and diabetes[142]. In the InCHIANTI study, individuals with peripheral arterial disease were shown to have reduced leg muscle power and muscle CSA and that poor nerve conduction velocity was related to slower 400m walk time and lower physical performance score as measured by the Short Physical Performance Battery[143].

Another very important physiologic change in skeletal muscle that occurs with age is increasing adiposity. There are two fat depots present in skeletal muscle, intramuscular or intramyocellular fat (IMF) – fat infiltration in the muscle fibers themselves and intermuscular fat (IMAT) – fat that is visible within the muscle fascia, surrounding skeletal muscles. These fat depots have both been shown to increase with age[122,144-147]. In a longitudinal study in HABC participants, Delmonico et al. showed, using CT, that intermuscular fat increased in older adults who lost, gained and maintained their overall body weight[122]. This suggests that increasing intermuscular fat is a feature of the aging process independent of overall weight gain. In this study 5-year increases in IMAT of 48.5 ± 59.6% and 29.0% ± 43.6 in men and woman respectively were observed. Goodpaster et al. observed a one year increase of 3.6% in IMF in the education control group of an intervention study[148]. Interestingly, although IMAT has been shown to be linked to obesity, it has been shown in a study of men of both Caucasian and African ancestry, that those with African ancestry had higher levels of IMAT in the calf, despite having lower levels of total adiposity compared to Caucasians[149]. Higher levels of intramyocellular fat have been linked to worse functional performance in older adults[30,144,150]. IMAT in the calf has been linked with poorer muscle power and physical performance[151] . However, a larger study by Buford et al. was unable to find and association between IMAT levels in the calf or thigh and physical performance[145].

It is important to note that these two fat depots are hypothesized to have two distinct etiologies. It has been postulated that intermuscular fat is a result of changes in the differentiation of quiescent skeletal muscle satellite or stem cells[152], as these cells can either mature into myoctyes or adipocytes. There is evidence that the adipocytic phenotype is increased with age[153]; however research in this area is conflicting and this process is still poorly understood. Increasing

intramyocellular lipid content with age is thought to be the result of a decreased oxidative capacity of muscle with age and increasing insulin resistance[154]. Fatty acid oxidation includes ~90% of the energy requirements at rest and the oxidative capacity of skeletal muscle has been shown to decrease with age[7,155,156]. Therefore, it is hypothesized that larger lipid droplets are present in aged muscle fibers because they are not being metabolized as efficiently or as readily. Furthermore, higher intramyocellular fat content has been associated with insulin resistance in humans[157-160]. Higher IMAT levels have also been linked to diabetes and insulin resistance in humans[52,146,161]. Additionally, higher IMAT levels have also been shown to be associated with lower mitochondrial function (r^2=0.32, p=0.004) in a study consisting of both older (75.7±1.0 years) and younger (27.2±1.0) adults[162]. However, this was an unadjusted correlation, so this could be an affect of age, decreased physical activity or another confounding factor and not higher IMAT per se.

In addition to increasing adiposity of skeletal muscle tissue, Obesity in general has been shown to exacerbate physical disability in older adults[65,68-70,163,164]. In addition to the loss of lean mass, aging is accompanied by an increase in fat mass[100]. Specifically, aging is associated with increases in central adiposity or visceral adipose tissue (VAT)[165,166] , intracellular deposition of fat and deposition of fat outside of adipose tissue[153,167].

Despite obesity being related to physical disability in older adults, there is controversy as to whether older adults should lose weight and whether overall body weight or particular aspects of body composition should be targeted[168-170]. Observational studies have shown a positive association between weight loss in old age and mortality[171]; whereas randomized controlled trials have shown that weight-loss (WL) in combination with moderate physical activity (PA) improves physical function in older adults to a greater degree than physical activity alone[49,172],

weight loss alone[49] and normal care[173]. Additionally, WL in conjunction with PA has been shown to improve components of frailty, including physical function, in obese frail older adults [174]. This may be attributable to the fact that intentional and unintentional weight-loss may target fat mass differently. Intentional weight loss combined with a moderate physical activity intervention has been shown to decrease fat mass to a greater degree than lean mass[48,174-176]. Intentional weight loss may also target particularly harmful fat depots. Few studies have shown specific composition data following intentional weight loss in older adults[173,175,177], but the few that do seem to support this hypothesis. Furthermore, Shea et al. have published findings from a randomized controlled trial showing that intentional weight loss in old age is not associated with increased mortality[49].

1.2.4 Changes in Muscle Performance with Age

Changes to the neuromuscular system lead to age-related changes in muscle performance in the form of decreased muscle strength and power. It is well documented that muscle strength decreases with age[79,134]. This has been shown both cross-sectionally and longitudinally in both the upper and lower extremities. Upper extremity muscle strength in older adults is using measured in the form of grip strength by a hand held dynamometer. Lower extremity muscle strength is usually measured by an isokinetic dynamometer. Lower extremity muscle strength can also be measured isometrically, where the participant's one repetition maximum is determined by having them push against increasing resistance until they are no longer able to move the load. This review will discuss changes in the lower extremities because lower extremity function is more important for mobility and most IADL and ADL tasks.

Cross-sectional studies estimate knee extensor strength to be between 20-40% lower in those in their 60s and 70s compared to those in their 20s and 30s for both men and women[111,112,115-117,127,178-185]. Additionally, Frontera et al. observed 20.0% and 17.6% lower knee extensor strength in men and women age 65-78 respectively compared to men and woman age 45-54[103]. Cross-sectional evidence from HABC showed 17.8% and 13.5% lower muscle strength in white and black men and 16.9% and 23.1% in white and black woman respectively, age 79 compared to age 70. There is also some evidence that strength declines at a steeper rate starting in the late 8th decade and into the 9th decade of life[184]. However, there is a lack of data concerning

Fewer longitudinal studies have been done. In a study by Frontera et al. where 9 of 12 men, age 71.1±5.4 years at baseline, where revaluated 9 years after participating in a resistance training intervention. Percent annual declines in knee extensor strength, measured using an isokinetic dynamometer at 60°/s, of 2.5%. These are similar estimates to those observed in the much larger epidemiologic Health ABC study. In Health ABC, percent annual declines of 4.12% and 3.42% in white and black men and 2.65% and 2.97% in white and black women respectively. It is important to note that rates of decline different significantly by sex within race and race within sex. In summary, longitudinal evidence suggests that the rate of decline may be more severe than previously estimated by cross-sectional studies and that men lose a greater proportion of strength compared to woman. However, men start with higher levels of strength than women, and thus have more to lose. Additionally white men and black woman appear to experience more severe declines in strength than their within gender racial counterparts. It should be noted that these longitudinal studies examined participants with a much greater minimum baseline age than cross-sectionally. Therefore, in the addition to the inherent design biases

between longitudinal and cross-sectional studies, the loss of strength with age may be accelerated in old age, partly accounting for the differences seen in cross-sectional compared to longitudinal studies. It should be noted that the above changes in knee extensor strength were absolute measures and not adjusted for lean or body mass. However, several studies well equipped to do so, including HABC[83,99,122] and the Baltimore Longitudinal Study of Aging[116,126] have shown a decrease in muscle quality, defined as muscle strength per unit muscle mass with age.

Muscle power, which is defined as force multiplied by velocity, decreases with age at a greater rate than muscle strength[77,186] which is thought to be due to the velocity component decreases with age along with the strength (force) component[187,188]. Muscle power can be measures several different ways, none of which have been agreed upon as a gold-standard method. These methods include: vertical jumping on a force plate, Nottingham power rig (fixed load leg extensor power), isokinetic dynamometry and leg extensor power using pneumatic resistance machines[77,189]. A recent study by Pojednic et al. showed that muscle power in healthy middle aged adults (mean age 47.2 ± 4.5 years) was 19.4% and 44.5% higher when compared to healthy older adults (mean age 73.6 ± 3.5 years) and mobility-limited older adults (mean age 77.9 ± 4.3 years) respectively[187]. It has also been shown by several studies that muscle power is more strongly related to certain physical limitations in older adults than muscle strength[186,190-192]. Similarly, the velocity component has been shown to be related to physical impairment in older adults independent of the strength component[187,193]; this may be why power has been shown to be a stronger predictor of physical limitations than strength.

1.2.5 Interventions Improving Muscle Function

Many studies have been conducted attempting to increase muscle performance in older adults. These studies include pharmacological, nutritional and physical activity interventions. The most common form of physical activity intervention for improving muscle strength in older adults is resistance training[194]. It is also important to note that interventions specifically designed to improve muscle power, as opposed to strength, have also been conducted. An overview of the efficacy and effectiveness of these interventions will be presented in this section.

Several studies have been designed to assess whether or not nutritional supplementation increases the benefits of resistance training on muscle performance in older adults. Protein, creatine and vitamin D supplementation are nutritional supplements that have been studied. In Health ABC, participants in the highest quintile of energy-adjusted protein intake lost about 40% less total and appendicular lean mass when compared to those in the lowest quintile over a 3-year follow-up period[195]. Over the same 3-year follow-up period and after adjustment for potential confounders, higher energy-adjusted protein intake was associated with smaller losses in total and appendicular lean mass, as measured by DXA[195]. Therefore, protein deficiency in older age may contribute to the loss of muscle mass and performance.

Several studies have been specifically designed to assess the added benefits of protein supplementation to resistance training in older adults[196-200]. In a study conducted by Meredith et al in which 11 older men underwent a 12-week lower extremity resistance training program, 6 of these men were given a daily dietary supplement of 560 +/- 16 kcal/day (17% energy from protein, 43% from carbohydrate, 40% from fat)[201]. In this study, the supplement group increases their muscle mass to a greater degree than the non-supplement group, but no differences in improvements in strength were observed between groups[201]. Fiatrone et al. published findings in

the New England Journal of Medicine from a randomized controlled trial comparing the effects nutritional supplementation (360 kcal in a 60% carbohydrate, 23% fat, 17% soy-based protein formula and one-third of the recommended daily allowance of essential vitamins and minerals), progressive resistance training, both and neither on muscle strength (1 repetition maximum (1RM)) in nursing home residents over the age of 70[202]. In this study, nutritional supplementation did not convey significant additional benefits to resistance training in improving muscle strength after 10-weeks[202]. The same group published findings from a subset of 26 individuals involved in the previously mentioned clinical trial, who were willing to undergo a muscle biopsy[203]. In these individuals, protein supplementation did significantly augment increases in muscle strength following the exercise intervention[203]. Esmarck et al. conducted a randomized controlled trial in 13 men, comparing the effects of protein supplementation immediately following, compared to 2 hours following an identical resistance training program[204]. In this study the group that ingested the supplement immediately following training improved both their 1RM and isokinetic strength, whereas the group that ingested the supplement 2 hours following training only improved their 1RM strength and to a lesser degree than the immediate ingestion group[204]. However, Candow et al. observed no effect of protein supplementation consumed before or after resistance exercise on muscle performance or size in men aged 59-76[200]. Similarly, no effect of protein supplementation, creatine supplementation or a combination of both immediately following resistance training was observed in men aged 57.0±1.9 years[205]. The studies by Candow et al. [200] and Carter et al. were conducted in much larger (n= 38 and 42) samples than the Esmarck study (n=13).

In summary, sufficient evidence does not exist suggesting protein supplementation is necessary to improve muscle function in older adults[198]. A comprehensive review of the effects

of protein supplementation on muscle composition performance in older adults can be found here[197]. Studies including women as well as older (aged ≥80) and more frail adults appear to be lacking. Although protein supplementation does not appear to augment the effects of resistance training on muscle performance, in a meta- analysis of trials examining the effects of protein supplementation and resistance training on fat-free mass a significant relationship between amount of protein supplementation and change in lean mass observed[197]. In fact, those who consumed the average daily dietary recommendation of 0.8g of protein/kg of body weight actually would be predicted lose about 0.2 kgs of lean mass over a 12-week period. Protein intake of 1.0 g/kg of body weight was associated with no change in lean mass. Thus, although protein supplementation does not appear to augment changes in muscle performance induced by resistance training, consuming adequate levels of protein seem to be important for preserving lean mass in old age.

Creatine supplementation is another nutritional agent of interest because of its involvement in skeletal muscle performance, specifically through increasing energy stores (Phosphocreatine (PCR)). Creatine may also aid in reducing the amount of protein breakdown and increase protein synthesis in skeletal muscle, as well as reduce oxidative stress[206]. Several studies have assessed the added benefits of creatine supplementation with resistance training in older adults[206]. In a study conducted by Brose et al. comparing the added benefits of creatine supplementation to a 14-week resistance training program, the creatine supplementation group showed a greater increase in isometric knee extension strength in men and women compared to the placebo group[207]. Interestingly, isometric dorsiflexion strength was improved in the supplement group to a greater degree than the placebo group, but only in men[207]. A similar study in men only conducted by Chursch et al. produced similar results, with significant group by time

interactions on knee extensor strength (1RM) and knee extensor power, with the creatine supplement group showing greater improvements following 12-weeks of resistance training[208]. In a study consisting of both men and women comparing the added benefits of creatine supplementation to 6-months of resistance training, both men and women in the supplement group showed greater improvements in both isokinetic knee extensor strength and 1RM compared to the placebo group[209]. In a study consisting of 32 older men and woman (age 67-80) comparing the effects of weight training with and without supplementation to control groups with and without training, no added benefit was observed in regard to lean mass or 1RM strength after 8 weeks[210]. In a study comparing creatine supplementation to placebo after 30 days, without resistance or exercise training, reported no difference between the two groups in regard to both muscle strength and muscle mass[211]. In two similar studies comparing the effects of creatine supplementation to placebo after 5 days, no differences between groups concerning elbow extensor strength or isokinetic knee extensor strength was observed[212,213]. Conversely a study in 15 men and woman comparing the effect of creatine supplementation vs. placebo showed significant increase in grip strength following 14 days of supplementation[214].

In summary, results are conflicting regarding the added benefits of creatine supplementation to resistance training, and creatine supplementation without exercise. In three of the four trials comparing creatine supplementation plus resistance training to resistance training plus placebo, an added benefit of creatine supplementation was reported. Two of the three trials showing an added benefit of creatine supplementation on muscle performance included both men and women[207,209], while one included men only[208]. The trial showing no benefit included both men and women[210]. Therefore, it is unlikely that the conflicting results are attributable to gender differences between studies. The trial that showed no benefit lasted 8 weeks, where the minimum

length of the three trials showing a benefit was 12-weeks. Perhaps 8-weeks were not long enough to see a benefit of creatine supplementation with resistance training compared to placebo with resistance training. Importantly, the only trial of the 4 to include functional or physical performance measures[207], timed chair-stand, timed walk and a timed stair-climb test, showed greater improvements for all 3 tests in the creatine supplementation group compared to the placebo group. Additionally, it appears as though resistance training is necessary to observe benefits of creatine supplementation, as 2 of the 3 trials comparing creatine supplementation to placebo without either group participating in an exercise intervention, showed to benefit. In summary, there does seem to be some promise to creatine supplementation while participating in a resistance training program. Larger double-blind placebo trials are warranted[206]. These trials should also include a measure of overall physical function, such as the SPPB and/or walking performance, such as the 400m walk or timed get-up-and-go test. It is important to determine whether or not improvements in muscle performance are also conveying improvements in physical function, i.e. gait-speed or SPPB score.

Vitamin D is another nutrient that has been suggested to possibly improve muscle function in older adults. Several studies assessing the benefits of Vitamin D supplementation with and without exercise training, have been conducted[206,215]. Two randomized controlled trials comparing the added benefit of Vitamin D supplementation to resistance training in older adults have been conducted. Neither of these trials showed any additional beneficial effect with Vitamin D supplementation compared to resistance training alone in regard to muscle function[216,217]. However the trial conducted in Vitamin D deficient individuals showed added benefits of Vitamin D, specifically greater improvements in physical function measured by the timed up and go test[216]. It should also be noted that a meta-analysis conducted examining the

effect of Vitamin D supplementation on fall risk showed a protective effect of supplementation, which may be due to increased muscle performance[218]. More work examining the effect of Vitamin D on muscle performance is needed. However, it has been suggested that Vitamin D supplementation should be considered for those with low levels or in populations who are likely to have low levels, such as nursing home residents[198].

Testosterone therapy has been hypothesized as a possible mechanism to prevent age-related declines in muscle performance in men, because testosterone levels have been shown to decrease in men age 40 and older. Similar to protein and creatine supplementation, results are mixed regarding the effectiveness of testosterone, especially in community-dwelling men[198,206]. A meta-analysis looking at the combined effect size in 11 randomized controlled trials comparing the effect of testosterone or dihydrotestosterone (DHT) replacement therapy in healthy men aged 65 and older was published by Ottenbacher et al in 2006[219]. A modest improvement in strength in those receiving testosterone compared to placebo was reported in this meta-analysis, with those trials implementing injection as opposed to topical or oral administration of testosterone showing larger effects[219]. One study in particular severely drove the effect size up as sensitivity analyses showed that excluding this study reduced the mean g-index from 0.53 to 0.23 (g-index of 0.0 = no effect).

Selection of participants (healthy men vs. hypogonadal men with low testosterone) is likely to affect results. In a more recent clinical trial of 237 men with testosterone levels below the 50th percentile of the population screened (13.7 nmol/L), 80 mg of testosterone or placebo were administered twice a day for 6 months[220]. This study reported greater increases in lean mass in the testosterone group, but this increases in lean mass did not result in improvements in muscle strength[220]. Conversely, in a randomized controlled trial in 274 frail men comparing the

effects of 50 mg per day of testosterone compared to placebo over the course of 6-months, greater improvements in muscle mass and strength were recorded in the testosterone group compared to the control group[221]. Testosterone supplementation also conveys certain health risks, as testosterone treatment has been reported to be associated with an increase in prostate disorders, raised hemocrit levels and adverse cardiovascular events[198,206,222].

In summary, testosterone supplementation is promising, but the long term risks and benefits need to be assessed before general use can be recommended. However, those with low muscle mass, strength and testosterone levels may benefit from supplementation. The fact that two large trials arrived at conflicting results, with one showing an improvement in strength[221] while the other did not[220], may be due to the fact that the latter enrolled frail men, while the former did not. It could be that testosterone supplementation is important for subsets of older men, especially those who are hypogonadal (have low testosterone), frail or both. Currently, there is a large trial being conducted in 800 men, across 12 sites in the United States designed to compare the effects of testosterone supplementation to placebo. To be enrolled in the trial men had to have low blood testosterone and also have one or more of the following: difficulty walking a quarter of a mile, less interest in sex, or less vitality than they used to have. This trial is going to examine the effects of testosterone compared to placebo on physical function, sexual function, vitality, cognitive function, and low hemoglobin concentration, as well as risk factors for cardiovascular and diabetes. The trial will also examine any adverse effects associated with testosterone. This trial should provide a wealth of information concerning the risk and benefits of testosterone supplementation. Although muscle performance was not directly evaluated, walking performance was and improved muscle function is ultimately of little importance unless it also coveys improvements in physical performance - e.g. walking.

Growth Hormone (GH) supplementation is another means by which muscle strength may be improved in older adults. Liu et al. published a review paper titled "Systematic Review: The Safety and Efficacy of Growth Hormone in the Healthy Elderly" in 2007[223]. The review included randomized clinical trials designed to assess the effects of GH therapy on body composition, exercise capacity, bone density, serum lipid levels and glucose metabolism as well as adverse events. The review included only those trials that compared injectable GH with no GH and GH plus a lifestyle intervention with a lifestyle intervention alone. The doses ranged from 1.7 µg/kg – 45 µg /kg, with a mean and standard deviation of 14.3 ± 14.7. The duration of GH therapy ranged from 2-52 weeks w/ a mean of 26.6 weeks w/ SD of 15.6[223]. Those receiving GH manifested a decrease in fat mass and increase in lean body mass compared to non-GH participants. The differences in these body composition measures were not statistically significant, but suggested that GH therapy can reduce fat mass and increase lean mass in the healthy elderly. Additionally, several trials have failed to show increases in muscle performance following GH supplementation, whereas others have found a positive effect[198,206]. Furthermore, significantly higher rates of soft tissue edema, carpel tunnel syndrome, joint pain and gynecomastia in GH participants as compared to non-GH participants were reported in the review by Lui et al[223].

Angiotensin II-converting enzyme (ACE) inhibitors have also been hypothesized to have positive effects on skeletal muscle. In Health ABC use of ACE inhibitors was associated cross-sectionally with larger lower extremity lean mass[224]. However, results of clinical trials looking at the effect of ACE inhibitors on muscle mass and performance have been conflicting[206]. In an extensive review by Rolland et al. from 2010 discussing pharmacological treatments for sarcopenia, 3 trials are discussed; two of which reported null results, while a 3rd reported

significantly greater improvements on the 6-minute walk test in the ACE inhibitor group, which may indicate increased muscle performance[206]. However, this could be due to cardiovascular affects and not improvements in skeletal muscle per se[206]. Other pharmacological agents discussed in this review include statins, beta-blockers, vasodilators and anti-inflammatory drugs. There is insufficient evidence regarding these drugs and their effects on muscle performance[198,206]. Larger, double-blind placebo clinical trials are needed to determine the effects of ACE inhibitors and other medications on muscle performance. These trials should also examine dose response effects and not just effects in general. Cardiovascular measures such as blood-pressure and ankle arm indexes, nerve function, blood lipid panels, metabolic profiles and inflammatory marker levels should be collected in these trials to examine underlying factors associated with improved muscle performance and medications.

The most well-documented and perhaps the most effective way to improve muscle performance, mainly strength, is resistance training. This is exemplified in an exhaustive 2009 Cochrane review by Liu and Latham et al. in which 121 randomized controlled trials of resistance training in older adults were reviewed[194]. The authors of this review found a large standard mean difference (SMD), or effect size, when comparing resistance training groups to control groups. In the 73 trials that were equipped to measure this, the SMD was calculated to be 0.84 (95% CI 0.67 to 1.00) using a random effects model. This indicates that the average person in a resistance training group showed larger improvements in strength than 79% of those in the control group. To minimize heterogeneity, the authors only used strength data concerning the most common muscle group used, which was the leg extensor, when calculating this SMD. This review also noted that high vs. low intensity resistance training resulting in slightly greater improvements in strength[194]. Also, of particular interest, this review found that 10 studies

(n=487) compared the effect of resistance to aerobic training on muscle strength and found that resistance training improves muscle strength to a greater degree than aerobic training. Also of interest, in the Lifestyle Interventions and Independence for Elders pilot (LIFE-P) study, the intervention group, which consisted mainly of over ground walking, supplemented with lower extremity resistance and balance training, lost knee extensor strength, but to a significantly lesser degree than the control group[148]. This loss in strength did not translate into overall loss of physical function or mobility[225]. Finally, strength training is also recommended to older adults by the American College of Sports Medicine[226]. Data concerning very old (aged ≥80) are lacking, however there have been a 2 trials in conducted in populations of older adults in their eighties. These studies both show that plasticity of skeletal muscle may diminish significantly in the 9th decade of age, with muscle showing a blunted response, compared to younger older adults, to training in both studies[227,228]. More work regarding those in their 9th decade of life and older is needed.

Specific interventions to improve muscle power have also been conducted. These interventions focus more on the speed of the exercise rather than the resistance. That is exercises are performed with lower resistance with an emphasis on speed[229]. Lower resistance exercises would induce less stain on muscle and joints may be particularly appropriate for older more frail adults. These interventions have been shown to increase both strength and power in older adults. A meta-analysis conducted by Tschopp et al. actually reported greater improvements to muscle strength and power with power training as opposed to resistance training[230], but results in the literature are conflicting[77,229]. It is important to note that with both traditional resistance training and power training, both strength and power will increase to some degree, as strength (force) is one of the two components of power, the other being velocity.

1.2.6 Changes in Muscle and Physical Performance: Its' not only the Mass

In Health ABC, Goodpaster et al. showed that change in muscle mass was only able to explain ~5% of the variability in strength decline[83]. A similar finding was presented by Hughes et al. where age-associated changes in muscle mass explained about 5% of the variance in the change in knee extensor strength decline[124]. Furthermore, Fleg et al. has shown, in the BLSA, that maximum aerobic capacity (VO_2 peak), normalized to fat-free mass, decreases with age at similar rates across those who report low, intermediate and high levels of physical activity[231]. That is, although those with high levels of physical activity start and end with higher levels of aerobic capacity, the rate of decline in aerobic capacity normalized to lean mass and across physical activity group. This suggests that the rate of decline in aerobic capacity cannot be fully explained by changes in lean mass and lower levels of physical activity. For these reasons it is important to examine other physiologic changes that occur with age. Mitochondrial function and biogenesis may be an important contributor to declining muscle and physical function with age. This is because the ability of skeletal muscles to extract and use the oxygen to produce energy is one of two major components of VO_2 peak. Additionally, mitochondria produce ~90% of the energy required for movement[7]; therefore, declines in mitochondrial function may be of major importance in explaining the loss of muscle performance and physical function with age.

1.2.7 Changes in Mitochondrial Energy Production with Age

The mitochondria as it exists today is thought to be the result of evolution and a symbiotic relationship between ancient bacteria and eukaryotes[232]. Mitochondria allowed for enhanced energy production via oxidative phosphorylation in ancient eukaryotic cells. Mitochondria

contain their own DNA (mtDNA), which is inherited maternally and includes genes that encode for proteins involved in oxidative phosphorylation, the mitochondria's energy producing process. Mitochondria are the primary producers of adenosine triphosphate (ATP) in the human body. In fact, mitochondria have been shown to produce about 90% of the energy needed for human ambulation[7]. This dissertation focuses on this energy production and how decreases in mitochondrial energy production may affect fatigability and physical function in older adults.

Skeletal muscle mitochondrial function is extremely complex and regulated at multiple biologic levels, including proteins encoded by both nuclear and mtDNA. For example, the replication, maintenance, and transcription of mtDNA is controlled by transcription factor A, mitochondrial (TFAM) and TFAM is regulated by nuclear transcription factors (NRF1, NRF2), which also regulates the expression of nuclear genes encoding mitochondrial proteins[233,234]. (PGC-1α) regulates NRF1 and NRF2 and is a key regulator of energy metabolism[235]. Additionally, alterations to lipid-membrane health and other factors including calcium release from the sarcoplasmic reticulum to maintain the energy gradient needed for proper function of the respiratory chain and oxidative phosphorylation can affect mitochondrial function[236]. Interestingly, Amara et al. found mild uncoupling in aged skeletal muscle, which did not impact ATP production in the tibialis anterior muscle and this uncoupling was hypothesized to be a compensatory mechanism protecting against oxidative damage from ROS production[237]. However, larger uncoupling was observed in the first dorsal interosseus muscle, which resulted in lower ATP production[237]. Thus, there are many factors they may affect and lead to dysfunctions in ATP production with age. A few of these are discussed in the section after next, but an in-depth discussion of the biochemistry and regulatory process and age-related changes to these processes is beyond the scope of this epidemiologic dissertation. This dissertation focuses

on the culmination of these changes, impaired ATP production, and whether or not decreases in mitochondrial ATP production affect physical function in older adults. It should be noted that mitochondria may be implicated in declining physical function with age by processes not directly related to ATP production, such as apoptosis. Mitochondria play a large role in regulating apoptosis and there is some evidence that apoptosis, induced by mitochondrial dysfunction, may lead to nuerodegeneration and declines in skeletal muscle mass with age[238,239]. Reviews of this can be found elsewhere[238-241].

Researchers have arrived at conflicting results regarding the independent relationships between age and physical activity level with mitochondrial function. Some research has shown an independent decline in mitochondrial function with age, while others have argued that these declines are simply a function of lower physical activity levels. These studies are described in Table 3 and in the following section.

Several studies using both in vitro and in vivo methods (information regarding measurements of mitochondrial function can be found in section 1.2.9), have shown that mitochondrial function decreases with, despite older and younger subject having similar physical activity levels. Conley et al. showed a significant cross-sectional difference in mitochondrial function and mitochondrial volume density in older compared to younger participants using ^{31}P MRS[156]. This difference was said to be independent of activity, as all participants were recreationally active, although there were no data presented to support this. Furthermore, Coggan et al. showed, using ^{31}P MRS dynamics, in activity matched older and younger adults, that older adults had significantly lower mitochondrial function. Short et al. showed a significant relationship ($r = -0.51$, $p=0.001$) between citrate synthase activity (CS), which is a marker of oxidative capacity and mitochondrial content, and age in a study consisting of 86 females and 60

males age 18-89. This same study showed a significant correlation ($r=-0.55$, $p<0.001$), between maximal ATP production, measure with Firefly luciferase, and age. Furthermore, Lanza et al. showed that regular endurance training only partly attenuates age induced declines in mitochondrial function (CS activity) in a cohort of young trained and untrained compared to older trained and untrained adults [242]. However, Lanza reported that MAPR was lower in older untrained compared to younger untrained individuals, but similar in older trained and younger trained participants. This evidence supports the notion that the aging process has detrimental effects on oxidative metabolism, independent of physical activity level, but physical activity is largely responsible for age-related declines in mitochondrial function.

Other research has shown that when physical activity level is adjusted for, no independent age effect on mitochondrial function exists. Brierly et al. revealed that when physical activity was adjusted for, overall *in vitro* mitochondrial respiratory activity was not significantly associated with age [243]. This same research group showed similar results in a study comparing mitochondrial function in young athletes (22-33 years) with older athletes (61-68 years) [244]. In this study, no difference in CS activity was observed between young and older athletes. It should be noted that physical activity was assessed via questionnaire in the two previously mentioned studies. Furthermore, no independent age effect on mitochondrial function was observed in a study of young and older participants with similar physical activity levels [245]. In this study by Kent-Braun and Ng, physical activity was measured objectively by accelerometry and mitochondrial function of the tibialis anterior was measured by ^{31}P MRS. Similar results using ^{31}P MRS and accelerometry have been observed by Lanza et al. Interestingly, Larsen et al. showed that mitochondrial function, measured by ^{31}P MRS, in the vastus lateralis (quad) but not the tibialis anterior was lower in older compared to younger

participants matched for physical activity levels[246]. In fact, this studied showed that mitochondrial function in the tibialis anterior was actually higher in older compared to younger adults. These and other studies are depicted in Table 3.

To summarize, cross sectional studies have led investigators to arrive at conflicting conclusions. One possible explanation for the conflicting results is that most of these studies assessed physical activity levels via questionnaire or self-report, with only a few of the cross-sectional studies implementing an objective measure of physical activity[245]. Additionally, the studies reporting no age-related changes in mitochondrial function, in participants matched for activity or when activity was adjusted for, had small sample sizes ranging from comparing 20 younger adults to 19 older adults to comparing 9 younger to 12 older adults. These studies may not have been adequately powered to detect differences. Conversely, the study by Conley et al. compared 9 younger to 40 older adults and the study by Short et al. contained 146 total participants; both of these studies detected age-related differences. Furthermore, studies in the oldest of older (aged ≥80) and frail adults are lacking. Studies including older more frail individuals may show greater age related declines, however these declines may be attributable to inactivity and disuse. Studies including lower function individuals would yield important information regarding declines in mitochondrial function. Perhaps a threshold of mitochondrial dysfunction exists, where mitochondrial function ceases to decline, while function continues to deteriorate. Conversely, mitochondrial function may continue to decline even in the oldest and frailest of older adults. Research is needed establishing subsets of older adult in which mitochondrial function may be a particularly limiting factor.

Additionally, studies examining declines in mitochondrial function with age measured different muscle groups, either the quad or vastus lateralis or the gastrocnemius or tibialis

anterior; and as illustrated by the Larsen study, age-related changes may differ between muscle groups. Finally, all these studies were cross-sectional; therefore they are subject to inherent biases. For example, healthy older adults are more likely to volunteer for studies, especially those involving muscle biopsy or performing an exercise bout in an MR magnet for ^{31}P MRS studies. Therefore, the older adults in these studies were healthier and may have experienced less mitochondrial dysfunction than the average older adult.

Despite differences in methodologies, conclusions and the recall bias introduced by subjective measures of physical activity, important conclusions can be drawn from these cross-sectional studies. It appears as though there are age-related declines in mitochondrial function and that physical activity is a very strong mediator in the relationship between age and mitochondrial function. An extensive review of studies examining the impact of age and physical activity of both enzymatic activity and 31P MRS parameters of mitochondrial function has been published by Russ and Kent-Braun[247]. In summarization, it seems as though both conclusions are partly right – independent age-related declines in skeletal muscle mitochondria exist and engaging in regular physical activity can attenuate these declines.

Additionally, mitochondrial dysfunction may be a contributor to as well as a consequence of age-related declines in physical activity. For example, mitochondrial dysfunction may lead to an increase in fatigability or exercise intolerance, which may in turn lead to a decrease in activity level in order to keep fatigability at a minimum. There is also evidence that skeletal muscle mitochondrial dysfunction activate apoptotic pathways; activation of these pathways may lead to nuerodegeneration and could contribute to declines in muscle mass with age[240,248]. These changes may also result in a decrease in physical activity level. Longitudinal studies measuring mitochondrial function both in vivo and in isolated mitochondria from muscle biopsy and that

include objective measures of free-living physical activity for a period of time (e.g. 7 days) are needed to truly established the relationship between age and mitochondrial function and how physical activity mediates or affects this relationship.

Table 3. Effects of Age and Physical Activity on Mitochondrial Function

Study	Y Age	O Age	Y N/Sex	O N/Sex	Muscle	Mito Parameter	PA Measure	Difference Between Y and O	Effect of PA
Short[249]	18-89		86F	60M	VL	CS activity MAPR	Excluded if active	CS - R_{age} = -0.51, p=0.001 MAPR – R_{age} = -55, p<0.001	Participants matched for PA
Conley[156]	39±8	69±6	6M 3F	18M 22F	Quad	PCr Recovery (mM ATP/s)	All considered recreation-ally active	O 47.4% < Y, S	Not directly Examined
Lanza[242]	18-30	59-76	-	-	VL	CS activity MAPR	Q	MAPR OT ≈ YT MAPR OUT < YUT CS OT <YT CS OUT <YUT	Training status attenuated age affects but effects remained
Lanza[250]	22±1	75±5	8M	8M	tibialis anterior	PCr Recovery (mM ATP/s)	AC	O ≈ Y	PA: O≈Y
Lanza[251]	27±1	70±1	10F 10M	8F 10M	tibialis anterior	PCr Recovery (mM ATP/s)	AC	O ≈ Y	PA: O≈Y
Brierly[243]	21-96		51		VL	CS activity	Q	R_{age} = -0.27, p=0.07	attenuates to p=0.17
Brierly[244]	22-33	61-68	3F 6M	2F 10M	VL	CS activity	All considered highly active-Q	O ≈ Y, S	All participants highly active-attenuated age effect
Kent-Braun[245]	34±5	76±5	9F 10M	9F 9M	tibialis anterior	$t_{1/2}$ PCr Recovery	AC	OF 6%< YF, NS OM 10% < YM,NS	No difference in PA between Y and O
Coggan[252]	20-29	60-69	10F 10M	10F 10M	gastro	CS activity	None	OF 14%< YF, S OM 28% < YM, S	N/A

Table 3 Continued

Coggan[253]	YU: 25±2 YT: 27±4	OU: 63±3 OT: 62±2	YU: 6M YT: 6M	OU: 6M OT: 6M	gastro	Pi/PCr slope	Q	OU < YU, S OT < YT, S	Matched for PA
Chilibeck[254]	26±2	70±4	6F 7M	2M 8F	gastro	IT from Pi/PCr and pH	All considered moderately active – Q	IT O < Y, S	Participants matched for PA
Chilibeck[255]	28±2	67±4	4F 6M	8F 2M	gastro	Time for PCr recovery	All considered moderately active – Q	O ≈ Y	Participants matched for PA
McCully[256]	28±7	66±6	4M	1F 5M	gastro	Max PCr recovery rate (mM/kg/min)	Range of Activity levels – Q	O 32.5% < Y, S	N/A
Schunk[257]	27±4	61±6	4F 6M	11F 11M	RF	$t_{1/2}$ PCr Recovery	Survey – all not involved in formal exercise	O ≈ Y	Participants matched for PA
Young[258]	20-29	70-83	-	-	gastro	$t_{1/2}$ PCr Recovery	None	O ≈ Y	N/A
Larsen[246]	21-35	65-80	8F 8M	8F 8M	TA and VL	Max PCr recovery rate (mM/kg/min)	AC	TA O > Y VL O < Y	Participants matched for PA

Abbreviations: y= young; o= old; Mito= mitochondrial; PA= physical activity; f= female; m= male; VL= vastus lateralis; CS= citrate Synthase; Quad= Quadriceps; PCr= phosphocreatine; mM= millimolar; ATP= adenosine triphosphate; S= significant; QU= questionnaire; MAPR = Mitochondrial ATP Production Rate; OT= old trained; YT= young trained; OUT= old untrained; YUT= young untrained; AC= accelerometry; $t_{1/2}$= half-time; OF= old female; YF= young female; NS= non-significant; OM= old male; YM= young male; gastro= gastrocnemius; Pi= free phosphorous; IT= intracellular threshold; kg= kilogram; min= minute; RF=rectus femoris; TA= tibialis anterior;

1.2.8 Reasons for Age-Related Declines in Mitochondrial Dysfunction

There a few theories with supporting empirical evidence for why mitochondria may become dysfunctional with age, independent of physical activity level. The main and probably most well-known theory postulates that accumulated oxidative damage due to prolonged ROS exposure, to mitochondrial DNA and RNA results in mtDNA mutations and deletions, which lead to dysfunctional mitochondria in aged skeletal muscle. As mitochondria are the primary producers of reactive oxygen species (ROS) in humans, mitochondrial DNA are inherently exposed to more ROS than nuclear DNA. Further exacerbating the oxidative damage are the facts that mitochondrial DNA lack histones which help to protect nuclear DNA from ROS damage and that mitochondrial DNA lacks the advanced repair system of nuclear DNA. A vicious cycle of aging has been hypothesized involving ROS induced mtDNA mutations and deletions, leading to dysfunctional mitochondria that produce more ROS and thus inflicting more damage to mtDNA which then leads to further mitochondrial dysfunction and eventually cellular apoptosis[238,239,241,259]. However, data from mouse models has put into question whether or not increased ROS production in dysfunctional mitochondria is implicated in or necessary to induce more damage leading to cellular apoptosis. More recently it has been hypothesized that general electron transport chain (ETC) dysfunction, leading to energy deficient mitochondria, is the culprit of mitochondrial dysfunction induced apoptosis. This is discussed in more detail in elsewhere[241]. Regardless, mtDNA damage leading to mutations and deletions in mtDNA, initially induced by ROS exposure, seems to be a primary cause in age-related mitochondrial dysfunction and decreased energy production.

Mitochondrial membrane uncoupling also seems also increase with age in certain muscles[237] and it has been shown that permeability of mitochondrial membranes to protons increases in aged muscle tissue[260]. This uncoupling would lead to a decrease in energy and ROS production, as the energy gradient needed for proper mitochondrial function would be impaired. It has been suggested that this uncoupling may precede significant accumulations of mtDNA mutations, and has been suggested to be a compensatory mechanism to reduce ROS production and prevent oxidative damage[236].

A more recent theory as to why mitochondrial become dysfunctional with age is called "Mitochondrial-Lysosomal Axis Theory of Aging"[261]. This theory has to do with impaired mitophagy (autophagocytosis of mitochondria) and is thought to be occurring simultaneously with occurring ROS damage. Mitochondria tend to become larger with age as they become more dysfunctional. This theory basically states that because aged mitochondria are larger, produce less energy and ROS, they are less likely to be marked for autophagocytosis[262]. The thought is that because these dysfunctional mitochondrial are producing less energy, and as a result less ROS, they are less autophagocytosized because their membranes haven't undergone as much oxidative damage[261]. There are other reasons for impaired autophagy of mitochondria with age, such as an increased inefficiency of the autophagosomal-lysosomal pathway with age. This in turn leads to an accumulation of large, dysfunctional mitochondrial in aged human skeletal muscle that should have been autophagocytosized [262]. This theory is discussed in detail in two recent reviews[238,262]. Similarly, the capacity for mitochondrial fission and fusion may decline with age. Fission and fusion are vital processes to ensure the health and function of mitochondria and to maintain proper mitochondrial turnover[263].

Mitochondrial function may also decline to a decrease in mitochondrial protein content. Mitochondrial protein content has been shown to decrease with age by both decreased enzyme activity and my more direct measures. Maintaining mitochondrial protein content is important for maintaining mitochondrial function, because it is the function of these proteins that drives the energy producing processes of the mitochondria. As mentioned above, it is important for mitochondrial function to maintain proper rates of protein degradation and protein synthesis. Regulatory proteins such as PGC1α, AMPK, COX, those in the SIRT family and other may also be implicated in decreasing mitochondrial energy production with a age. Research concerning mitochondrial protein maintenance is discussed in more detail in this review[263].

Experimental evidence has shown that mutations or deletions in mtDNA do in fact lead to mitochondrial dysfunction and reductions in ATP production and subsequently activate pathways that lead to apoptosis[238,239,241,259] . For example, two labs observed three to eight fold increases in the rate of mtDNA mutations in mice where the mtDNA proofreading subunit polgA, of the mtDNA polymerase γ (pol- γ) (POLG mice) was deficient[264,265]. Subsequent impairments in ATP production in the heart and skeletal muscle were observed, along with a sarcopenic phenotype[266]. Additionally, in rhesus monkeys and rats, mtDNA deletions have been shown accumulate with age and are associated with decreased ATP production in skeletal muscle[267-269]. In humans, correlations between accumulated mtDNA mutations and deletions with a decreased capacity for oxidative capacity in skeletal muscle have been observed[270-272]. However, longitudinal evidence is needed to establish the causal role of mtDNA mutations in humans. There is also some evidence that over time, activation of apoptotic pathways, attributable to mitochondrial dysfunction, may lead to nuerodegeneration and could contribute to declines in muscle mass with age[239-241,248]. These mtDNA mutations have been shown to appear in humans between the age of

60 and 70[273]. There are several very nice reviews on the role of mitochondrial DNA mutations in apoptosis, sarcopenia and aging that discuss the potential underlying process and biochemistry in more detail[236,238,239,241,274].

1.2.9 Methods for Assessing Mitochondrial Function

Briefly, there are several markers that researchers can measure to quantify mitochondrial function including: mRNA content for mitochondrial proteins, mitochondrial RNA (mtRNA) and mitochondrial DNA (mtDNA) content, mitochondrial protein synthesis, mitochondrial enzyme activity, mitochondrial respiration and energy production. The most common markers used to measure mitochondrial function are the activity of certain oxidative enzymes including, citrate synthase, succinate dehydrogenase, cytochrome c oxidase and others. The activity of these enzymes is an indirect measure of the oxidative capacity of the mitochondria because they reflect electron transport chain (ETC) activity. They also reflect mitochondrial content. Citrate synthase is a common marker and is usually said to reflect mitochondrial content, however, some researchers use it as a measure of mitochondrial function. The important thing to note is that enzyme's such as citrate synthase are markers of mitochondrial oxidative capacity and are not directly measuring ATP production. Enzyme activity is measured *in vitro* using specific assays in isolated skeletal muscle tissue obtained from needle biopsy.

A more direct measure of mitochondrial function is mitochondrial respiration (oxygen consumption) and ATP production. Traditionally, mitochondria's ability to consume oxygen or produce ATP is measured using *in vitro* assays applied to isolated mitochondria or permeabilized muscle fibers from muscle samples. This *in vitro* technique measures the mitochondria's ability to consume oxygen or produce ATP in the presence of certain fuels. This method can directly

assess respiratory capacity at different location in the ETC and can also be used to assess uncoupling. There is also a method called Firefly luciferase, which can be used to directly measure ATP production in isolated mitochondria. Firefly luciferase uses a luminometer to measure maximal ATP production rate by catalyzing reactions[249].

The main limitations of in vivo methods are actually the parameters they are designed to measure. Their physiologic relevance, because they are conducted in isolated mitochondria or muscle tissue obtained from muscle biopsy, is often questioned. That is, how well do these markers reflect actual ATP production in vivo. Studies have shown that ATP production from ^{31}P MRS methods is closely related to oxidative enzyme activity. However, in vivo measures of ATP production are considered to be more physiologically relevant. That being said, in vitro methods provide important mechanistic information concerning mitochondrial function that in vivo methods cannot provide, such as abundance of mtDNA, deletions and mutations present in mtDNA, the activity of specific regulatory enzymes and proteins, as well ROS production and markers of apoptosis[263].

1.2.9.1 ^{31}P Magnetic Resonance Spectroscopy

This method is an *in vivo* technique which directly measures mitochondrial function noninvasively and was utilized to measure the primary outcome for two of the three papers described in this dissertation. This method is a companion technique to traditional magnetic resonance imaging (MRI) scan and maximum mitochondrial ATP production in vivo ($ATP_{max,}$ mM ATP/s produced) can be calculated. This technology involves little participant burden, whereas the traditional method of muscle needle biopsy is highly invasive, induces considerable participant burden and must be performed by a physician. Additionally, mitochondrial

measurements using biopsy may not accurately reflect actual mitochondrial function in the living human body.

^{31}P MRS measures the regeneration of phosphocreatine (PCr) after a short bout of exercise to quantify mitochondrial ATP production. PCr is a high energy phosphate that can be readily converted to ATP and vice-versa via the creatine kinase reaction (PCr + ADP H^+ ↔ ATP + Cr). PCr is the initial full-source of skeletal muscles. It has been shown that a linear relationship links change in PCr with exercise to mitochondrial oxidative phosphorylation rate[275,276]. The model shows that at full PCr depletion, skeletal muscle mitochondria should be functioning at their maximum oxidative capacity[156]. Therefore, measuring the dynamics of the PCr shuttle provides a method to estimate muscle oxidative capacity *in vivo*[156,277]. Furthermore, this method has been confirmed to be a good measure of oxidative capacity in rodent and human studies showing that ATP_{max} varies in direct proportion to the activity of oxidative enzymes in healthy muscle [278,279]. It has also been shown to reflect mitochondrial content in human skeletal muscle[280]. The amount of ATP production from glycolysis (which does not involve the mitochondria) has been shown to be minimal in resting skeletal muscle (<8%)[237]. Regardless, this method can tease out the amount of ATP production by glycolysis by determining the pH from the chemical shift of the P_i peak relative to the PCr peak[281]. H^+ ions, which would lower the pH, are a byproduct of glycolysis.

Briefly, the exercise protocol utilized by the two studies in this dissertation takes place in a typical MRI magnet. Participants lay flat on their backs with the knee of the right leg (unless contraindicated) supported so that the leg is slightly elevated at a ~30 ^{o} degree angle. Straps are place over their legs and a 2.5" surface RF coil tuned to ^{31}P is placed over the right quadriceps. At two different points during the scan, they are asked to kick repeatedly as hard and as fast as

they can, producing fast contractions at the highest rate possible for ~30s, followed by a 6 minute rest period. The protocol is designed to deplete PCr stores by at least 33-66% without lowering pH. PCr recovery rate is measured during this rest period. A figure depicting a cross-sectional spectra and PCr depletion and recovery can be seen in Figures 2 and 3.

This method has several strengths. First, as mentioned previously, this technique involves little participant burden compared the traditional method of muscle needle biopsy, which is highly invasive, induces considerable participant burden and must be performed by a physician. It is a measure of functional mitochondrial performance in the sense that it is a measurement of what is actually going on in living tissue as opposed to mitochondrial performance under perfect conditions in mitochondria isolated from skeletal muscle. This technique has also been shown to be both valid and reliable and has been confirmed to be a good measure of oxidative capacity in rodent and human studies showing that ATP_{max} varies in direct proportion to the activity of oxidative enzymes in healthy muscle [278,279]. It is also easy to obtain a measure of muscle mass or volume and fat depots by implementing a MR image sequence at the end of the protocol. This method can also be paired with oxygen occlusion measurements, to study the effects of oxygen delivery on mitochondrial function. Similarly, near infrared techniques can be used to measure oxygen delivery to skeletal muscle; these techniques can be used to measure in vivo oxygen delivery to skeletal muscle and can be used to identify pathologies underlying mitochondrial dysfunction.

This technique has limitations as well. First, the actual mathematical modeling techniques used to glean important physiologic information from the spectra can be questioned. Secondly, participants with bilateral knee replacements cannot participate, even if these replacements are MR safe, the implants interfere with spectra acquisition. Claustrophobia is also exclusion, as

participants of average height are almost fully position in the bore of the magnet. Additionally, participants with tattoos or any MR unsafe implants would be excluded from participation. This particular exercise protocol may not be conducive to more frail older adults, as they are required to kick as hard and as fast as they can for 36s, which may not be possible in the oldest and frailest of adults. However, this method has been conducted using electrically stimulated muscle contractions, which may provide a way to implement this in extremely disabled populations. This method is subject to all other exclusions associated with MRI, such abundance of mtDNA, deletions and mutations present in mtDNA, the activity of specific regulatory enzymes and proteins, as well ROS production and markers of apoptosis or efficiency cannot be measured using this technique.

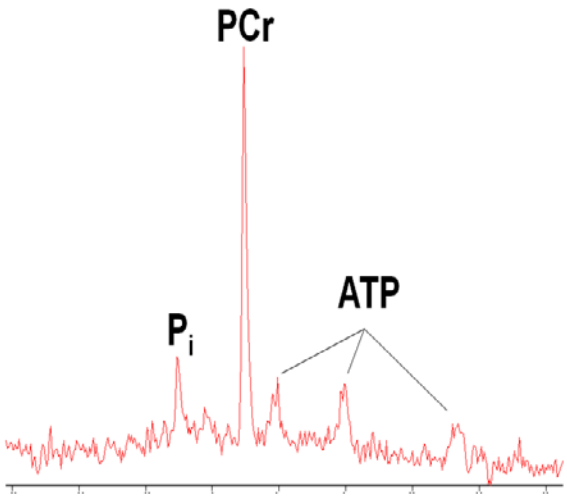


Figure 2. Cross-sectional 31P MRS spectra with Labeled Free Phosphorous (P_i), PCR and ATP Peaks

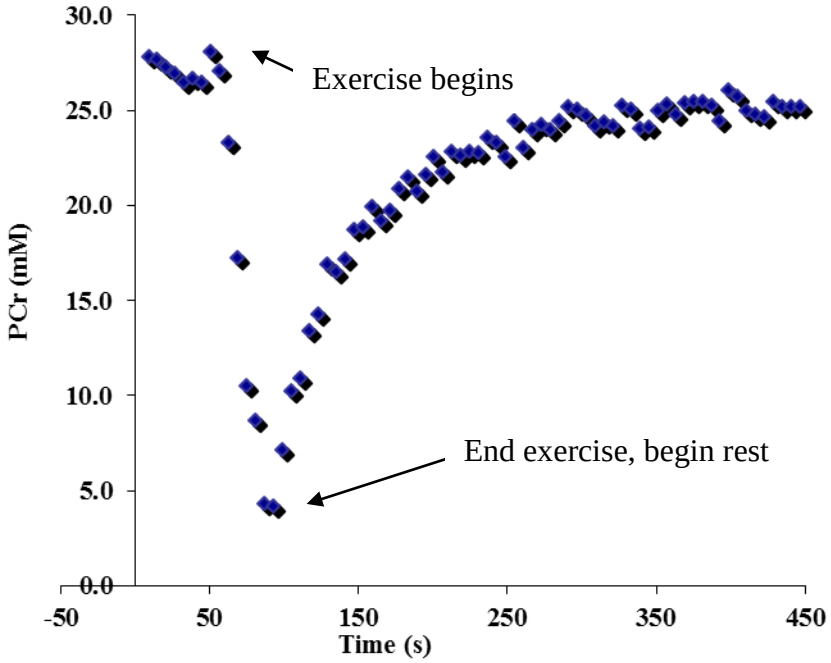


Figure 3. Longitudinal Depiction of PCr Dynamics at Rest, During Exercise (Kicking Bout), and during Recovery

1.2.10 Other Factors that may potentially affect Mitochondrial Function

Several other health factors and diseases, other than specific mitochondrial disorders and diseases, have been shown to negatively affect mitochondrial function. This information is presented below. The administration of antiretroviral medication in HIV patients has been shown to negatively affect mitochondrial function by affecting the replication of certain genes involved in glycolysis and lipid oxidation which can lead to mitochondrial dysfunction[282,283]. Additionally, it has been shown in vitro that HIV patients have a significantly lower ratio of mitochondrial to nuclear DNA in the peripheral blood cells than non-infected controls[284]; which suggested mitochondrial necrosis due to HIV viral infection.

Mitochondrial dysfunction has been hypothesized to be both a cause and effect of certain neurological disorders including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and Huntington's disease[248]. It has been shown that specific interactions and overlap exist between disease related proteins and proteins involved in mitochondrial function[248]. Mitochondrial respiration, in mice with advanced peripheral arterial disease (PAD) was shown to be lower when compared to mice with PAD[285]. Participants with multiple sclerosis (MS), have been shown to have slowed mitochondrial biogenesis using 31P MRS compared to controls without MS; indicating impaired oxidative capacity of skeletal muscle in those with MS[286]. It is fairly well established that mitochondrial function is lower in individuals with worse insulin resistance, type 2 diabetes and obesity[287-290] and similar to neurological diseases – mitochondrial dysfunction has been hypothesized to be both a cause and effect of insulin resistance and type 2 diabetes. Severe burn trauma has been shown to result in mitochondrial dysfunction[291]. Patients with severe chronic heart failure have been shown to have 20% lower mitochondrial volume and surface density of mitochondrial cristae as well as significantly

decrease cytochrome oxidase activity, indicating lower mitochondrial function compared to health participants[292]. Mitochondrial function in circulating lymphocytes and in platelets was shown to be lower in smokers compared to non-smokers[293,294]. In the study examining lymphocytes, participants were matched for age, sex and physical activity level[171].

Alcoholism, specifically the effects of ethanol, has been shown to induce morphological changes in skeletal muscle mitochondria[295]. It has also been shown that men experience greater mitochondrial dysfunction than women. This is because mitochondrial in women produce less ROS than men, due to the mediating role of estrogen[**296-299**]. This work has mostly been conducted in rodents and the role of estrogen in regulating mitochondrial function is relatively poorly understood[296]. However, some studies have shown no difference in oxidative stress and mitochondrial function between men and women in both human and rodents[298,300].

The effects of current and previous smoking and alcohol exposure as well as some of the health conditions mention that may affect mitochondrial function into old age should be examined in large epidemiologic studies of aging such as Health ABC and BLSA. Previous work in this area as tended to be in rodents or in very small samples of humans or in specific diseased populations. These large epidemiologic prospective cohort studies should begin to add measures of mitochondrial function. Implementing noninvasive techniques, such as ^{31}P MRS (which will be discussed in great detail later), might be the best approach, as noninvasive techniques would be more conducive to an older adults population as opposed to the more invasive techniques of muscle biopsies. However, ideally, both noninvasive and biopsy techniques would be implemented, as certain things, such as such as abundance of mtDNA, deletions and mutations present in mtDNA, the activity of specific regulatory enzymes and proteins, as well ROS production and markers of can currently only be measured in isolated mitochondria. Either way,

measures of mitochondrial function should start being implemented in longitudinal prospective cohort studies, especially those that will follow or are following participants into old age.

1.2.11 Physical Activity to Improve Mitochondrial Function in Aged Skeletal Muscle

The following section will briefly describe the population, mode of intervention and pertinent results for exercise intervention trials designed to measure change in mitochondrial function in older adults. It is important to describe the population and mode of intervention used in each trial, as these aspects may have immense implications regarding the observed changes in mitochondrial function. This information can also be found in Table 4. It is important to note that mitochondrial function refers to the capacity of the mitochondria to produce energy in the form of ATP and whereas mitochondrial biogenesis refers to the production of new mitochondrial.

The first trial was designed to measure changes in mitochondrial function following either a 6-month resistance or endurance training intervention in older adults (69.2 ± 0.6 years) [301]. This study also included a control group for comparative purposes. The resistance training was primarily performed using a two-legged leg press machine and also included exercises involving the arms and shoulders. Participants in this group completed 3 sessions per week. Training intensity was increased in phases as the trial progressed; resistance was increased while the number of sets was decreased. The endurance training group also attended 3 sessions per week. They trained primarily on a stair-stepper machine and a kayaking-type exercise machine. Participants progressed as rapidly as possible to 20 minutes for each exercise and to an intensity of 80-85% of heart rate reserve. The investigators found large increases in oxidative capacity (as measured by MRS – PCr recovery rate) in both the resistance (57% increase, $p<0.05$) and endurance (31% increase, $p<0.05$) training groups. The investigators also observed a decrease in

glycolytic ATP synthesis in the endurance training group, following the intervention. This decrease in glycolytic (anaerobic) activity is indicative of an increase in oxidative energy production. Additionally, this study by Jubrias et al. showed that mitochondrial volume density (Vv(mt,f)) but not oxidative capacity per Vv(mt,f) was increased following resistance training. Conversely, in the endurance training group no increase in Vv(mt,f) was observed but an increase in oxidative capacity per Vv(mt,f) was recorded. This study established that muscle energetics can change significantly in elderly skeletal muscle following regular physical activity of different modes.

This next study included healthy men and woman with a wide age-range (21-87 years) and randomized who were randomized into either a 16-week aerobic exercise intervention or control group [302]. The 16-week intervention started with 3 sessions per week, eventually progressing to 4, and was performed on a stationary cycle. The intensity and duration of the training was gradually increased over the course of the intervention. This study was designed to measure changes in mitochondrial enzyme activity (citrate synthase (CS) and cytochrome c oxidase) and mRNA levels of genes involved in mitochondrial biogenesis (cytochrome c oxidase (COX4), NADH dehydrogenase subunit 4 (ND4), PGC-1α, NRF-1 and TFAM). This study showed that mitochondrial enzyme activity and biogenesis, indicated by higher levels of mitochondrial mRNAs that regulate mitochondrial biogenesis, increased significantly following the 16-week intervention with no age-dependent effect. Thus, the response of skeletal muscle mitochondria to physical activity was similar across all age ranges.

The primary aim of this next study was to determine the effect of an 8-week exercise program on mitochondrial function in a cohort (n=10) of older adults (71.0 ± 2.0 years) with chronic obstructive pulmonary disorder (COPD)[303]. The investigators measured mitochondrial

function *in vivo* using MRS. The primary measure of mitochondrial function was defined as PCr recovery rate. The exercise intervention consisted of cycling, walking and leg extensions. Sessions were conducted 2 times per week for approximately 75 minutes each and intensity was increased gradually. The investigators included a group of healthy control participants (n=10), who did not participate in the exercise intervention. At baseline, the COPD group showed impaired mitochondrial function as compared to the healthy control group (19 ± 8% lower, $p<0.05$). Following 8 weeks of structured exercise, the COPD group increased their mitochondrial function, as measured by PCr recovery rate, by 38 ± 12% ($p=0.003$). This study showed that with exercise, older adults with COPD can increase their mitochondrial function to equal or exceed that of healthy control participants – who did not participate in structured exercise.

Menshikova et al. conducted a study consisting of eight healthy, sedentary older adults (67.3 ± 0.6 years) deigned to measure changes in mitochondrial function following a 12-week structured exercise program [304]. Participants in this study were required to complete four to six exercise sessions weekly, of which 3 were supervised. The exercise was performed using treadmills, stationary cycles or walking outside. Intervention sessions were 30 minutes to start and progressed in both duration and intensity over the course of the trial. Mitochondrial function was measured *in vitro* in tissue obtained from muscle biopsy. Investigators measured Nicotinamide adenine dinucleotide (NADH) oxidase as a measure of overall electron transport chain activity and succinate oxidase as a measure of complex II-IV activity. The investigators also chose to examine mitochondrial function among distinct mitochondrial subpopulations within skeletal muscle, namely subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria. In this study, succinate oxidase activity increased significantly, with more prominent increases

observed in SS compared to IMF mitochondria. Overall ETC activity, as measured by NADH oxidase activity, also increased significantly following a structured exercise program.

Williams et al. conducted a trial in older adults (70±8 years) in which 7 chronic heart failure patients participated in a resistance training intervention for 11 weeks[305]. This study showed a significant increase in *in vitro* mitochondrial ATP production, CS activity, as well as increases in VO_2 peak. The investigators also showed very strong correlation between change in mitochondrial ATP production and change in VO_2 peak ($r = 0.875$; $p<0.0001$). This shows that increases in mitochondrial energy production explain about 76.6% of the variance in improvements in VO_2 peak; which suggests that mitochondrial function may play a large role in age-related declines in aerobic capacity or fitness. However, it is important to keep in mind that these are chronic heart failure patients, and these results may not be generalizable to healthier populations of older adults.

Two separate studies conducted by Parise et al. showed no improvements in CS activity following both 12-[306] and 14-weeks[307] of resistance training in older adults. However, following 14-weeks of resistance training, complex IV activity of the electron transport chain was significantly increased, which suggests an increase in electron transport chain activity without an increase in mitochondrial volume[307]. The 12-week intervention study showed a significant increase in antioxidant enzyme activity[306]. This suggests that resistance training can increase the antioxidant capacity of aged skeletal muscle. However, this study did not measure ROS production, so this may be a counter-measure to increased ROS activity, due to an increase in mitochondrial activity in response to the increase in physical activity. Future studies are needed to examine the antioxidant effects of resistance training in aged-human skeletal muscle. It is worth noting that in POLG mice (described previously), who possess a defect in the mtDNA

proofreading enzyme polymerase gamma, endurance training has been shown to induce an extraordinary phenotypic rejuvenation of many mitochondrial processes. These included increases mitochondrial biogenesis (production of new mitochondria), prevented mtDNA depletion and mutations compared to unexercised POLG mice, increased mitochondrial oxidative capacity and prevented pathological levels of apoptosis[308]. These results are very promising and suggest that participating in endurance exercise can prevent age-related mitochondrial function caused by mutations and deletions to mtDNA. These findings need to be replicated in humans.

Additionally, other studies have shown an increase in markers, specifically cytochrome c oxidase (COX) and PGC1-α, of marker of mitochondrial biogenesis following both endurance training. In fact, Konopka et al. actually showed a decrease in PGC1-α protein content[309], while Short et al. showed an increase PGC1-α mRNA level[302]. However, both of these studies found an increase in COX-4 levels, suggesting an increase in mitochondrial biogenesis. Perhaps PGC1-α is not necessary to induce mitochondrial biogenesis in aged human skeletal muscle[309]. Marcuello et al. found increased in PGC1-α and a decrease in the ratio of mtDNA/nDNA [310]. They suggested that this decreases in mtDNA/nDNA may be a signaling mechanism to induce mitochondrial biogenesis. This finding concerning an increase in COX levels and activity is interesting, as COX negative fibers are one of the hallmark manifestations in ragged red skeletal muscle fibers present in those with mitochondrial myopathies[311]. This is further evidence that exercise training can rejuvenate mitochondria in aged skeletal muscle.

In summary, increases in *in vitro* mitochondrial enzyme activity[302], *in vivo* oxidative capacity ($PCr_{1/2t}$), mitochondrial volume [301], rate of PCr recovery [303] and mitochondrial mRNAs [302] were all reported. Participants were primarily healthy older adults in their 60s and 70s. The

studies in Table 4 indicate that different aspects of mitochondrial function ranging from the mtDNA to the energy (ATP) production level can be improved with increased physical activity. There is also evidence that physical activity may increase mitochondrial biogenesis in human skeletal muscle, indicated by increases in PGC-1α and COX-IV mRNA and protein levels. The work Parise et al. also suggests that resistance training may covey certain antioxidant benefits in aged human skeletal muscle. However, this needs to be studied in more detail, employing direct measures of both antioxidant enzyme activity and ROS production. Studies that include lower function and older adults aged 85 and older are also lacking. Studying skeletal muscle energetics in lower function and older adults aged 85 and older is important. Studies in lower function and older adults aged 85 should examine whether or not mitochondrial function is lower than adults in their 60s and 70s and how it affects physical performance.

As illustrated in table 4, improvements in mitochondrial function were achieved despite differences in both mode and intensity of the physical activity interventions. These trials suggest that mitochondrial function can be improved with a resistance, endurance or combination intervention. Jubrias et al. showed that mitochondria respond differently to resistance training and endurance training [301] (described above). However, overall oxidative capacity measured by PCr recovery was increased in both groups but to a greater degree in the resistance training group. Mitochondrial response to different modes and intensities of activity needs to be studied in more detail. In summary, it is clear that both mitochondrial content and function can improve with different modes, intensities and durations of exercise training in the elderly, but studies in the oldest (age ≥85) and frailest of older adults are lacking.

Future studies should include both *in vivo* and *in vitro* measures of mitochondrial function and incorporate the most valid and up-to-date methods of data collection and

calculation. Including both *in vivo* and *in vitro* measures of mitochondrial function will allow researchers to obtain information concerning both physiologically relevant ATP production, as well as underlying mechanisms impacting in vivo energy production. Including in vitro measures will permit the quantification of changes to mtDNA, markers of antioxidant activity, ROS production and apoptotic activity following exercise. Also, the composition of the inner mitochondrial membrane needs to be studied in further detail, as it has been shown that permeability of mitochondrial membranes to protons increases in aged muscle tissue [260]. This could further contribute to decreases in ATP production with age, as a stable proton gradient is imperative for proper oxidative phosphorylation activity.

It is imperative that any future longitudinal or controlled trials include validated performance measures of physical function. There have been no trials relating improvements in mitochondrial function to improved mobility or physical function. There have only been three studies that have examined the cross-sectional relationship between mitochondrial function and physical function in older adults[312-314]. All three of these found a positive relationship. Longitudinal studies that incorporate the many aspects of mitochondrial function, from mtDNA to energy (ATP) production are needed. These studies are needed to determine which changes in mitochondrial function occur the most rapidly and have the highest potential to cause cellular damage. Intervention studies incorporating a variety of mitochondrial measures would provide insight into which of these aspects are the most preventable or reversible and the most closely associated with improved physical function. Finally, there is a great need for additional intervention studies designed to measure changes in mitochondrial function following different modes and intensities of physical activity. For example, perhaps a period of resistance training, resulting in increased mitochondrial biogenesis and volume, followed by a period of aerobic

exercise to train these mitochondrial would be ideal. It is vital that researchers design practical interventions to maximize the generalizability of their studies. Interventions that include exercises or interventions that can be conducted in the home or include over-ground walking are ideal.

Table 4. Physical Activity to Improve Mitochondrial Function and Markers/Genes of Mitochondrial Function

Author	Age	N	Sex	Mode of Intervention	Length of Intervention	Mitochondrial Measurement	Muscle	Percent Change
Jubrias[301]	69±0.6	17M, 23M		Resistance Training Endurance Training	6-months/3x per week	31P MRS, PCr recovery rate	VL	+57% ($p<0.05$) +31% ($p<0.05$)
Mckeough[303]	71±2	6M, 4F w/ COPD		Cycling, walking and leg extensions	8-weeks/2x per week	31P MRS, PCr recovery rate	quad	+38% ($p<0.05$)
Short[302]	21-87	65	M,F	Cycling	16-week/3x per week	CS COX activity mRNA Levels: COX4, ND4, PGC 1α, NRF-1 and TFAM	VL	CS +45% COX +76% COX4/ND4 +66% PGC 1α +55% NRF-1 +15% TFAM 85%
Menshikova [304]	67±0.6	5M, 3F		Treadmill/out-door walking and cycling	12-weeks/4-6x per week	NADH Oxidase and Succinate Oxidase Activity	VL	NADH +50% SO +62%
Williams[305]	67±9	7 CHF patients	n/a	Resistance Training	11-weeks	ATP Production rate and CS activity	VL	MAPR +30% ($p<0.05$) CS +40% ($p<0.05$)
Parise[307]	68±5	15m, 15F		Resistance Training	14-weeks/3x per week	CS activity	VL	8.2%, NS
Parise[306]	71±7	12M		Resistance Training	12-weeks/3x per week	CS activity	VL	-3.1%, NS
Puente-Maestu[315]	67±8	11w/ COPD	n/a	Cycling	1-2-weeks	mtDNA/nDNA ratio PGC-1α mRNA content	VL	Decreased mtDNA/nDNA Increased PGC-1α mRNA
Konopka[309]	70±2	9F		Cycling	12-weeks	COX IV protein, TFAM mRNA and PGC-1α protein	VL	COX IV +33% PGC-1α -20% No change in TFAM mRNA

Abbreviations: N= number; m= male; x= number of times; PCr= phosphocreatine; VL= vastus lateralis; P= phosphorous; MRS= magnetic resonance spectroscopy; quad= quadriceps; f= female; CS= citrate synthase; COX= cytochrome c oxidase; mRNA= Messenger RNA; ND4= NADH dehydrogenase subunit 4; PGC 1 α= Peroxisome proliferator-activated receptor γ coactivator 1; NRF-1= nuclear transcription factor1; TFAM= transcription factor A, mitochondrial; NADH= Nicotinamide adenine dinucleotide; SO= succinate oxidase; mtDNA= mitochondrial DNA; nDNA= nuclear DNA; ATP= adenosine triphosphate;

1.3 FATIGUE AND FATIGABILITY IN OLDER ADULTS

This dissertation deals directly with fatigability, whole-body fatigue, referenced to an activity of a specific intensity and duration. The term whole-body is used here to emphasize that fatigability, in the context of this dissertation, is an overall feeling of fatigue following a specific activity of a specified intensity and duration, as opposed to skeletal muscle fatigability. A review of muscle fatigability in aged skeletal muscle can be found here[316]. Most of the research concerning fatigue in older adults has focused on global fatigue, which can be thought of as a general overall lack of energy, not referenced to a specific task or activity. Thus, the epidemiology of global fatigue will be summarized briefly. Additionally, the rationale for the field moving towards measuring fatigability in lieu of global fatigue, especially when interested in physical performance and disability, will be discussed.

1.3.1 Epidemiology of Fatigue in Older Adults

Fatigue is common complaint among older adults. However, prevalence rates vary considerably from as low as 5% to as high 68%, mostly due to the lack of a standardized measurement tool and definition[11,317-319]. Fatigue has been defined in a number of ways. These include, a sense of diminished energy and the increased need to rest, perceived lack of physical energy and weariness resulting from exertion and subjective lack of physical and/or mental energy, or excessive weakness or tiredness, perceived to interfere with usual and desired activity[11,12]. In a study by Hardy et al. in community dwelling older adults, 43% reported tiredness, 40% reported

sleepiness, 42% reported a lack of energy and 29% reported weakness[320]. For the purposes of this dissertation, these definitions can be thought of as global fatigue. As mentioned previously, global fatigue is measured using a variety of instruments, batteries and questions[11,238,239,319]. A review of tools used to measure global fatigue can be found elsewhere[321,322].

Fatigue is a common symptom of many chronic and acute illnesses[323,324]. Specifically fatigue is a symptom of cancer, inflammatory or auto-immune disorders, stroke, cardiorespiratory disorders and neurologic diseases[11]. In older adults from the Jerusalem Longitudinal Study of Aging, those with who report fatigue were also more likely to report poor sleep, lower levels of physical activity, back or joint pain, impaired cognitive function, depression and had less education[318]. A majority of fatigue can be attributed to underlying diseases; however a large portion cannot[9]. Therefore, there are also central causes that have been hypothesized to cause fatigue in older adults. These include but are not limited to neurotransmitter deregulation, inflammation and oxidative stress[11]. This dissertation will examine the relationship between fatigability and mitochondrial function in older adults, as mitochondrial dysfunction may underlie age-related increases in fatigue and fatigability. This relationship will be discussed in more detail in the section after next.

1.3.2 Fatigue vs. Fatigability and the Disablement Pathway

Research has shown that fatigue is significantly related to physical function and disability independent of disease status[8]. More recently, it has been suggested that researchers attempting to understand the relationship between fatigue and physical function should measure fatigability as opposed to fatigue[12]. As defined previously, fatigability is a whole-body sensation induced by an activity of a specific intensity and duration, e.g. brisk walking for 30 minutes. It has been

suggested that referencing fatigue to specific activities is necessary to control for self-pacing bias. Eldadah uses the finding by Willis et al., who showed that adults are likely to select a preferred walking speed that is very close to minimum levels of perceived exertion as an example of self-pacing[325]. Similarly, older adults may titrate their activity levels to minimize their fatigability levels. Self-pacing bias may explain why no consistent relationship between fatigue and age has been established, as some research shows an increase[318,326,327], no change[328] and some even a decrease[319,329] in global fatigue levels with age.

In the same vein, fatigability, as opposed to global fatigue, may be a better indicator of the degree to which an individual is limited functionally due to fatigue. The following example can be used to illustrate this. An older adult master's athlete who regularly participates in athletic competition and an older adult who is mostly sedentary may both report usual energy levels of 9 out of 10 or neither may report a lack of energy. Then, when the relationship between fatigue and physical function is examined, it is possible that none would be observed. If fatigability were measured, in lieu of global fatigue, e.g. by asking how fatigued would you feel after walking 30 minutes at a brisk pace, it is unlikely the two individuals would give the same response. As a result, a relationship between fatigability and physical performance may in fact be observed and greater insight into the degree to which fatigue is limiting one functionally can be ascertained. Similarly, fatigability's role in the disablement pathway may be in contributing to lower levels of activity as an attempt to limit one's usual fatigue level. This would then lead to lower levels of fitness, and in turn lead to functional decline and ultimately physical disability. While fatigability has been shown to be associated with lower levels of fatigability cross-sectionally[330], longitudinal studies are needed to established temporality and causality.

Fatigability may also be an important domain to measure in intervention studies, lifestyle or pharmaceutical[12]. It is possible that an intervention may appear to increase global fatigue levels, which would then deem the intervention as having a side-effect of fatigue. However, if the intervention is also aimed at increasing activity levels or improving energy levels or pain with the goal of increasing activity or function, this increase in fatigue may be an artifact of increased activity. Conversely, this intervention may have actually decreased fatigability, or increased exercise tolerance, which would be a positive effect of the intervention[12]. A more accurate measure of the degree to which one is limited functionally can be ascertained if investigators measure fatigability, in lieu of global fatigue. Furthermore, fatigability may also be a more useful parameter to determine, because if one is able to tolerate more activity, one may be more likely to do more activity. Increasing physical activity levels has many well documented benefits, including improved and preventing declines in muscle performance and physical performance[148,194,199,331]. Longitudinal studies are needed to determine if lowering fatigability levels results in a lasting increase in physical activity levels.

Few intervention studies have actually examined fatigability in older adults. However, there have been some that have measured changes in fatigue in older adults. For example, the effects of L-carnatine, which is a component of the inner mitochondrial membrane that facilitates beta oxidation of fatty acids, on change in fatigue measured by questionnaire have been studied. L-carnatine supplementation has been shown to decrease fatigue in centenarians[332] and in older adults meeting criteria for chronic fatigue syndrome[333]. Interestingly, the l-carnatine group in the centenarian study significantly improved their 6-minute performance and those with chronic fatigue syndrome (CFS) significantly improved their self-reported physical function. Additionally, 12-weeks of lipid replacement antioxidant therapy (specifically a dietary

supplement called NTFactor®) has been shown to decrease fatigue levels by about 35.5% on the Piper Fatigue Scale in older adults (age 68.9±4.2) who had moderate to high levels of fatigue at baseline[334]. Interestingly, mitochondrial function, determined by transport and reduction of the dye Rhodamine-123, was also increased by 23.7% in these participants[334]. Yoga has also been reported to decrease fatigue to a greater degree than walking and usual care[335]. Fatigue was measured using the SF-36. Eldadah also reported that no differences in change in self-reported fatigue between the exercise group and successful aging health education control group in the Lifestyle Interventions and Independence for Elders Pilot Study, were observed after a year[12]. However, none of these studies measured fatigability. Future intervention studies in older adults should, if possible, employ measures of both performance and self-reported fatigability.

There are several ways to measure fatigability including performance, perceived and a combination of the two, which will be referred to as perceived performance fatigability. First, there is self-reported fatigability. This would be a questionnaire that asks how fatigued an individual feels after activities of specific intensities and duration. There are currently no published and validated instruments to measure self-reported fatigability in older adults. Although one has been developed and validated by our group at the University of Pittsburgh and should be in press this year, 2013[336,337]. There are however, examples of a few instruments that are designed to measure fatigability in other populations or that measure a certain domain of fatigability, but fail to specify intensity or duration of activities. Avlund et al. developed a tiredness scare (MOB-T), which asks participants if they get tired while performing each of 15 ADL activities[338]. While these activities all correspond to functional limitations from which older adults may suffer, the MOB-T is probably inappropriate for high functioning older adults. Also, questions are simply answered with a yes or no response, which limits the ability of the

instrument to establish degrees of fatigability. Similarly, the Dutch Exertion Fatigue Scale asked participants if they feel fatigued after conducting 9 different tasks[339]. However, only 2 of these 9 items have a duration attached to them and all of the tasks are subject to self-pacing biases as intensity is not specified. The Situational Fatigue Scale (SFS) contains well-designed questions to assess fatigability; however, this questionnaire was designed for younger populations, as its 15 items included activities such as ball sports and jogging[340]. The SFS also contains questions that do not specify intensity, which is a major component of fatigability and helps to eliminate self-pacing biases.

Fatigability can also be measured following a standardized performance measure and is defined as perceived performance fatigability. For example, Schnelle et al. established a measure where the participant is asked how tired they feel after walking on a treadmill at their self-selected pace for 10 minutes[330]. Their tiredness level was then divided by the amount of distance they walked, which yielded a fatigability score[330]. Similarly, Simonsick et al (in press) validated a method, in the BLSA, where participants are asked their Rating of Perceived Exertion (RPE), using the Borg scale[341], after walking on a treadmill for 5 minutes at 0.67m/s (1.5mph). A cut-point of a RPE >9 was established to categorize older adults has having high fatigability. The perceived performance fatigability method developed by Simonsick et al. was utilized in the third paper presented in this dissertation.

Finally, fatigability can be measured objectively during a performance test and is defined as performance fatigability. Schnelle et al. also validated a method where the distance individuals walked during the 10 minute treadmill bout was assessed in 2.5 minute intervals[330]. This allowed the investigators to calculate walking speed in 2.5 minute intervals. Percent change in walking speed was then calculated and divided by total distance walked to “calculate change

in performance in the context of physical activity"[330]. This number was then used a performance fatigability score. Similarly, Simonsick et al., (in press), validated a method in the BLSA to measure performance fatigability during the long distance corridor walk, a 400m walk done as quickly as possible. This walk is done in 10 40m lap segments and split times for each lap were recorded. Percent change between the 2nd and 9th lap is then calculated; those whose lap 9 times were 6.5% or more slower than their second lap were considered to have slowed, and thus were characterized has having a high level of performance fatigability. Performance measures of fatigability are ideal, as they are completely objective and activity level and duration can be controlled and standardized. But, they are time-consuming, require trained staff, as well as in-person clinic visits. They also may not be appropriate for more frail older adults. Studies involving older adults should employ, when possible, both performance and perceived measures of fatigability to avoid healthy participant biases and losses to follow-up.

Fatigability likely shares the same risk factors as fatigue, as fatigability is a measure of fatigue, simply normalized to activity. Thus, it is likely that underlying diseases cannot fully explain age-related increases in fatigability. Age-related mitochondrial dysfunction may contribute to higher levels of fatigability. This dissertation will examine this hypothesis directly. The possible relationship between fatigue and fatigability and mitochondrial function is discussed below.

1.3.3 Fatigue, Fatigability and Mitochondrial Function

A majority of fatigue can be attributed to underlying diseases; however a large portion cannot. For example, in a study of ambulatory primary care patients aged 60 years and over, 38.3% of fatigue cases could not be attributed to comorbidities or physiological disorders[9]. Similarly, in

the InCHIANTI study (n= 1,055, age 65-102), fatigued compared to non-fatigued men and women had a similar average number of comorbidities: 2.1 vs. 1.7 in men and 1.7 vs. 1.7 in woman[8]. Although this was a statistically significant difference among men (p=0.02), it further illustrates how a large proportion of fatigue in older adults cannot be attributed to underlying diseases.

Fatigue is primarily considered an energy disorder, thus it has been hypothesized that age-related decreases in mitochondrial function may contribute to higher levels of fatigability in older adults[11,12]. The capacity for oxidative phosphorylation in skeletal muscle and mitochondrial function has been shown to decrease significantly with age, mainly due to mutations and/or mtDNA from prolonged exposure to reactive oxygen species[4]. Diseases involving mutations or deletions to mtDNA result in increased levels of fatigability and exercise intolerance[311]. For example, mitochondrial gene ANT1-knockout mice, which are a model for chronic ATP deficiency, have been shown to display exercise intolerance and fatigability[342,343]. Additionally, one of the hallmark symptoms of humans with mitochondrial myopathies is significantly higher levels of exercise intolerance[311,344]. A review of mitochondrial myopathies and exercise intolerance has been published elsewhere[311]. Similarly, age-related mitochondrial dysfunction may contribute to age-related increases in fatigability.

As mentioned previously, VO_2 peak has also been shown to decrease significantly with age, independent of muscle loss and decreases in physical activity levels[231]. The ability of skeletal muscle mitochondrial to produce ATP with the oxygen delivered to them via the cardiorespiratory system is one of two major components comprising $V0_2$ peak. VO_2 peak is also one of the hallmark manifestations of mitochondrial disorders[345,346]. Thus, mitochondrial dysfunction may contribute to higher levels of fatigability via lower aerobic capacity due partly

to decreases in mitochondrial energy production. Oxidative damage resulting in mutations to mtDNA may also play a role in this pathway. The relationship between mitochondrial energetics and fatigability in older adults has not been previously examined. This dissertation will address this important gap in knowledge by examining the relationship between oxidative capacity of mitochondria present in the quadriceps (ATPmax) and oxidative capacity of the quadriceps (ATPmax*muscle volume), measured by ^{31}P MRS, and perceived performance fatigability.

1.4 SPECIFIC AIMS

1.4.1 Relationship between Changes in Body Composition and Physical Function

Controversy exists as to whether older adults should lose weight and whether overall body weight or particular aspects of body composition should be targeted[168-170]. Intentional and unintentional weight-loss may target lean and fat mass differently. Unintentional and age-related weight loss is associated with greater decreases in lean than fat mass[100]. Conversely, intentional weight loss combined with a moderate physical activity intervention has been shown to decrease fat mass to a greater degree than lean mass[48,174-176]. Intentional weight loss may also target particularly harmful fat depots. Specifically, aging is associated with increases in visceral (VAT)[165,166] and intermuscular (IMAT) adipose tissue independent of overall body mass change[83,122,145]. VAT is strongly associated with insulin resistance independent of overall adiposity and higher proinflammatory cytokine levels, which negatively affect muscle performance[52,347,348]. IMAT has been shown to be related to insulin resistance and the metabolic syndrome independent of overall adiposity and has been described as being similar to VAT in

size[52,146,161]; however it has not been consistently linked to muscle performance. Intentional weight loss may target these particularly harmful fat depots. Few studies have shown specific composition data following intentional weight loss in older adults[173,175], but the few that do seem to support this hypothesis.

Specific Aim 1: To examine the relationship between changes in regional body composition measured by computed tomography (CT) and physical performance in older adults, who participated in a one year intervention study of physical activity combined with weight loss (PA+WL) compared to physical activity combined with a successful aging health education program (PA+ SA).

Hypothesis: Decreases in IMAT, VAT and intramuscular adipose tissue (IMF) will be associated with improvements in physical function following one year of intervention, regardless of intervention group.

1.4.2 Mitochondrial Function and Walking Performance in Older Adults

Although many risk factors for disability in older adults are known, the role of changes in the neuromuscular system in decreasing physical performance and physical disability in older adults is still unclear. Therefore, exploring the cellular and physiologic bases of mobility and functional decline in older adults is important. Studies of mitochondrial function have tended to be small and lack performance measures of physical function. Three studies in older adults have examined the relationship between mitochondrial function and physical function in older adults[312]. All three showed a relationship between mitochondrial function and physical function. One was in patients with PAD, one included a small number of older adults, some of whom were hospitalized and the other was in high functioning older adults. These results need to be

replicated in a non-disease specific group of community older adults with a wide-range of function.

Specific Aim 2: To examine the relationship between mitochondrial function, as measured by ^{31}P Magnetic Resonance Spectroscopy, and time to walk 400m in older adults with a wide range of function

Hypothesis: Higher levels of mitochondrial energy production will be related to faster walk-times.

1.4.3 Relationship Between Mitochondrial Function and Fatigability in Older Adults

Fatigue is often assumed to be the manifestation of underlying co-morbid conditions. However, a large proportion of self-reported fatigue cannot be attributed to underlying diseases[9,10]. Research has also shown that fatigue is significantly related to physical function and disability, independent of disease status[8]. Therefore, fatigue is an independent risk factor for age-related declines in physical function and disability. It is important to study the etiology of age-related increases in fatigue in order to determine the most effective ways to treat it. Fatigue is primarily considered an energy disorder, thus it has been hypothesized that decreased mitochondrial function with age may be a major contributor to the onset of fatigability[12]. Yet, there is no objective data to support this.

Specific Aim 3: To determine the relationship between mitochondrial function, as measured by ^{31}P MRS, and fatigability, assessed following a standardized exercise bout, in older adults.

Hypothesis: Higher levels of mitochondrial energy production will be related to lower levels of fatigability.

2.0 INTERMUSCULAR ADIPOSE TISSUE CHANGE IS RELATED TO IMPROVED PHYSICAL PERFORMANCE IN OLDER ADULTS

The prevalence of obese (BMI ≥30) older adults was shown to be 30.5% in the National Health and Nutrition Examination Survey data from 2005-2006. The high prevalence of obesity in older adults is a major public health concern, as obesity worsens age-related declines in physical function, which increase the risk of institutionalization and increases health care costs. There are currently no agreed upon guidelines for intentional weight loss in older adults. Studying the relationship between changes in specific fat depots following intentional weight-loss and physical activity and how they impact physical function in older adults is important. The aim of this study was to determine the relationship between changes in body composition and physical function in older adults following a one-year physical activity + weight loss (PA+WL) or physical activity + successful aging health education (PA+SA) intervention. Thirty-six overweight to moderately obese, sedentary older adults were randomized into a one-year PA+WL or PA+SA intervention. The PA intervention focused on walking, supplemented with leg resistance and balance exercises. The WL intervention focused on caloric restriction, specifically calories from fat. Measurements were conducted at baseline (BL) and 12-months (12) and included body composition by computerized tomography (CT), short physical performance battery (SPPB), and gait-speed (GS). Visceral adipose tissue (VAT) of the abdomen, intermuscular fat (IMAT) and muscle density (HU), and indirect measure of

intramyocellular fat, were quantified. The relationship between concurrent changes in body composition and physical function, from BL-12, were determined using multivariate linear regression. After adjustment for age, sex, race, baseline body composition and SPPB, 6- and 12-month change in IMAT, HU, SUBQ adipose of the thigh, total thigh fat, VAT and total abdominal fat were all significantly related to 6- and 12-month change in SPPB. Changes in IMAT, HU and VAT were significantly associated with improvements in change in function. Despite losing some lean mass, participants were able to improve their function (SPPB). This suggests that intentional weight-loss, supplemented with moderate intensity PA, is beneficial to older adults.

2.1 INTRODUCTION

In 2010, men and woman age 60 and older possessed the highest prevalence rates of obesity compared to any other age group, with rates of 42.3% and 36.6% respectively[349]. Obesity has been shown to exacerbate physical disability in older adults[163,164] and is associated with other comorbidities including diabetes, heart disease and osteoarthritis[350]. Therefore, studying the effects of weight loss in old-age is important. Previously, weight-loss in old age was thought to be detrimental to health, as findings from observational studies showed a positive association between weight-loss in old age and mortality[171]. However, more recently, Shea et al. have published findings from a randomized controlled trial showing that intentional weight-loss in old age is not associated with increased mortality[49]. Additionally, randomized controlled trials have shown that weight-loss (WL) in combination with moderate physical activity (PA) improves physical function in older adults to a greater degree than physical activity alone[49,172], weight-loss alone[49] and normal care[173]. Additionally, WL in conjunction with PA has been shown to improve components of frailty, including physical function, in obese frail older adults [174]. Controversy still exists as to whether older adults should lose weight and whether overall body weight or particular aspects of body composition should be targeted[168-170].

Aging is associated with increases in adipose tissue (VAT)[165,166] and IMAT independent of overall body mass change[83,122,145]. VAT, found within the abdominal cavity surrounding organs, is strongly associated with insulin resistance independent of overall adiposity and with higher levels of proinflammatory cytokines, which can negatively affect muscle performance[52,347,348]. IMAT, fat present inside the muscle fascia surrounding skeletal muscle, has been shown to be related to insulin resistance and the metabolic syndrome independent of overall adiposity and has been described as being similar in size to VAT [52,146,161]. However, higher

IMAT levels in the calf and thigh have been inconsistently linked to lower muscle power and physical performance[145,151,351,352]. For example, Buford et al. showed that when muscle CSA of the thigh is accounted for, IMAT of the thigh and calf are not independent predictors of SPPB score or gait-speed[145]. However, in two other studies, IMAT levels of the calf were shown to be strongly related muscle power[151] and physical function[151,352] in older adults. In addition to IMAT, higher levels of intramyocellular fat (HU), lipid droplets present within muscle fibers, have been linked to insulin resistance and worse functional performance in older adults[30,144,150,351].

Intentional weight-loss and physical activity may target these particularly harmful fat depots. Few studies have specifically examined change in regional body composition following intentional weight-loss in older adults and/or physical activity[148,173,175], but the few that do seem to support this hypothesis. Intentional weight-loss, both with and without moderate physical activity interventions, has been shown to decrease fat mass to a greater degree than lean mass[48,174-176]. A study comparing a weight-loss only (WL) to a weight-loss plus resistance training group (WL-RT) showed statistically similar decreases in IMAT in both groups, with the decrease in the WL-RT group reaching significance (-18.5 vs. -9.6%). In this study, both groups improved similarly in terms of physical performance measures[173]. This may have been due to improvements in regional body composition, specifically decreases in IMAT, HU and VAT, but these mechanistic relationships were not explored. To understand the mechanisms underlying improvements in physical function following weight-loss and physical activity interventions, it is important to explore the relationships between improvements in physical function and changes in regional body composition.

The purpose of the research described in this paper was to determine the relationship between changes in regional body composition measured by computed tomography (CT) and physical performance in older adults who participated in a one year intervention study comparing physical activity combined with weight-loss (PA+WL) to physical activity combined with a successful aging health education program (PA+ SA). We hypothesized that decreases in IMAT, VAT and a marker of intramyocellular fat (HU) will be associated with improvements in physical function following one year of intervention, regardless of intervention group. Additionally, we hypothesized that subcutaneous, or gluteofemoral fat loss as well as change in muscle mass will not be associated with changes in physical function. This research will provide important insight into the mechanisms by which intentional weight-loss improves physical function in older adults. This research will provide clinicians and public health professionals with important information they can utilize to prevent age-related physical disability.

2.2 MATERIALS AND METHODS

2.2.1 Participants

Community dwelling older men and woman age 60 and over, who were overweight to moderately obese (body mass index between 28.0 and 39.9 kg/m2) and living a sedentary lifestyle (formal exercise less than 3x/week for a total of less than 90 min/week), were recruited from the greater McKeesport, PA area to participate in a one-year randomized clinical trial. Initial eligibility criteria included the self-reported ability to walk ¼ mile (2-3 blocks), completion of a 400-meter walk in less than 15 minutes without assistance from another person

or the use of an assistive device, successful completion of a behavioral run-in, which included an activity log and food diary, the willingness to be randomized to either intervention group as well as attend meetings and physical activity sessions in McKeesport, PA. Participants were excluded if they failed to provide informed consent, had diabetes requiring insulin, history of diabetic coma, uncontrolled diabetes (defined as a fasting blood sugar greater than 300 mg/dl), severe kidney disease that requires dialysis, or severe hypertension (systolic blood pressure > 180 mmHg or diastolic blood pressure > 100 mmHg). Further, significant cognitive impairment (known diagnosis of dementia or a Modified Mini-Mental State Exam score < 80) and other conditions impairing understanding and communication were also exclusion criteria. Other significant co-morbid diseases severe enough to impair one's ability to participate in an exercise-based intervention resulted in exclusion. Any person who developed chest pain or severe shortness of breath during 400m walk test was also excluded. Participants were also ineligible if a member of their household was already enrolled in the study, if they were currently participating in another intervention trial, planned to move in the next year, had lost more than 10 pounds in the past 4 months, or were taking any drugs for the treatment of obesity.

Participants who met the above were randomly assigned into one of two intervention programs: physical activity plus weight-loss (PA+WL) or physical activity plus a successful aging health education program (PA+SA). Randomization was done using a Microsoft Access-based random-number generating algorithm with stratification by age and sex to further ensure balance between groups (Microsoft® Redmond, Washington). All of the methods described in this paper were implemented following approval by the University of Pittsburgh's Institutional Review Board.

All participants, with the exception of one in the PA+SA group, were followed up to their 6FU visits. The participant dropped out of the study for personal reasons before starting the intervention and is excluded from all longitudinal and change analyses. Three additional participants, all three in the PA+WL group were lost to follow-up before their 12-month clinic visit. One moved out of the study area to take care of a family member, one stopped coming to intervention sessions and did not return phone calls from study staff and one dropped out due to dissatisfaction with the program. In summary 14/15 (93.3) participants in the PA+SA group, 18/21 in the PA+WL group (85.7), for a total of 32/36 (88.9%) were followed until study end.

2.2.2 Physical Activity Program

All participants, regardless of the randomized group assignment, participated in an identical physical activity program. The PA program combined aerobic, strength, balance and flexibility exercises [37]. The PA program focused on treadmill walking for at least 150 min/wk as the primary mode of activity. To complement the walking, participants completed lower extremity resistance training, balance training exercises and stretching.

The program was divided into three phases: adoption (weeks 1-8), transition (weeks 9-24), and maintenance (weeks 25-52), which were designed to gradually transition exercise out of the clinic setting and into the participant's daily routine. During the adoption phase, all participants were required to attend three center-based exercise sessions per week, which averaged 60 minutes per session. For the transition phase, center-based sessions were reduced to two sessions per week. During this phase, the center-based sessions were supplemented with one or more home-based sessions. The home-based sessions were to be similar to the center-based sessions. During the maintenance phase of the program, participants were invited to attend an

optional exercise session at the center once per week, but were expected to engage in physical activity at least three times per week.

The intervention was designed to transition into the home after the first 6-months. Participants were not required to attend any clinic based exercise sessions, but were permitted to come once per week, after the first 6-months. At 6FU, self-reported moderate PA increased uniformly in both intervention groups (by 222.9 ± 329.2 min/week in PA+WL and 199.0 ± 319.1 min/week in PA+SA), indicating an equally strong adherence to the PA program by both groups at 6FU.

2.2.3 Weight-loss Program

Those randomized into the PA+WL arm participated in a healthy-eating WL intervention, in addition to the PA program described above. Participants attended 24 weekly, 2 bi-monthly, and 5 monthly sessions, which were lead by the study nutritionist. During these meetings, strategies to achieve the recommended caloric intake were discussed and performance in the weight-loss intervention was assessed. The nutritionist scheduled one-on-one sessions if a participant was having difficulty adhering to the WL intervention.

The WL intervention was designed to promote weight reduction and decrease lipid levels. The calorie and fat gram goals were developed by the Diabetes Prevention Program [353]. Participants were assigned one of the following daily goals based on baseline weight,: 1200 calories and 33 fat grams, 1500 calories and 42 fat grams, 1800 calories and 50 fat grams, or 2000 calories and 55 fat grams. Total daily fat intake was limited to approximately 25% of total calories. An emphasis was put on the consumption of mono- and polyunsaturated fats while limiting saturated fat and cholesterol. In addition, participants were asked to include at least 5

servings of fruits or vegetables and 6 servings of grains, especially whole grains, in their daily diets. To ensure that participants met daily nutrient recommendations, age-appropriate multivitamin/mineral and calcium/vitamin D supplementation was recommended.

The goal of the WL intervention was a 7% reduction in body weight at the rate of 1 to 2 pounds per week during the first six months of the intervention. The goal for the remaining six months was to assist participants in achieving and maintaining their weight goal. Participants were required to keep food diaries at least six days per week during the first six months of the intervention and then for a minimum of once a month for the remainder of the study. Self-monitoring of caloric intake was emphasized and participants were encouraged to weigh themselves weekly at home. In addition, participants were weighed once a week by the study nutritionist at the start of the nutrition sessions. Overall adherence to this arm of the intervention was gauged by examining the percentage of participants who met the weight-loss goal.

At 6FU, 8 of the 21 (38.1%) participants in the PA+WL group, followed to 6FU, had achieved their 7% weight-loss goal. Upon study end, the PA+WL group maintained a -5.5%± weight reduction on average, which was 1.5% shy of the 7% weight-loss goal. At 12FU, 7 of the 18 (38.9%) participants in the PA+WL group, followed to 12FU, had achieved and maintained their 7% weight-loss goal.

2.2.4 Successful Aging (SA) Health Education Intervention

Participants randomized into the PA+SA intervention participated in a successful aging health education workshop series in addition to the PA program described above. The workshops were based on "The Ten Keys to Healthy Aging™" [354], and the SA intervention used in the Lifestyle Interventions and Independence for Elders Pilot Study (LIFE –P)[37]. Topics included cholesterol,

diabetes, blood pressure, bone and muscle health, smoking, cancer screening, social contact, depression, immunizations, and physical activity. Participants enrolled in this study arm attended 1 session per month, for a total of 12 sessions in addition to their physical activity sessions.

2.2.5 Clinical Measurements

At the baseline (BL) screening visit, 6- and 12-month follow up visits (6FU and 12FU), body height (cm), measured using a wall-mounted stadiometer, and body weight (kg), measured using a standard certified calibrated scale, were used to calculate BMI (weight (kg)/height (m^2)). Waist circumference (cm) was also measured at BL and follow-up using the Gulick II Tape Measure (Country Technology Inc., Gray Mills, WI). Waist circumference was measured twice and rounded to the nearest 0.1 cm; if the two measurements had a difference greater than 5 cm, then a third measurement was obtained. The Short Physical Performance Battery (SPPB), a validated measure of lower extremity functional disability in older adults, was performed and included a 4m walk, chair stands and a balance. More details concerning the SPPB can be found elsewhere [23]. Participants also completed questionnaires on socio-demographic data, medical and hospitalization history, and the Community Healthy Activities Model Program for Seniors (CHAMPS) physical activity questionnaire [355]. The CHAMPS questionnaire was used to quantify physical activity and assess adherence to the PA program [331]. Activities performed at or above 3.0 metabolic equivalents (METs) were defined as moderate physical activity; the type of physical activity the program was designed to deliver. A resting ECG, physical exam and interview with a nurse practitioner were conducted before subjects were medically cleared to participate in the physical activity intervention by the study physician.

2.2.6 Dual Energy X-ray Absorptiometry (DXA)

At BL, 6FU and 12FU, total body fat mass, percent body fat, total lean body mass, appendicular lean body mass, total body bone mineral density (BMD) and total hip BMD were assessed using DXA(Hologic QDR 4500, software version 12.3; Bedford, MA). Bone mineral content was subtracted from the total and appendicular lean mass to define total non-bone lean mass, which represents primarily skeletal muscle in the extremities [356]. Appendicular lean mass was defined as the sum of upper and lower extremity lean mass [95]. Two participants, one in each group, did not complete hip scans due to hip replacements. Two participants are missing 6-month DXA data. One in the PA+SA group dropped out before starting the intervention and one in the PA+WL group refused because of concerns over radiation exposure. Four participants are missing 12-month DXA data, all were lost to follow-up.

2.2.7 Computed Tomography (CT)

At BL, 6FU and 12FU, axial CT scans (9800 Advantage, General Electric, Milwaukee, WI) were obtained and used to measure cross-sectional abdominal visceral and subcutaneous adipose tissue (VAT and SAT) areas using an established method[357]. Briefly, a cross-sectional scan at 10 mm thickness was obtained, centered at the L4-L5 vertebral disc space using 170 mÅ with a scanning time of two seconds and a 512 matrix. The visceral and subcutaneous AT boundary was defined using a manual cursor, and adipose tissue areas were determined using commercially available software (Slice-O-Matic, Tomovision, Montreal, Canada).CT was also used to measure cross-sectional area (CSA) of mid-thigh muscle and adipose tissue and to characterize muscle attenuation. An anterior-posterior scout scan of the entire femur was used to localize the mid-

thigh position. With the subject supine, a 10 mm cross-sectional scan of the dominant leg was obtained at the midpoint. The scanning parameters for this image were 120 kVp and 200–250 mÅ. This protocol has been utilized elsewhere[148]. Image analysis of adipose tissue and skeletal muscle CSAs of the thigh were calculated from the axial CT images using commercially available software (Slice-O-Matic, Tomovision, Montreal, Canada). Briefly, the mean attenuation coefficient values of muscle within the regions outlined on the images were determined by averaging the CT number (pixel intensity) in Hounsfield units (HU). The methodological variability of this measure is quite small[85]. Skeletal muscle and adipose tissue areas were calculated by the range of attenuation values for skeletal muscle (0 to 100 HU), normal density muscle (35–100 HU), and adipose (–190 to –30 HU) tissue. Intermuscular adipose tissue (IMAT), fat that is visible within the muscle fascia, surrounding skeletal muscle, was distinguished from the subcutaneous (SUBQ) adipose tissue by manually drawing a line along the deep fascial plane surrounding the thigh muscles. Quadriceps muscles were separated from hamstring muscles with manual tracing. Two additional reviewers' analyzed thigh and abdominal scans from five randomly selected participants from this project and inter-rater reliability was assessed using a two-way mixed effects ANOVA model with SPSS 17.0 (SPSS Inc., Chicago, IL). The interclass correlation coefficient (ICC) was non-significant (P =0.99).

At baseline, two participants, one in each group, are missing abdominal scans due to metal deposits in the body. Similarly, two participants, one in each group are missing baseline thigh scans due to metal deposits in the body. At 6-months, four participants are missing abdominal and thigh scans; two due to metal deposits, one refused and one was lost to follow-up. At 12-months, seven participants are missing thigh scans, two due to metal, one refusal, and four

were lost to follow-up. Six participants are missing 12-month abdominal scans, two due to metal deposits and four were lost to follow-up.

2.2.8 Isokinetic Strength Testing

At baseline and follow-up visits, isokinetic strength of the knee extensors was determined at 60°/s with a dynamometer (model 125 AP, Kin-Com, Chattanooga, TN). The right leg was tested unless it was injured or weaker by self-report or restricted in motion. After instruction on the procedure, the participant was positioned so that the lateral femoral epicondyle of the knee joint was aligned with the rotational axis of the dynamometer. The participant's leg was weighed for gravity correction, and start-stop angles were set at 90° and 30°. Two practice trials were performed at 50% effort to familiarize the participant with the procedure and to provide a warm-up period. Each participant performed at least three maximal efforts. Beginning with the first maximal effort, the torque production over the entire range of motion was plotted, and the plot of each subsequent effort was overlaid on the previous efforts until three similar curves were obtained. Participants were not asked to perform more than six trials. Maximal torque production was recorded as the mean peak torque production from three similar trials. This methodology was used in the Health ABC Study[144]. Additionally, specific torque was calculated for each participant (knee extensor strength per unit cross-sectional area of the quadriceps) and used as a measure of muscle quality in the quadriceps.

Strength data is missing for one participant at baseline due to bilateral total knee replacement. Four participants are missing strength data at 6-months, one due to bilateral total knee replacement, two due to an examiner error and one was lost to follow-up. Six participants

are missing 12-month strength data, one due to bilateral total knee replacement, one due to severe pain in both knees and four were lost to follow-up.

2.2.9 Statistical Analyses

Univariate statistics including means and standard deviations were generated for each variable. Changes from baseline to 12-months (12-baseline) were calculated and the significance of changes both within groups and between groups were calculating using t-tests, paired t-test, chi-squared tests and nonparametric tests where appropriate. Generalized Estimating Equations were used to assess the relationship between 6- and 12-month changes in body composition with change in physical function as measured by the SPPB. Six and twelve month change scores, as opposed to value at each time point by time interaction terms, were used in models due to the small sample size of this study. Intervention groups were pooled for these analyses to address the specific aim of determining the mechanisms, in terms of changes in body composition, underlying improved physical function regardless of intervention group. The effect of adjusting for intervention assignment and the significance of the beta coefficient associated with randomization assignment were assessed. Adjusting for intervention assignment had little to no effect on the relationship between changes in specific body composition depots. Additionally, the beta coefficient associated with randomization assignment was not significant in any model. Analyses were conducted using SAS v9.3.

2.3 RESULTS

2.3.1 Baseline

Study participants (N = 36) were 70.3 ± 5.9 years of age, weighed 87.9 ± 8.9 kg with a BMI of 32.9 ± 3.2 kg/m^2, classifying them as overweight to moderately obese at baseline[358]. All participants were nonsmokers. Study participants were 16.7% black and 16.7% male (Table 5). There were no statistically significant differences between the PA+WL and PA+SA groups in regard to baseline demographic, anthropometric, body composition, bone, strength, and functional characteristics, except for total SPPB score and total abdominal fat CSA (Tables 5 and 6), which were marginally higher in the PA+WL group at baseline. Also, no significant differences at BL between intervention groups in self-reported PA levels as measured by the CHAMPS questionnaire (P = 0.17) were found.

2.3.2 Change in Anthropometrics and Body Composition (DXA)

The PA+WL intervention was designed to induce weight-loss within the first 6-months of the trial and subsequently maintain this weight-loss for the remainder of the study period. This is displayed in Table 8, which shows change that occurred from the six-month follow-up visit (6FU) to twelve months (12FU). No significant changes in body composition were observed in the PA+WL group and borderline significant decreases in IMAT and an increase in HU were observed in the PA+SA group from 6FU to 12FU. Baseline to six-month follow-up results have been published elsewhere[14]. This paper will focus on changes that occurred over the entire 12-month study period, which are displayed in Table 7.

Participants in the PA+WL group significantly decreased their body weight (-4.9 ± 6.1 kg, p =0.002) and BMI (-1.7 ± 2.3 kg/m^2, p=0.002) from BL to 12FU whereas the PA+SA group did not. Significant between group differences for 12-month change in body weight and BMI (Table 7) were found. Participants in both groups did not significantly decrease their waist circumference (Table 7).

Participants in the PA+WL significantly reduced their percent body fat (-2.9 ± 3.4%, p <0.001) from BL to 12FU as measured by DXA whereas the PA+SA group did not (0.8% ± 1.6%, p=0.09). Similarly, total body fat mass decreased by 13.1% (p=0.003) in the PA+WL group over the course of the study, which was significantly greater than the 3.2% decrease observed in the PA+SA group (p=0.06). Total body lean mass also decreased significantly by 2.5% (p=0.01) in the PA+WL group, but not in the PA+SA group (-0.2%, p=0.81, Table 7); however, these changes did not differ significantly between groups. The PA+WL group experienced over a 5-fold greater percent decrease from BL to 12FU in total body fat mass compared to lean mass (-13.1% vs. -2.5%, Table 7).

2.3.3 Change in Body Composition (CT)

Mean and percent changes, by group assignment, in regional body composition measured by CT can be found in Table 7. Significant 12-month decreases in total (-81.5 ± 104.8 cm^2, p= 0.006), SUBQ (-56.6 ± 62.8 cm^{2,} p=0.02) and visceral (-34.8 ± 40.4 cm^2, p= 0.004) adipose tissue were observed in the PA+WL group but not in the PA+SA group (Table 7). Additionally, significant between-group differences were observed in 12-month change in VAT and total abdominal adipose tissue CSA, but not abdominal SUBQ adipose tissue. The PA+WL lost a greater

proportion of VAT (-16.0%) compared to SUBQ abdominal adipose tissue (-12.8%). This is depicted in Figure 4.

Significant 12-month decreases in IMAT, SUBQ and total adipose tissue CSA of the thigh were observed in the PA+WL group, but no significant between group differences were observed for these measures. As depicted in Figure 4, the PA+WL experienced a 2-fold greater percent decrease from BL to 12FU in IMAT compared to SUBQ adipose tissue in the thigh (-24.6% vs. 11.6%). A significant 12-month decrease in IMAT (-1.8 ± 2.6 cm^2, -14.5%, p=0.03) was also observed in the PA+SA group; none of the other thigh fat depots decreased significantly in this group. Neither group significantly increased thigh muscle HU (Table 7). Decreases is thigh muscle CSA were observed in both groups, with both the between group difference and decrease in the PA+WL group reaching significance (-5.0 ± 6.4 cm^2 vs. -2.1 ± 5.7 cm^2), between group p=). From BL to 12FU, the PA+WL group lost over twice the proportion of total fat CSA compared to muscle CSA in thigh (-12.7% vs. -4.9%).

2.3.4 Relationship between 6- and 12-month Change in Body Composition with Change in Physical Function

Intervention groups were combined for these analyses. Pooled change in total and regional body composition, strength, specific torque and physical function can be found in Table 9. After adjustment for age, sex, race, baseline body composition and SPPB, 6- and 12-month decrease in IMAT, SUBQ adipose of the thigh, total thigh fat, VAT and total abdominal fat were all significantly related to 6- and 12-month improvements in SPPB (Table 10). An increase in HU HU (muscle density), indicating a decrease in intramyocellular fat, was also associated with an increase in SPPB score after adjustment for age, sex, race, baseline body composition and SPPB

(Table 10). Models for IMAT and HU were also adjusted for total thigh muscle CSA. Decreases in SUBQ abdominal fat and total thigh muscle CSA were not significantly related to improvements in SPPB. Decreases in IMAT (stb -0.3829, p=0.005) and VAT (stb -0.4407, p=0.0013), in terms of standardize beta size, had the strongest relationship with improved SPPB. Adjustment for randomization assignment had minimal effects on these relationships and actually increases the standardize beta coefficients and lowers p-values for IMAT and VAT. Additionally, the intervention term was non-significant in all models. Similarly, adjustment for body height has minimal to no effect on these relationships and was not significant in any of the models.

Additionally, after adjustment for age, sex, race, baseline body composition and SPPB, 6- and 12-month change in specific torque, or muscle quality, of the quadriceps (stb. 0.2876, p=0.005) and change in total fat mass (stb. -0.3268, p=0.0156) from DXA were significantly related to 6- and 12-month change in SPPB. Six- and twelve-month change in total body mass from DXA was borderline significantly associated with change in SPPB (stb. -0.2260, p=0.056). Change in total lean mass from DXA was not related to change in SPPB (Table 10).

Table 5. Baseline and Demographic Characteristics

	Weight-loss + Physical Activity (N= 21)	Physical Activity + SA Education (N=15)	p-Value
Age	70.6 (± 5.9)	69.9 (± 5.9)	0.80
Gender			0.68
Male	4 (19.0%)	2 (13.3%)	
Female	17 (81.0%)	13 (86.7%)	
Race			0.20
White	19 (90.5%)	11 (73.3 %)	
African American	2 (9.5%)	4 (26.7 %)	
Education			0.84
High School/GED	13 (65.0%)	10 (66.7%)	
College	6 (30.0 %)	2 (13.3%)	
Other	1 (5.0%)	3 (20.0%)	
Household Income ($thousand/year)			0.99
<$50K	13 (61.9%)	9 (60.0%)	
>$50K	3 (14.3%)	3 (20.0%)	
Don't Know/Refused	5 (23.8%)	3 (20.0%)	

Table 6. Baseline Anthropometric, Body Composition, Muscle Strength and Physical Function by Intervention Group

	Weight-loss + Physical Activity (N= 20)	Physical Activity + SA Education (N=14)	p-Value
Anthropometric			
Waist Circumference, cm	108.8 (7.2)[#]	105.1 (8.8)	0.22
Body Weight, kg	89.8 (10.0)	85.4 (6.5)	0.21
Height, cm	164.1 (8.4)	163.2 (5.2)	0.77
BMI, kg/m^2	33.6 (3.3)	32.1 (3.0)	0.30
DXA			
Percent Body Fat	43.0 (5.4)	42.5 (6.1)	0.73
Total Fat Mass, kg	38.0 (5.9)	35.9 (6.5)	0.61
Total Lean Mass, kg	48.2 (7.6)	46.1 (5.2)	0.55
Appendicular Lean Mass, kg	20.6 (3.7)	19.7 (2.8)	0.47
Total body BMD, g/cm^2	1.14 (0.12)	1.11 (0.15)	0.53
Total Hip BMD, g/cm^2	0.93 (0.11)	0.93 (0.15)	0.91
Abdominal CT			
Total, cm^2	661.5 (± 134.1)	569.5 (97.6)	0.04*
Visceral Fat, cm^2	217.7 (± 61.3)	179.8 (47.9)	0.06
Subcutaneous Fat, cm^2	443.7 (± 124.5)	389.1 (93.4)	0.17
Right Thigh CT			
Total Fat, cm^2	150.8 (± 52.4)	137.9 (47.8)	0.47
Subcutaneous, cm^2	133.2 (± 52.8)	119.8 (47.4)	0.45
Inter-muscular Fat, cm^2	12.5 (± 3.6)	13.4 (5.5)	0.57
Muscle Mass (CSA), cm^2	102.3 (± 23.2)	102.5 (90.2)	0.99
Muscle Density, HU	39.6 (± 3.1)	40.1 (3.3)	0.62
NDM, cm^2	68.8 (± 18.9)	71.7 (21.1)	0.64
Knee Extensor Strength			
Peak Torque, N·m	105. 9 (32.2)	110.8 (23.7)	0.66
Specific Torque, $N \cdot m/cm^2$	2.2 (± 0.3)	2.2 (0.5)	0.65
SPPB			
Total	9.7 (1.4)	10.7 (1.1)	0.05*

± Standard Deviation, *Significant at $p<0.05$

Table 7. Mean Change in Anthropometric, Body Composition, Bone Mass, Muscle Strength and Physical Function from Baseline to 12 Month by Intervention Group

	Weight-loss + Physical Activity (N= 18)			Physical Activity + Successful Aging (N=14)		
	Mean Change (12FU-BL)	% Change from BL	p-Value	Mean Change (12-BL)	% Change from BL	p-Value
Anthropometric						
Waist Circumference, cm	-2.5 (7.8)[#]	-2.1%	0.18	0.1 (10.5)	0.2%	0.62
Body Weight, kg	-4.9 (6.1)§	-5.5%	0.002*	-0.8 (3.0)§	-1.0%	0.32
BMI, kg/m^2	-1.7 (2.3)§	-5.2%	0.002*	-0.2 (1.1)§	-0.6%	0.71
DXA						
Percent Body Fat	-2.9 (3.4)§	-7.2%	<0.001*	-0.8% (1.6)§	-1.8%	0.09
Total Fat Mass, kg	-4.8 (4.6)§	-13.1%	<0.003*	-1.2 (2.2)§	-3.2%	0.06
Total Lean Mass, kg	-1.2 (1.7)	-2.5%	0.01*	-0.1 (1.2)	-0.2%	0.81
Abdominal						
Total Fat, cm^2	-81.5 (104.8)§	-11.0%	0.006*	-26.5 (77.8)§	-5.9%	0.24*
Visceral Fat, cm^2	-34.8 (40.4)§	-15.1%	0.004*	-1.0 (29.3)§	2.1%	0.91
Subcutaneous Fat, cm^2	-56.6 (62.8)	-8.2%	0.02*	-24.8 (63.8)	-8.7%	0.19
Right Thigh						
Total Fat, cm^2	-19.1 (24.4)	-13.7%	0.007*	-3.4 (7.8)	-2.4%	0.15
Subcutaneous, cm^2	-15.4 (23.7)	-12.3%	0.02*	-1.5 (7.5)	-0.7%	0.49
IMAT, cm^2	-3.2 (2.2)	-23.6%	<0.001*	-1.8 (2.6)	-14.5%	0.03*
Muscle Mass, cm^2	-5.0 (6.4)§	-5.0%	0.008*	-2.1 (5.7)§	-2.5%	0.21
Muscle Density, HU	0.7 (1.5)	1.8%	0.11	0.2 (1.4)	0.6%	0.55
NDM Mass, cm^2	-1.7 (5.3)	-2.3%	0.32	-0.8 (6.0)	-1.7%	0.50
Knee Extensor Strength						
Peak Torque, N·m	-17.6 (22.3)	-14.8%	<0.001*	-7.9 (16.6)	-7.7%	0.17
Specific Torque, N·m/cm^2	-0.2 (0.4)	-7.2%	0.12	-0.2 (0.3)	-5.8%	0.27
SPPB Score	0.8 (1.4)	10.0%	0.02*	0.1 (1.6)	1.7%	0.86
400m Walk Time, s	-20.56 (63.07)	-3.9%	0.32	11.07 (61.6)	2.9%	0.51

± Standard Deviation, * Significant change from baseline, p<0.05, § Denotes a significant difference between intervention groups, p<0.05

Table 8. Mean Changes in Anthropometric, Body Composition, Bone Mass, Muscle Strength and Physical Function Measures from 6 to 12 Month Follow-up by Intervention Group

	Weight Loss + Physical Activity (N= 19)			Physical Activity + Successful Aging (N=13)		
	Mean Change (12FU-6FU)	**% Change**	**p-Value**	**Mean Change (12FU-6FU)**	**% Change**	**p-Value**
Anthropometric						
Waist Circumference, cm	2.2 (7.1)[#]	2.3%	0.17	-0.6 (8.7)[#]	-0.5%	0.81
Body Weight, kg	0.5 (4.5)	0.6%	0.81	0.2 (2.7)	0.2%	0.71
BMI, kg/m^2	0.2 (1.7)	0.6%	>0.99	0.1 (1.0)	0.6%	0.50
DXA						
Percent Body Fat, %	-0.7 (2.2)	-1.8%	0.35	-0.7 (2.2)	-1.7%	0.17
Total Fat Mass, kg	-0.5 (2.6)	-1.8%	0.58	-0.6 (2.5)	-1.6%	0.39
Total Lean Mass, kg	0.5 (1.9)	1.0%	0.32	0.6 (1.2)	1.3%	0.12
Abdominal						
Total Fat, cm^2	-9.0 (49.6)	-0.7%	0.47	-31.7 (73.5)	-6.1%	0.39
Visceral Fat, cm^2	1.9 (26.5)	1.2%	0.77	-6.5 (24.3)	-1.1%	0.91
Subcutaneous Fat, cm^2	-10.9 (36.3)	-2.1%	0.23	-25.2 (71.7)	-7.3%	0.23
Right Thigh						
Total Fat, cm^2	-0.2 (12.2)	0.6%	0.96	2.1 (8.5)	1.8%	0.15
Subcutaneous, cm^2	0.4 (13.9)	1.6%	0.90	3.4 (8.6)	3.7%	0.19
IMAT, cm^2	-0.8 (1.7)	-5.5%	0.13	-1.2 (1.9)	-9.8%	0.048*
Muscle Mass, cm^2	-2.0 (7.1)	-2.4%	0.27	-3.6 (5.7)	-3.9%	<0.01*
Muscle Density, HU	-0.7 (1.4)	-1.5%	0.07	-0.7 (1.2)	-1.6%	0.08
NDM Mass, cm^2	-2.7 (5.9)	-3.7%	0.09	-3.5 (4.1)	-5.7%	0.01*
Knee Extensor Strength						
Peak Torque, N·m	-3.7 (14.1)	-2.5%	0.08	-4.4 (17.2)	-5.3%	0.39
Specific Torque, N·m/cm^2	-0.0 (0.4)	0.7%	0.81	-0.1 (0.3)	-5.8%	0.11
SPPB Score	0.2 (1.3)	6.7%	0.56	-0.4 (1.2)	-3.6%	0.28
400m Walk Time, s	24.44 (33.23)	3.4%	0.01*	32.21 (49.09)	9.1%	0.03*

[#] ± Standard Deviation, * Significant change from baseline, p<0.05, § Denotes a significant difference between intervention groups, p<0.05

Table 9. Mean Changes in Anthropometric, Body Composition, Bone Mass, Muscle Strength and Physical Function Measures from Baseline to 12-month Follow-up

	Entire Cohort		
	Mean Change (12FU-BL)	**% Change**	**p-Value**
Anthropometric			
Waist Circumference, cm	-1.4 (9.0)	-1.1%	0.40
Body Weight, kg	-3.1 (5.3)	-3.5%	0.002
BMI, kg/m^2	-1.0 (2.0)	-3.2%	0.004
DXA			
Percent Body Fat, %	-2.0 (2.9)	-4.8%	<0.0001
Total Fat Mass, kg	-3.2 (3.9)	-8.8%	0.89
Total Lean Mass, kg	-0.7 (1.6)	-1.5%	0.58
Abdominal			
Total Fat, cm^2	-57.7 (96.6)	-8.8%	0.003
Visceral Fat, cm^2	-20.1 (40.7)	-7.7%	0.01
Subcutaneous Fat, cm^2	-37.2 (69.2)	-8.4%	0.006
Right Thigh			
Total Fat, cm^2	-12.1 (20.2)	-8.6%	0.0002
Subcutaneous, cm^2	-9.1 (19.3)	-7.1%	0.005
IMAT, cm^2	-2.6 (2.5)	-19.5%	<.0001
Muscle Mass, cm^2	-3.7 (6.2)	-3.9%	0.003
Muscle Density, HU	0.5 (1.5)	1.3%	0.02
NDM Mass, cm^2	-1.3 (5.5)	-2%	0.59
Knee Extensor Strength			
Peak Torque, N·m	-13.7 (20.5)	-11.9%	0.0001
Average Torque, N·m	-9.6 (16.1)	-10.1%	0.001
SPPB Score	0.5 (1.5)	6.4%	0.07
400m Walk Time, s	-6.7 (63.4)	-1.0%	0.55

Table 10. 12-Month Change in Regional Body Composition Predicting 12-Month Change in SPPB Score using GEE

Variable	Std ß estimate	SE	p
Δ Intermuscular Fat**	-0.3829	0.1365	0.0050
Δ Muscle Attenuation**	0.2758	0.1094	0.0117
Δ Thigh SUBQ Fat*	-0.2802	0.0956	0.0034
Δ Total Thigh Fat	-0.3069	0.0943	0.0011
Δ Abdominal SUBQ Fat*	-0.1660	0.1029	0.1065
Δ Visceral Adipose Tissue*	-0.4407	0.1374	0.0013
Δ Total Abdominal Fat	-0.3335	0.1184	0.0049
Δ Muscle CSA*	0.0994	0.1692	0.5202
Δ Specific Torque*	0.2876	0.1270	0.0236
Δ Lean Mass, DXA*	-0.0730	0.0792	0.3561
Δ Fat Mass, DXA*	-0.3268	0.1352	0.0156
Δ Total Mass, DXA*	-0.2260	0.1180	0.0555

*Models are adjusted for Age, Sex, Race, baseline Body Composition and SPPB Scores
** Models are also adjusted for baseline thigh muscle cross-sectional area.

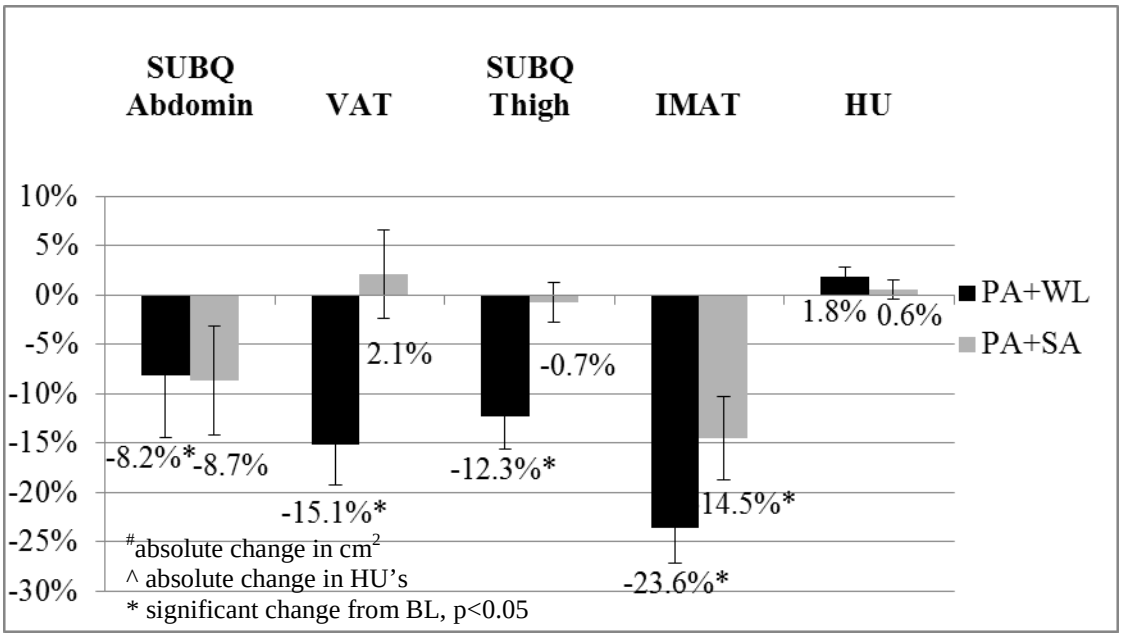


Figure 4. 12-Month Percent Changes in Specific Fat Depots of the Thigh and Abdomen

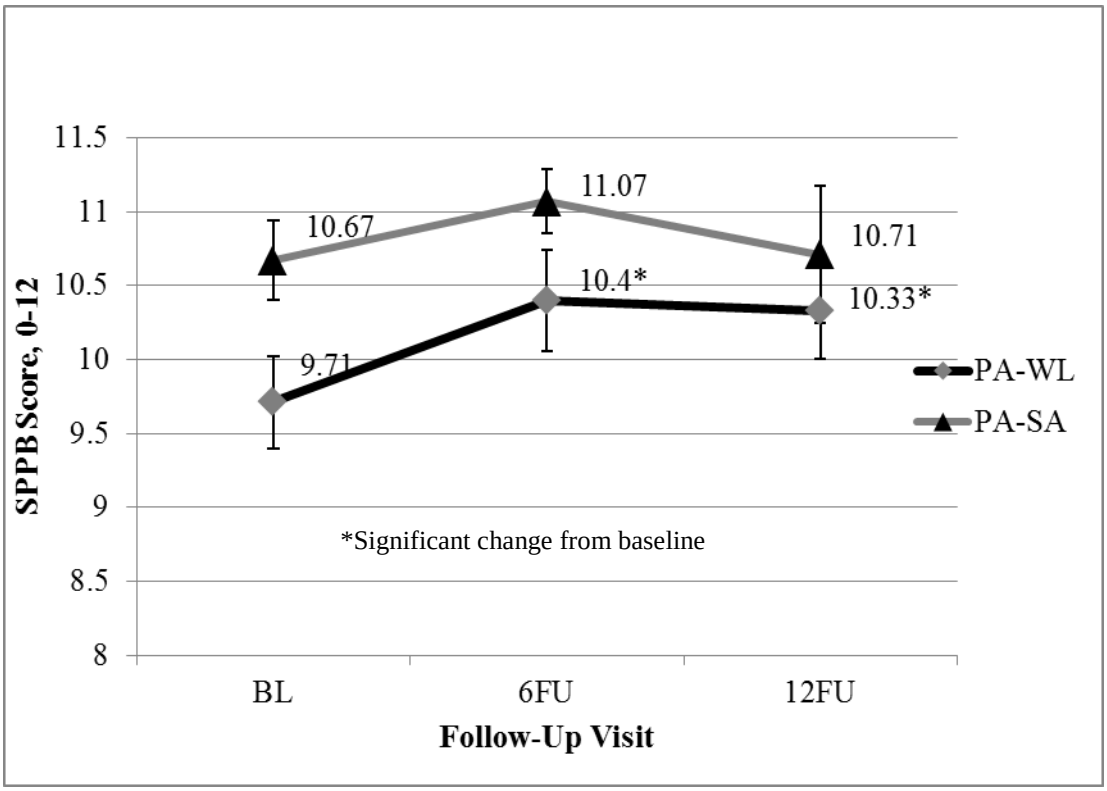


Figure 5. Change in SPPB score by Time Point

2.4 DISCUSSION

The primary aim of the current study was to determine the relationship between changes in regional body composition and physical performance in order to gain insight into the possible mechanisms underlying improvements in physical performance, regardless of intervention group. Consistent with our hypotheses, decreases in IMAT, HU and VAT were all significantly associated with improvements in physical performance as measured by the SPPB (Table 10). To our knowledge, this is the first study to show that changes in specific fat depots, in older adults, are associated with improvements in physical function. Decreases in SUBQ abdominal fat and total thigh muscle CSA were not significantly related to change in SPPB; however decreases in SUBQ thigh fat were related to increased physical function. Thigh SUBQ, or gluteofemoral fat, has been shown to possess certain protective properties including independent associations with lower total and low-density lipoprotein cholesterol as well as vascular health benefits, such as decreased aortic calcification and arterial stiffness[359-362]. The fact decreases in SUBQ fat were shown to be associated with improvements in physical function may be due to the fact that the spearman correlation coefficient between 12-month change in total and SUBQ thigh fat was 0.97 ($p < 0.0001$). Thus, almost all of the variance in change in SUBQ fat is explained by change in total thigh fat, suggesting that a more optimal lean to fat mass ratio in the thigh is underlying improvements in physical function and not a specific decrease in SUBQ thigh fat. This study is consistent with and expands upon previous cross-sectional work showing that IMAT, VAT and HU are associated with worse muscle or physical performance and adverse health conditions that may negatively affect muscle or physical performance[52,161,347,348,363]. In a study by Villareal et al., obese older adults participating in a 12-month weight-loss plus physical activity intervention participants in the weight-loss plus exercise group improved their physical function to a greater

degree than the weight-loss only, exercise only and control groups. The authors suggested that positive changes in body composition may be underlying these improvements in physical function[48]. The results of this study corroborate this hypothesis.

The results of this study also show that a dietary weight-loss intervention in conjunction with a moderate physical activity intervention results in decreases in total body lean and fat mass. The PA+WL group experienced a 5-fold greater percent decrease in total body lean compared to fat mass (Table 7). This is consistent with other studies that used DXA to measure total body lean and fat mass following a weight loss and physical activity intervention[48,173]. For example, a study by Avila et al. showed that older adults (age 67 ± 4 years) participating in a weight-loss plus resistance training intervention lost ~11.6% of their baseline fat mass while gaining a slight amount of lean mass (1.5%)[173]. The fact that participants in the Avila study gained lean mass and those in the PA+WL group in the current study lost a significant amount of lean mass, is likely attributable to differences in the PA intervention. The PA intervention in the Avila study consisted exclusively of upper and lower body resistance training, whereas the study described in this paper implemented a PA intervention consisting primarily of treadmill walking, supplemented with lower extremity resistance and balance training. In the study by Villareal et al., described previously, obese older adults participating in a 12-month weight-loss plus physical activity intervention lost 16% of their fat mass compared to 3% of their lean mass over the course of the trial[8].

Results of this study also show that weight-loss in conjunction with moderate physical activity appears to preferentially target the harmful fat depots of IMAT compared to SUBQ fat in thigh (-23.6% vs. -12.3%) and VAT compared to SUBQ abdominal adipose tissue (-15.1% vs. -8.2%). This is consistent with previous studies showing that both weight-loss induced via caloric

restriction, exercise and in combination result in decreases in IMAT and VAT[147,173,175,177]. For example, a study by Murphy et al. showed that both exercise induced weight-loss and caloric restriction reduced weight loss result in a greater reduction of IMAT compared to SUBQ thigh fat [177]. However, this same study showed that exercise induced weight-loss, but not caloric restriction induced weight-loss, resulted in greater reductions in VAT compared to SUBQ abdominal adipose tissue[177]. Finally, it is important to note that the Murphy et al study showed that exercise induced weight-loss, compared to caloric restriction reduced weight-loss, resulted in greater decreases in IMAT and VAT. The physical activity intervention implemented in the study described in this paper was not designed to induce weight-loss. However, the results from the current study are consistent with the Murphy et al. study, as the PA+SA group lost a significant amount of IMAT (-14.5%), despite not losing significant amounts of any other fat depot. The current study was not equipped to test whether or not physical activity in addition to weight-loss is necessary to induce reductions in these fat depots, as there was no weight-loss only group. However, these results while taken into consideration with the results of the Murphy et al. study, suggest that physical activity, in addition to weight-loss, may be necessary in order to optimize body composition remodeling[177]. Additionally, in a randomized controlled trial comparing a moderate physical activity intervention, not designed to induce weight-loss, to an education control group, the education control group gained a significant amount of intermuscular fat (IMAT), whereas the moderate physical activity group did not. Another study highlighting the effect of physical activity on IMAT showed that healthy humans, following 4-weeks of single leg immobilization, gained significant amounts of IMAT, in both the calf and thigh, in the immobilized leg despite a statistically significant decrease in total body mass[363]. Additionally, in a randomized controlled trial comparing a moderate physical activity

intervention, not designed to induce weight-loss, to an education control group, the education control group gained a significant amount of IMAT, whereas the moderate physical activity group did not[148].

The biological mechanisms by which decreasing these specific fat depots may improve physical performance are unclear. VAT is strongly associated with insulin resistance independent of overall adiposity and with higher levels of proinflammatory cytokines[52,347,348]. Therefore, decreases in VAT may result in improvements in metabolic and inflammatory states, which may positively affect muscle and physical performance. Similarly, IMAT and HU have been shown to be related to insulin resistance and the metabolic syndrome[52,146,161,351]; therefore, decreasing IMAT and HU levels may result in a more optimal metabolic state, resulting in improved physical performance. A few recent trials conducted in younger adult men (age 30-65) and women (age 20–41) have shown that decreases in VAT are associated with improvements in insulin resistance[364] and decreases in inflammatory markers[365]. Additionally, in HIV+ patients receiving Tesamorelin, a growth hormone-releasing hormone analogue, percent decrease in VAT was associated with changes in lipid levels and glucose homeostasis[366]. These studies did not examine how these changes affected muscle or physical performance. To our knowledge, the relationship between decreases in the fat depots present in skeletal muscle, IMAT and HU, and metabolic and inflammatory markers has not been examined. Further intervention trials are needed examining the specific relationships between decreases in IMAT, HU and VAT with inflammatory and metabolic markers and how they impact physical function in older adults.

The current study has several strengths. First, both interventions were effective, as participants in the PA+WL group lost a significant amount of total body weight on average and both groups substantially increased their physical activity levels during the adoption and

transition periods of the intervention. Physical performance was measures objectively using the well validated and widely used SPPB, rather than self-reported physical function. This eliminates any potential recall bias. The assessment staff was blinded to the intervention assignments of the participants. The gold standard method of CT was used to measure regional body composition depots. This study also measured body composition with DXA. DXA is widely used and well validated; implementing it allowed comparison of results with many other studies. This study also has a few limitations. First, this study lacks a weight-loss only group and a true control group. Including these two groups would have allowed the exclusive affects of weight-loss to be compared to those of physical activity and the combination of both to a control group. The participants were mostly white females (84.3%), fairly healthy and high functioning at baseline, thus the findings may not be as relevant to minority, male or more frail older adults. Finally, this study had a relatively small sample size.

In conclusion, weight-loss in conjunction with physical activity resulted in significant improvements body composition, which were related to improved physical function in older adults. Physical activity, consisting primarily of treadmill walking, supplemented with lower extremity resistance and balance training, seems to target IMAT. Decreases in the regional fat depots of IMAT, HU and VAT, regardless of intervention assignment, were significantly related to improved physical performance in older adults. Therefore, targeting these fat depots may be of particular importance when aiming to improve physical function or prevent physical disability in older adults. The results of this study have important public health implications as men and woman age 60 and older have higher prevalence rates of obesity compared to any other age group[349] and obesity is a well-known independent risk factor for age-related physical disability[163,164]. This study provides important and novel insights into the mechanisms by which

intentional weight-loss in combination with moderate physical activity may improve physical performance in older adults. Weight-loss and physical activity appear to target the particularly harmful fat depots of IMAT and VAT and we've shown for the first time that decreases in these depots were related to improvements in physical function. The results of this research provides clinicians and public health professionals with important information they can utilize to prevent age-related physical disability.

3.0 THE RELATIONSHIP BETWEEN MITOCHONDRIAL ENERGY PRODUCTION AND MOBILITY IN OLDER ADULTS WITH A WIDE RANGE OF FUNCTION

A portion of age-related declines in gait-speed may be attributable to mitochondrial dysfunction. Mitochondria produce over 90% of ATP needed for movement and the capacity for oxidative phosphorylation decreases with age. We examined the association between mitochondrial function and walking performance in older adults. Phosphocreatine (PCr) recovery in the quadriceps was measured following an exercise-bout using 31P magnetic resonance spectroscopy and ATPmax (mM ATP/s) was calculated. Participants were from an ancillary to the Lifestyle Interventions and Independence for Elders (LIFE) Study (n=36), which recruited functionally impaired participants (Short Physical Performance Battery (SPPB, 0-12), 7.9±1.2) and also from the Study of Energy and Aging (SEA, n=29), which enrolled higher functioning participants (SPPB, 10.9±1.4).Walking performance was defined as time (s) to walk 400m over level-ground at usual-pace and participants were asked if anything was bothering them upon completion. Participants were aged 77.6±5.3 years, 64.2% female, 67.2% white and SPPB scores ranged from 3-12 (9.2±2.0). In linear regression analyses, walk-time was significantly related to ATPmax in the SEA cohort (β -209.02, p=0.02), but not in the combined (β -65.31, p=0.32) nor the LIFE (β 42.70, p=0.74) cohorts. When we examined those who reported no discomfort at the end of the walk separately, walk-time was significantly related to ATPmax in the combined

cohort (β -160.02, p=0.04) and the relationship in LIFE was β -127.38, p=0.27.This suggests that oxidative capacity of skeletal muscle mitochondria may limit walking performance in higher functioning older adults and functionally impaired older adults able to walk 400m without experiencing discomfort.

3.1 INTRODUCTION

Mitochondria produce over 90% of ATP needed for movement[7]. The capacity for oxidative phosphorylation in skeletal muscle, mitochondrial function and mitochondrial content has been shown to decrease significantly with age[156,249,290,367]. Gait-speed has also been shown to decrease with age by about 0.21-0.29 m/s per decade with considerable individual variation and is slower gait is associated with an increased risk for mortality[41,368]. Age-related decreases in skeletal muscle mitochondrial energy production may contribute to declines in muscle and ultimately walking performance in older adults[367]. Experimental evidence has shown that the accumulation of mutations or deletions in mtDNA lead to mitochondrial dysfunction and reductions in ATP production[239,259]. Additionally, poorer mitochondrial function has been shown to be independently related to lower physical function in a study involving hospitalized and community-dwelling older adults[312]. More recently, Coen at el. showed that both in vivo mitochondrial function measure by ^{31}P magnetic resonance spectroscopy (MRS) and mitochondrial efficiency calculated from ^{31}P MRS and state 3 respiration measured in isolated mitochondria obtained from muscle biopsy, were significantly associated with walking speed in older adults[314].

Many reasons exist for age-related slowing and why some decline more than others; however the role of age-related changes to skeletal muscle is still unclear. Sarcopenia, the loss of muscle mass with age, was thought to be the primary cause of decreasing mobility and physical function with age; however, longitudinal evidence suggests that decreasing muscle mass only modestly predicts mobility in older adults[369] and other muscle qualities are related to mobility independent of muscle mass[30]. Furthermore, Fleg et al. has shown in the Baltimore Longitudinal Study of Aging that maximum aerobic capacity (VO_2 peak) normalized to fat-free mass decreases with age at similar rates across those who report low, intermediate and high levels of physical activity[231]. This suggests that decreases in aerobic capacity cannot be fully explained by changes in body composition and lower levels of physical activity. One of the major components of aerobic capacity is the ability of skeletal muscle mitochondria to convert oxygen to energy in the form of ATP. This finding by Fleg et al. suggests the importance of exploring the cellular and energetic bases of mobility and functional disability in older adults.

The purpose of this paper is to expand upon the findings presented by Coen et al. by examining the relationship between mitochondrial energetics and functional performance in older adults with a wide range of functional capacities. Two separate study cohorts were utilized. The first cohort is from the Study of Energy and Aging (SEA), which contained high functioning, physically active older adults and was the focus of the study conducted by Coen et al[314]. The second is a subset of participants participating in The Lifestyle Interventions and Independence for Elders (LIFE) Study, which contains functionally impaired sedentary older adults[39]. Combining these two cohorts provides a unique opportunity to examine the relationship between skeletal muscle mitochondrial energetics and physical performance in a population of older adults with a wide-range of functional abilities. Both studies implemented the non-invasive

and direct measure of ^{31}P Magnetic Resonance Spectroscopy (MRS) to measure ATP generation by mitochondria present in the quadriceps. This research is an important step towards the development of a non-invasive biomarker of declining function and may identify a new therapeutic target to treat and prevent declining physical function in older adults.

3.2 METHODS

3.2.1 Participants

For these analyses, participants from two separate studies were examined. The cohorts were examined both separately and in combination. Rationale for examining these two cohorts in combination is presented below. The first study was the cross-sectional Study of Energy in Aging-Pilot (SEA), conducted from May through August 2011. The second study was the randomized controlled trial: The Lifestyle Interventions and Independence for Elders (LIFE) Study. A subset of LIFE participants who completed an ancillary study is included in the current study. LIFE baseline data only, collected between June and August of 2011, are included in these analyses. The reason these studies were examined in combination are two-fold. First, because the SEA population was highly active and high functioning and the LIFE participants were functionally limited and sedentary by design. Combing the two cohorts would allow for the examination between ATPmax and time to walk 400m in older adults with a wide range of functional capacity. Second, the primary outcome of time to walk 400m and the primary predictor of oxidative capacity of the quadriceps (ATPmax), measured by ^{31}P MRS were

identical. These two studies as well as definitions for combined measures will be described below.

SEA participants were community-dwelling (n=37) men and women aged 70–89 years from the Pittsburgh, PA area. Eligibility was determined via a standardized telephone screening interview. The inclusion criteria were age 70–89 years; body weight less than or equal to 285 lbs. for men and less than or equal to 250 lbs. for women; body mass index 20–32 kg/m^2; ability to walk without the assistance of a device or another person; free of basic activities of daily living disability, defined as no difficulty getting in and out of bed or chairs, and no difficulty walking across a small room; no history of hip fracture; no heart attack, angioplasty, or heart surgery within the past 3 months, no cerebral hemorrhage within the past 6 months, stroke within the past 12 months, or chest pain during walking in the past 30 days; no symptomatic cardiovascular or pulmonary disease; no regular pain, aching, or stiffness in the legs, hips, knees, feet, or ankles when walking; no bilateral difficulty bending or straightening fully the knees; not regularly taking Coumadin, Plavix, Aggrenox, Ticlid, or Agrylin/Xagrid. They had to be able to have a magnetic resonance scan (able to lie still on back for 1 hour; and no metal or other implants, artificial joint replacements, or tattoos. They had to be able to wear the armband activity monitor (no disability in right arm; no swimming or water aerobics $\geq$ 3 times/week; and no supplemental oxygen use in household); and have ability to understand and sign an informed consent. Additionally, to qualify as an enrollee, participants had to provide written informed consent and complete the first day of the clinic visit, including the clinic questionnaire, height and weight, blood pressure and pulse, leg extensor strength (1-repetition maximum) and power, physical exam, short physical performance battery (SPPB), and the 400 meter walk. All participants

provided written informed consent. The study was approved by the University of Pittsburgh Institutional Review Board.

The LIFE Study is a multi-center randomized controlled trial; design, methods and rationale have been described in detail elsewhere[39]. Participants included in this study completed an ancillary study exclusive to the Pittsburgh site (n=39). Briefly, the LIFE Study eligibility criteria were designed to target older persons (age 70–89) who (a) were sedentary, as defined as spending less than 20 min/wk in the past month getting regular PA and reporting less than 125 min/wk of moderate PA[370]; (b) were at high risk for mobility disability based on objectively assessed lower extremity functional limitations assessed by the Short Physical Performance Battery (SPPB; score of ≤9)[25,39,67]; (c) could walk 400 m in 15 minutes without sitting, leaning, or the help of another person; and (d) could safely participate in the intervention. These analyses include baseline data for cross-sectional examination only. Potential participants were screened by telephone. Those who remained eligible following the telephone screening were invited to attend a group or individual prescreening visit during which the LIFE Study was presented in a lecture or individual format. Following a question and answer session, attendees were invited to review and sign a prescreening consent form. Those still eligible after administration of the SPPB and the Community Healthy Activities Model Program for seniors (CHAMPS-18) PA questionnaire[370] were invited to attend the first screening visit. If still eligible, participants attended a second and final screening visit, at the end of which they were randomized.

Approximately halfway through LIFE recruitment, participants from the Pittsburgh site only were informed about the opportunity to participate in an ancillary study following their randomization into the LIFE study. The ancillary study included one separate visit to perform a ^{31}P Magnetic Resonance Spectroscopy scan to measure oxidative capacity of the quadriceps

(ATPmax). This method will be described in detail below. To be eligible for the ancillary, participants had to be enrolled in the LIFE study, and be able to have the magnetic resonance (MRI) scan (not claustrophobic, able to lie still on back for 1 hour; and no metal or other implants, or bilateral artificial joint replacements, or tattoos). Participants had to be able to understand and sign a separate consent in order to participate in the ancillary study. The ancillary study was approved by the LIFE study's emerging science committee and data management and coordinating center as well as by the University of Pittsburgh's Institutional Review Board.

3.2.2 Clinic Examination and Measurements

Participants from both studies were examined at the Health Studies Research Clinic at University of Pittsburgh, Center for Aging and Population Health. As mentioned previously, LIFE study design and methods have been described in detail elsewhere[39]. In both SEA and LIFE participants, body height (cm) was measured using a wall-mounted stadiometer and body weight (kg)with a standard certified calibrated scale and were used to calculate BMI (weight (kg)/height (m^2)). Participants in both studies completed questionnaires concerning demographic information and self-reported medical history. Questions were asked in a similar manner in both studies, with a few exceptions. These exceptions will be described immediately below. History of arthritis was measured slightly differently, in SEA the question was "has a doctor ever told you that you had arthritis or rheumatism", in LIFE it was "have you seen a Dr. in the past 6-months for arthritis or rheumatism. For self-reported health status, SEA participants were asked "compared to people your own age, would you rate your overall health as, excellent, good, fair , poor or very poor" whereas LIFE used the more standard "would you say your health is excellent very good, good fair or poor" from the SF-12[371]. To describe the combined cohort, this question was

dichotomized into those who responded fair or worse and those who responded good or better. As a measure of global fatigue, SEA participants were asked “during the past 4 weeks how much of the time do you feel tired from 0-6”, where 0 is all of the time and 6 is none of the time; whereas LIFE participants were asked “during the past week how often have you felt tired, all of the time, most, a good bit, some or a little bit of the time. To describe the combined cohort, global fatigue was dichotomized into those who were tired some of the time or more in LIFE or answered 4 or less in SEA, compared to those who responded a little bit of the time in LIFE or 5 or 6 in SEA. Alcohol consumption, in drinks per week, was categorized in SEA and open-ended in LIFE. To describe the combined cohort, alcohol was dichotomized into those who consume 6 or more drinks per week and those who do not.

Finally, the last discrepancy has to do with how physical activity was measured in the two studies. 7-day free living physical activity was measured objectively in both studies. In the LIFE study, the Actigraph™ accelerometer, model GT3X, was used and in the SEA study, the multi-sensor Sensewear™ (Bodymedia Pittsburgh, PA) armband was used. The instruments were used to calculate minutes per-day spent in different modes of activity – sedentary, light and moderate and above physical activity. NHANES cut-points were used to categorize Actigraph counts into minutes per day and the Sensewear’s manufacturer devolved proprietary algorithm was used to categorize minutes per-day. In order to describe the combined cohort, minutes per day spent in different intensities of physical activity were combined.

As mentioned previously, objective lower extremity function was measured by the SPPB in both studies. The SPPB includes a 4m walk, chair stands and a balance[23].

3.2.3 400-Meter Walk

Time to walk 400m is the primary outcome for these analyses. The 400m walk protocol for both SEA and LIFE have been described elsewhere[39,314]. Briefly, the test was conducted on level ground using a 20-meter course. Participants were instructed walk at their usual pace and without overexertion for10 laps (20m up and back). Participants were reminded to walk at their usual pace every lap. Participants were required to complete the walk in less than 15-minutes. Seated blood pressure and pulse were reviewed for safety before the walk. There was one discrepancy in regard to the walk protocol between LIFE and SEA. In SEA, participants were not permitted to stop and rest during any portion of the test. However, since LIFE was designed to recruit functionally limited older adults, participants were permitted to rest standing in place, without touching the wall. Participants were still required to complete the walk in 15 minutes. Participants in both studies were asked if "anything was bothering them at the end of the walk". Discomfort following the walk was defined as responding yes when asked by the examiner "Is anything bothering you?" upon completing the walk. In SEA, the question was categorical, and in LIFE it was opened ended. In LIFE, 13 (36.1%) participants reported discomfort at the end of the walk (Table 11): three reported back pain or hip pain/discomfort, 2 reported knee pain, two reported light headedness, one reported being tired and experienced foot pain and one person reported discomfort during the walk and requested their straight cane (groups not mutually exclusive). In SEA, 5 (17.2%) reported discomfort at the end of the walk: reported calf or back pain, two reported shortness of breath, one reported hip, foot or knee pain and four reported other (groups not mutually exclusive).

Total time in seconds was used for these analyses. In order to eliminate the skewedness caused by participants stopping during the test, quartiles of 400m walk time were generated and

time to walk 400m was treated as both a categorical and continuous variable. One participant from the SEA study is excluded from analyses due to being unable to complete the 400m walk.

3.2.4 Determination of ATP_{max} by ^{31}P MRS

^{31}P MRS measures the regeneration of phosphocreatine (PCr) after a short bout of exercise to characterize mitochondrial ATP production. PCr is a high energy phosphate that can be readily converted to ATP and vice-versa via the creatine kinase reaction (PCr + ADP H+ ↔ ATP + Cr). PCr is the initial full-source of skeletal muscles. A linear relationship links change in PCr with exercise to mitochondrial oxidative phosphorylation rate[275,276]. Therefore, measuring the dynamics of the PCr shuttle provides a method to estimate muscle oxidative capacity in vivo[156,277]. Furthermore, this method has been confirmed to be a good measure of oxidative capacity in rodent and human studies showing that ATPmax varies in direct proportion to the activity of oxidative enzymes in skeletal muscle [278,279]. It has also been shown to reflect mitochondrial content in human skeletal muscle[280]. The amount of ATP production from glycolysis has been shown to be minimal in resting skeletal muscle (<8%)[237]. Regardless, this method can tease out the amount of ATP production by glycolsis by determining the pH from the chemical shift of the free phosphorous peak relative to the PCr peak[281]. H+ ions, which would lower the pH, are a byproduct of glycolysis.

Briefly, the exercise protocol took place in a typical MRI magnet (3T TIM Trio magnetic resonance scanner, Siemens' Medical System). Participants laid flat on their backs with the knee of the right leg (unless contraindicated) supported so that the leg was slightly elevated at a ~30 degree angle. Straps were place over the legs and a 2.5" surface RF coil tuned to ^{31}P is placed over the right quadriceps. At two different points during the scan, they were asked to kick

repeatedly as hard and as fast as they can, producing fast contractions at the highest rate possible for ~30s, followed by a 6 minute rest period. The protocol was designed to deplete PCr stores by at least 33-66% without lowering pH below 6.80 during the recovery period. PCr recovery rate was measured during this rest period.

Phosphorus spectra was collected using a 3T TIM Trio magnetic resonance scanner (Siemen's Medical System, Erlanger, Germany), this has also been described elsewhere[314]. A standard one pulse experiment was used to determine the levels of PCr, ATP, Pi, and pH throughout exercise and recovery. PCr, Pi, and ATP peak areas in the fully relaxed spectra were measured by integration using Varian VNMR 6.1C software (Varian Medical Systems, Palo Alto, CA). Areas of the PCr and Pi peaks were expressed relative to the ATP peak and quantified using a resting PCr value of 27mM as determined from biopsies of human vastus lateralis muscle[156]. Changes in PCr and Pi peak areas during the tests were analyzed as previously described[372,373].

Six participants from SEA and three participants from LIFE are missing ATPmax data due to either inadequate PCr breakdown or pH levels dropping too low during the recovery period. SEA and LIFE participants were tested using the same magnet and the scans were conducted by the same technician in both studies.

3.2.5 Statistical Analyses

Baseline characteristics including means and standard deviations for continuous variables and frequencies and percent's for categorical variables were generated for each study separately and combined. T-test, non-parametric, chi-squared and Fischer's exact test were used, where appropriate, to test for any between study differences. The primary aim of these analyses was to

determine the relationship between ATPmax and time to walk 400m, in each study separately and then combined. This was done using multiple linear regression to predict time to walk 400m using ATPmax. Univariate and models adjusted for age, sex, race and BMI. Since it is well documented that those with type 2 diabetes have significantly lower mitochondrial function, we also examined the affect that diabetes has on the relationship between ATPmax and time to walk 400m. Adding diabetes to the model containing ATPmax, age, sex, race and BMI has very minimal effect and the p-value corresponding to the beta coefficient for diabetes was 0.95. Additionally, the interaction term containing ATPmax and diabetes status was not significant (p=0.21). Finally, we examined models stratified by diabetes; ATPmax was not a significant independent predictor of ATPmax in either model.

Since the relationship between ATPmax and walk-time were drastically different in the SEA and LIFE cohorts, we conducted further analyses to gain insight into possible confounding factors present in the LIFE cohort. To identify possible confounders, we compared those LIFE participants with the unexpected relationship of being in the highest two quartiles of ATPmax and the lowest two quartiles of walk-time to those in both the highest two quartiles of ATPmax and walk-time (Table 14). These analyses prompted us to examine the relationship between ATPmax stratified by discomfort at the end of the 400m. All analyses were performed with SAS version 9.3.

As mentioned previously, six participants from SEA and three participants from LIFE are missing ATPmax data due to either inadequate PCr breakdown or pH levels dropping too low during the recovery period. One SEA participant was also unable to complete the 400m walk. This yields a final analytic dataset of 29 SEA participants and 36 LIFE participants.

3.3 RESULTS

3.3.1 Baseline Composition of SEA and LIFE Studies

Baseline characteristics of both the SEA and LIFE studies are depicted in Table 11. As evidenced by the data presented in Table 11, these two studies were designed to recruit dissimilar populations of older adults. Participants in the LIFE study were significantly more overweight, more fatigued, less active, lower functioning, as determined by the SPPB, and had slower 4m gait speed (Table 11). The LIFE study also contained a significantly larger proportion of those with diabetes, less with a history of cancer and less who reported drinking 6 or more drinks per week, compared to the SEA participants. Finally, the LIFE study contained a significantly larger proportion of both females and African Americans compared to the SEA study. There were no statistical differences between the two study population in regard to age, self-reported discomfort at the end of the 400m walk, history of peripheral arterial disease, congestive heart failure, myocardial infarction, chronic pulmonary disorder or arthritis. Finally, the two study populations had very similar ATPmax values.

3.3.2 Relationship between ATPmax and 400m Walk-time

ATPmax was not significantly related to time to walk 400m ($p=0.43$) in the combined SEA and LIFE cohorts. However, ATPmax was significantly related to time to walk 400m in the higher functioning, normal weight, physically active SEA population ($p=0.02$), but not in the functionally limited, obese sedentary LIFE population ($p=0.74$, Table 12). In fact, a reciprocal relationship between ATPmax and walk-time was evident (ß= -209.02), indicating higher

ATPmax levels were associated with shorter (faster) walk-times (as hypothesized) in SEA; but the opposite relationship (ß=42.70) was observed in LIFE. These relationships are depicted graphically in Figure 6. When age, sex, race and BMI were adjusted for, ATPmax remained borderline significantly associated with time to walk 400m (p=0.08) in the SEA population, but not in the LIFE or combined SEA and LIFE populations (p=0.12 and 0.85). In the SEA population, ATPmax, age, sex, race and BMI collectively explained 30% of the variance in time to walk 400m (Table 12).

ATPmax was also examined across quartiles of walk-time (Table 13). In contrast to the linear regression models, a test for trend in ATPmax across quartiles walk-time was not significant in SEA (p=0.16), LIFE (p=0.40) or combined (p=0.48). However, when examining mean ATPmax across quartiles in the SEA population, the trend of increasing ATPmax with faster walk-time is evident, with the exception of those in the second fastest quartile having the highest ATPmax. Similarly, in LIFE, the trend of increasing ATPmax with faster walk-time is also evident; however, the slowest quartile of walk-time actually had second highest ATPmax levels on average (Table 13). These relationships are also presented graphically in Figure 7.

The relationship between ATPmax was very different in the SEA compared to the LIFE population (Table 12). Either a protocol difference or study effect is unlikely, since identical protocols were used and the participants were seen in the same research clinic. MRS scans were also conducted by the same technician and in the same magnet for both studies. Additionally, tests were excluded if in adequate PCr breakdown or too low pH levels were observed. However, the drastically different results may be attributable to confounders concerning participant characteristics present in the LIFE but not SEA cohort.

In order to gain further insight into why the relationship between ATPmax and time to walk 400m was drastically different in the LIFE compared to SEA population, we compared those LIFE participants with the unexpected relationship of being in the highest two quartiles of ATPmax and the lowest two quartiles of walk-time to those in both the highest two quartiles of ATPmax and walk-time (Table 14). LIFE participants in the highest two quartiles of ATPmax and lowest two quartiles of walk-time reported a significantly higher proportion of discomfort following the 400m walk compared to the rest of the LIFE participants (87.5% vs. 21.4%., p =0.01) and those in both the highest two quartiles of ATPmax and walk-time (87.5% vs. 20.0%, p=0.02).

Models stratified by experiencing or not experiencing discomfort at the end of the walk, by study and in combination, can be found in Table 15. In SEA participants without discomfort, ATPmax was borderline associated with time to walk 400m (p=0.06), but not in LIFE participants (p=0.27). However, in the LIFE participants not experiencing discomfort higher ATPmax levels were related to shorter walk-times (ß=-127.38, Table 15), compared to the entire LIFE population (ß=42.70, Table 13) and those experiencing discomfort (ß=113.11, Table 15) where ATPmax was associated with longer walk-times. These relationships are depicted graphically in Figure 8. This is important, because we hypothesized that a higher ATPmax would be associated with a shorter (faster) walk-time. Additionally, in the SEA and LIFE combined population, higher levels of ATPmax were significantly related to shorter walk-times (ß=-160.02, p=0.04). After adjusting for age, race, sex, BMI and study cohort, higher levels of ATPmax remained borderline significantly related to shorter walk-times (ß=-132.98, p=0.07), in those without discomfort. In both the LIFE participants experiencing discomfort and in the combined SEA and LIFE population experiencing discomfort, ATPmax was not significantly related to

time to walk 400m. In fact, in the LIFE participants experiencing discomfort, higher levels of ATPmax were associated with slower walk times (ß=113.11, p=0.12).

Table 11. Baseline Characteristics by Study and in Combination

	SEA-Pilot (N=30) Mean (SD) or N (%)	LIFE (n=36) Mean (SD) or N (%)	Combined (n=66) Mean (SD) or N (%)
Age, yrs	78.5 (5.0)	76.5 (5.5)	77.6 (5.3)
Sex , female	14 (46.7)*	29 (78.4)*	43 (64.2)
Race, white	16 (93.3)*	17 (46.0)*	45 (67.2)
BMI, kg/m^2	25.9 (2.7)*	30.8 (5.2)*	28.6 (4.9)
Smoker Current/Former	10 (33.3)	11 (29.7)	21 (31.3)
Alcohol Intake, 6+ drinks•week^{-1}	8 (27.7)*	2 (5.4)*	10 (14.9)
How often Felt Tired, some or more	7 (23.3)*	24 (64.9)*	31 (46.3)
Diabetes, yes	1 (3.33)*	10 (27.0)*	11 (16.4)
History of PAD	0 (0)	0 (0)	0 (0)
History of CHF	1 (3.3)	1 (2.7)	2 (3.0)
History of MI	3 (10.0)	1 (2.7)	4 (6.0)
History of COPD	1 (3.3)	4 (10.8)	5 (7.5)
History of Arthritis	11 (28.9)	9 (23.7)	
History of Cancer	16 (53.3)*	9 (24.3)*	25 (37.3)
Health Rating, Fair or worse	2 (6.7)	4 (10.8)	6 (9.0)
Sedentary Activity, min•day^{-1}	685.3 (123.3)*	627.0 (110.1)*	654.1 (118.5)
Light Activity, min•day^{-1}	245.8 (70.9)*	204.8 (66.2)*	223.0 (71.2)
Moderate Activity, min•day^{-1}	71.2 (66.5)*	1.5 (2.0)*	34.1 (57.2)
All Activity, min•day^{-1}	320.9 (104.4)*	204.3 (66.6)*	259.0 (103.9)
SPPB, 0-12	10.9 (1.4)*	7.9 (1.2)*	9.2 (2.0)
5 Chair-Stand Time, s	11.4 (3.3)*	16.7 (5.8)*	14.3 (5.4)
Gait Speed, , m•s^{-1}	1.2 (0.2)*	0.8 (0.2)*	1.0 (0.3)
Time to Walk 400m, s	343.8 (65.5)*	467.5 (107.1)*	410.3 (108.0)
Discomfort at End of 400m Walk, yes	5 (17.2)	13 (36.1)	18 (27.7)
ATPmax, mM•s^{-1}	0.52 (0.13)	0.54 (0.14)	0.53 (0.14)

***between group difference at p<0.05**

Table 12. Association between ATPmax and Time to Walk 400 meters by Study and in Combination

Model	Beta	SE	STB	p-value	Model R^2
SEA Only - Unadjusted	-209.02	82.79	-0.43	0.02	0.19
SEA Only - Adjusted for age, race, sex and BMI	-176.96	95.29	-0.37	0.08	0.30
LIFE Only - Unadjusted	42.70	128.69	0.06	0.74	0.003
LIFE Only - Adjusted for age, race, sex and BMI	168.40	106.56	022	0.12	0.44
Combined, Unadjusted*	-65.31	82.0	-0.08	0.43	0.32
Combined - Adjusted for age, race, sex and BMI*	-14.73	76.90	-0.02	0.85	0.46

*adjusted for study

Table 13. Mean 400m Walk-time by Quartiles of ATPmax – SEA/LIFE Combined

ATPmax Quartile (n)	400m Walk-Time	Standard Error	F-Test for Trend (p-value)
SEA			0.22
Lowest ATPmax (8)	361.9	22.5	
3rd Highest ATPmax (6)	368.2	26.0	
2nd Highest ATPmax (8)	344.6	22.5	
Highest ATPmax (7)	301.0	24.0	
LIFE			0.82
Lowest ATPmax (10)	444.6	34.6	
3rd Highest ATPmax (8)	491.8	38.7	
2nd Highest ATPmax (9)	469.3	36.5	
Highest ATPmax (9)	454.9	36.5	
Combined			0.19
Lowest ATPmax (17)	383.5	25.8	
3rd Highest ATPmax (16)	457.1	26.6	
2nd Fastest Walk-time (16)	414.5	26.6	
Fastest Walk-time (16)	387.6	26.6	

Table 14. Comparison of LIFE participants in Highest two Quartiles of ATPmax and Slowest two Quartiles of 400m Walk-Time with those in Highest two Quartiles of both ATPmax and 400m Walk-Time

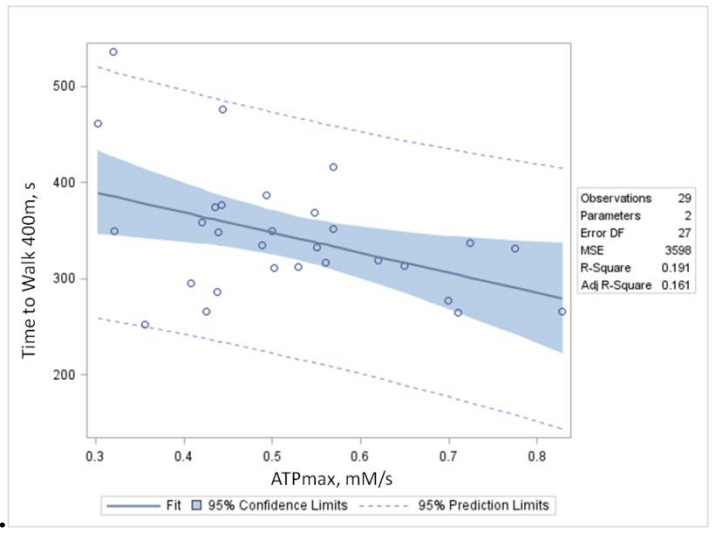
	High ATPmax and Slower Walk-time (n=8)	High ATPmax and Faster Walk-time (n=10)	P-value
Age, yrs	74.9 (6.2)	76.5 (5.8)	0.49
Sex , female	8 (100.00)*	5 (50.0)*	0.04
Race, white	3 (37.5)	5 (50.0)	0.66
BMI, kg/m^2	32.8 (5.9)	29.1 (4.6)	0.14*
Smoking Status, Current/Former	0 (0.0)	4 (40.0)	0.09
Alcohol Intake, 6+ drinks/week	0 (0.0)	1 (10.0)	>0.99
Tired, some or more	5 (62.5)	8 (80.0)	0.61
Diabetes	1 (12.5)	6 (60.0)	0.07*
Dr. for Arthritis last 6-months, yes	2 (25.0)	2 (11.1)	>0.99
Muscle/Joint Stiffness last 6-months,yes	7 (87.5)*	2 (20.0)*	0.02*
Muscle Strain/Soreness last 6-months,yes	4 (50.0)*	2 (20.0)*	0.32
Foot pain last 6-months, yes	2 (25.0)	2 (20.0)	>0.99
History of PAD	0 (0.0)	0 (0)	>0.99
History of CHF	0 (0.0)	0 (0.0)	>0.99
History of MI	0 (0.0)	1 (10.0)	>0.99
History of COPD	0 (0.0)	0 (0.0)	0.55
History of Cancer (non skin)	0 (0.0)*	2 (20.0)	0.48
Health Rating, Poor/V Poor/Fair	0 (0.0)	3 (30.0)	0.22
Minutes of Mod PA	1.3 (1.5)	1.7 (1.5)	0.29
Minutes of Light PA	223.2 (53.9)	195.1 (78.4)	0.33
Minutes of Sed. PA	591.4 (47.8)	639.0 (124.2)	0.25
SPPB, 0-12	7.4 (2.1)	8.4 (0.5)	0.37
5 Chair-Stand Time, s	22.9 (9.1)*	14.3 (3.8)*	0.09*
Unable to do 5 Chair Stands	1 (12.5)	2 (20.0)	>0.99
Gait Speed, m/s	0.77 (0.18)	0.91 (0.19)	0.28
Time to Walk 400m, s	542.4 (142.2)	398.0 (34.2)	0.003
Discomfort at End of 400m Walk, yes	7 (87.5)*	2 (20.0)*	0.02*
ATPmax, mM ATP/s	0.65 (0.13, 0.51 - 0.90)	0.65 (0.10, 0.54 - 0.84)	>0.99

***between group difference at p<0.05**

Table 15. Association between ATPmax and time to Walk 400 meters by study and in Combination Stratified by Discomfort at the end of the 400m Walk

Model	Beta	SE	STB	p-value	Model R^2
In those without Discomfort					
SEA Only - Unadjusted	-194.60	99.28	-0.39	0.06	0.15
SEA Only - Adjusted for age, race, sex and BMI	-185.52	117.11	-0.37	0.13	0.26
LIFE Only - Unadjusted	-127.38	112.47	-0.24	0.27	0.06
LIFE Only - Adjusted for age, race, sex and BMI	-75.85	125.30	-0.14	0.55	0.34
Combined, Unadjusted*	-160.03	74.32	-0.26	0.04	0.37
Combined - Adjusted for age, race, sex and BMI*	-132.98	72.67	-0.22	0.07	0.47
In those with Discomfort					
SEA Only - Unadjusted	-340.02	183.16	-0.73	0.16	0.53
SEA Only - Adjusted for age, race, sex and BMI	-671.31	0.0	-1.44	<0.01	1.0
LIFE Only - Unadjusted	135.03	174.44	0.24	0.46	0.06
LIFE Only - Adjusted for age, race, sex and BMI	195.86	159.10	0.23	0.21	0.61
Combined, Unadjusted*	-18.94	213.48	-0.02	0.93	0.32
Combined - Adjusted for age, race, sex and BMI*	184.37	156.43	0.20	0.26	0.77

*adjusted for study



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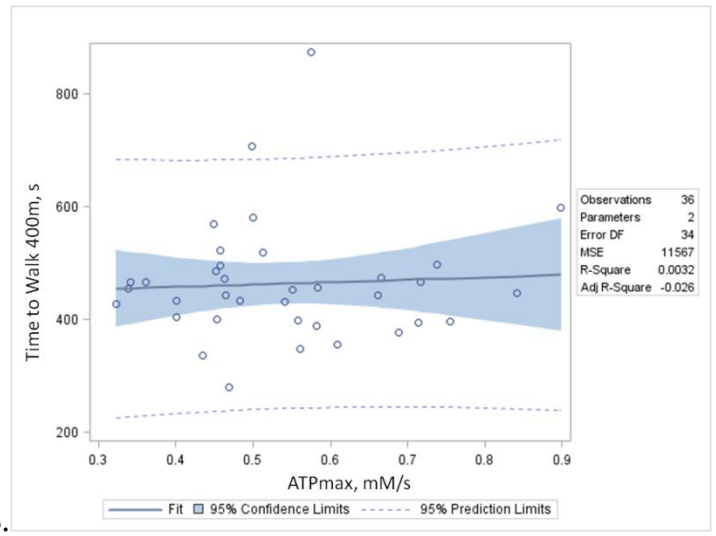

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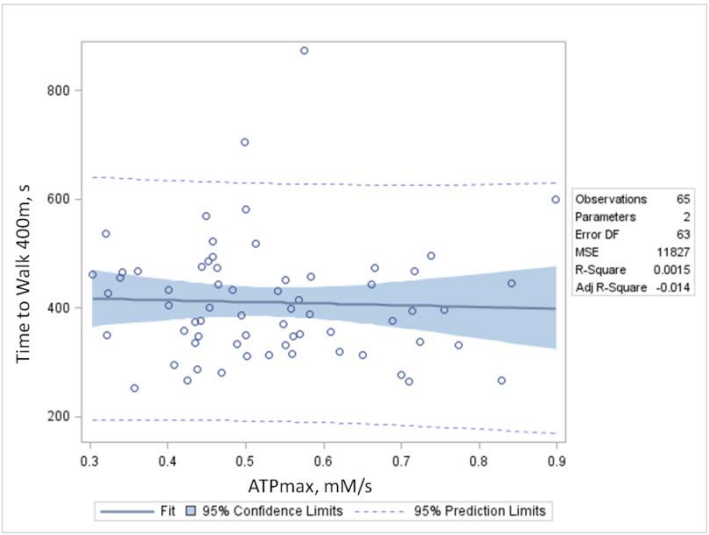


C.

Figure 6. Relationship between ATPmax and 400m Walk in SEA (A), LIFE (B) and Combined (C)

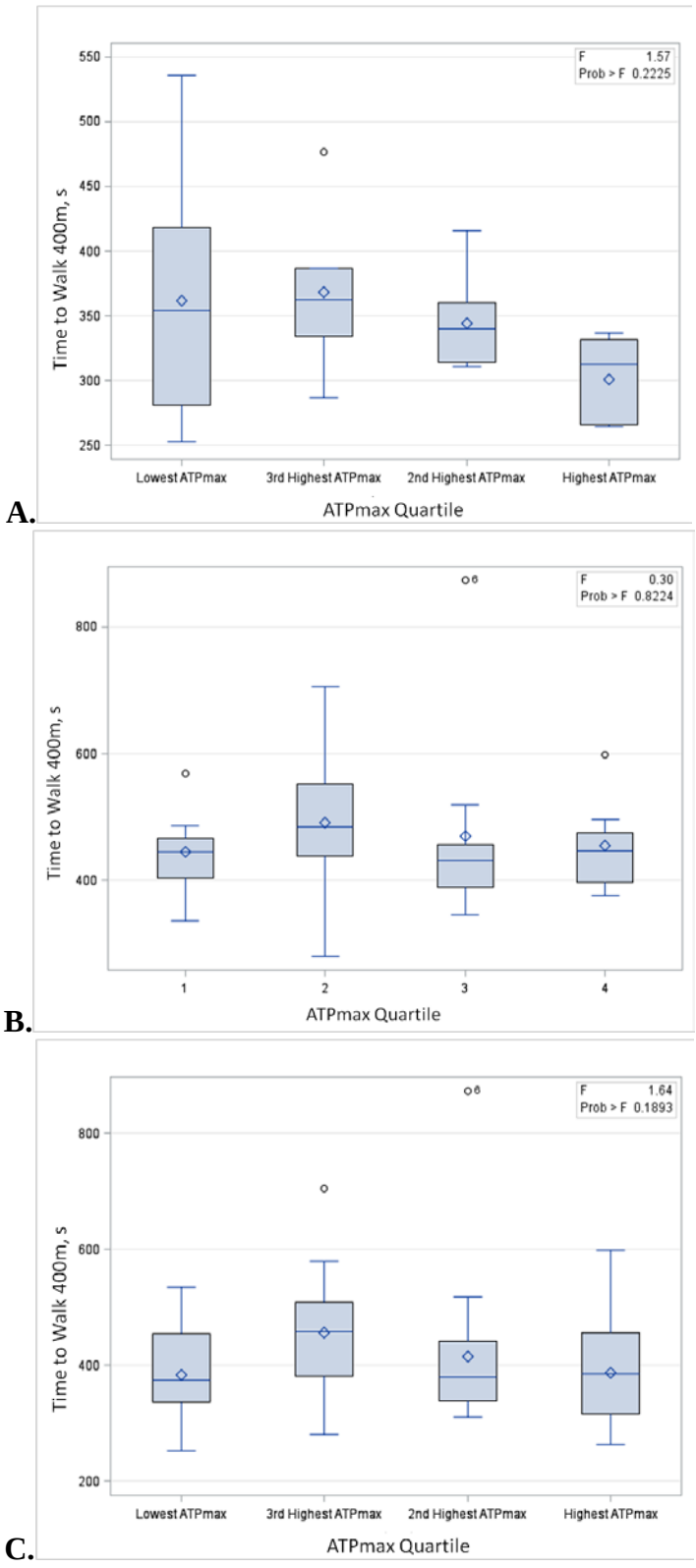
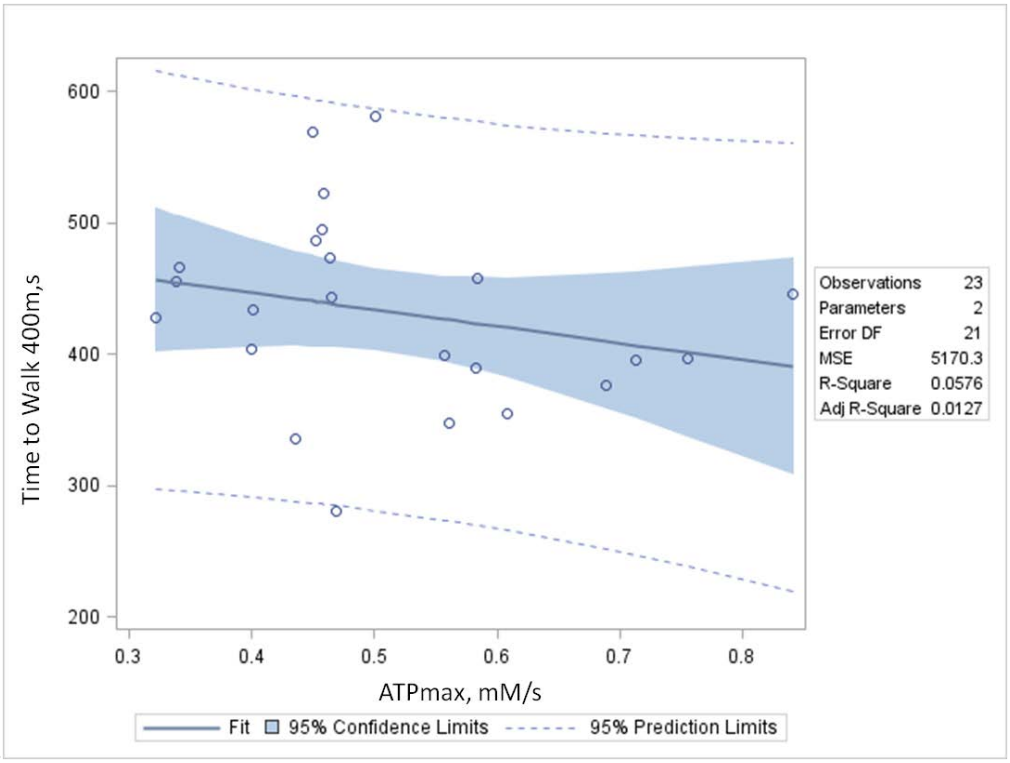


Figure 7. 400m Walk-time across Quartile of ATPmax in SEA (A), LIFE (B) and Combined (C)

A.



B.

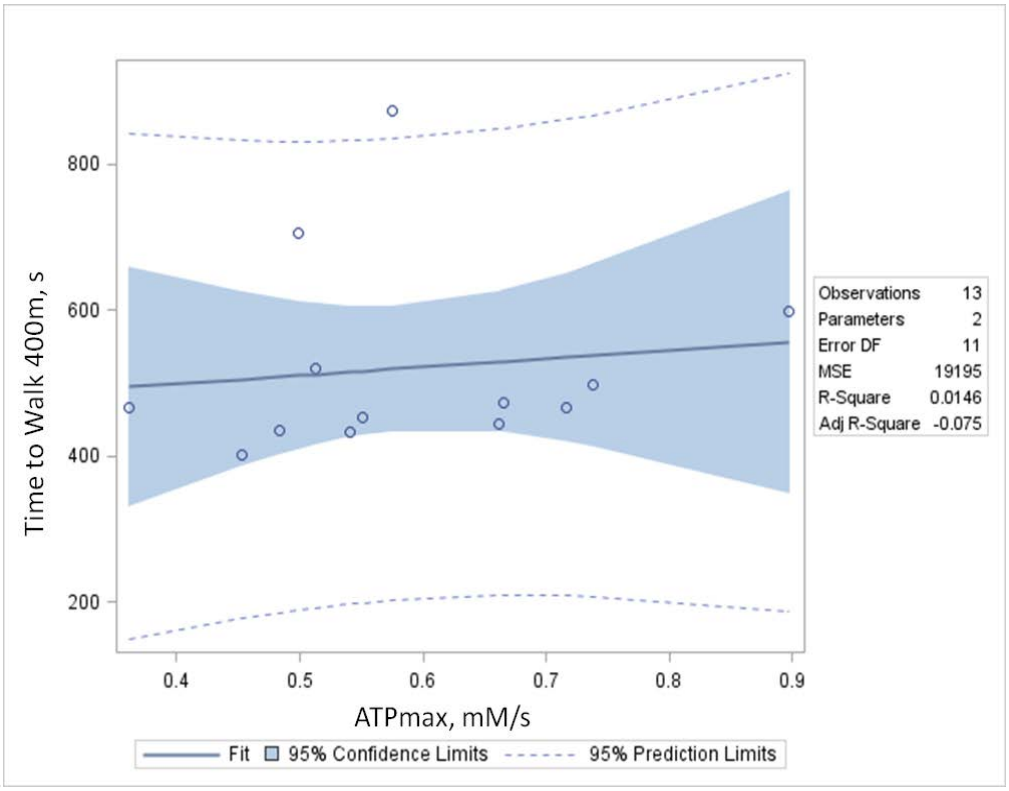


Figure 8. Relationship between ATPmax and Time to Walk 400m in those in LIFE without discomfort (A) and with Discomfort (B) at the End of the Walk

3.4 DISCUSSION

We demonstrated that the relationship between ATPmax and walking performance in older adults varies by physical function and walking ability. This study was designed to extend and expand upon previous work from our group showing that functional oxidative capacity of mitochondria present in the quadriceps (ATPmax) was significantly associated with usual walking speed over a 400m in higher functioning, physical active older adults (SEA study) [314]. Contrary to our hypotheses ATPmax was not associated with time to walk 400m in neither the LIFE population nor the combined SEA and LIFE population (Table 12 and Figure 6). Coen et al. showed in the SEA study, that ATPmax was significantly associated with walking speed during the 400m walk[314]. This is shown in a slightly different manner in Table 12 and Figure 6, Panel A.

These results conflict with previous studies in addition to the work conducted by Coen et al. For example, a study conducted by Bourdel-Marchasson et al[312] showed that shorter half-time for PCr recovery (higher mitochondrial function), measured by ^{31}P MRS, was related to shorter times to complete a modified get-up and go-test in hospitalized and community dwelling older adults. Similarly, a study conducted in PAD patients showed that higher mitochondrial ATP production rate measured in isolated mitochondria obtained from the gastrocnemius medialis muscle via biopsy was related to a longer duration of time walked during a maximal treadmill test[313]. However, both of these studies measured mitochondrial function in the gastrocnemius muscle, whereas the current study used the quadriceps. Additionally, walking performance was measured differently in each of the three studies. Although these are important differences, the drastically different results observed in the current analyses are unlikely attributable to them.

Since identical protocols, MR magnets, technicians and MR spectroscopy analysts were used for the LIFE and SEA studies, we hypothesized that confounding characteristics were present in the LIFE cohort. To indentify these potential confounding factors, we examined those with the unexpected relationship of high ATPmax and slow walk-times in comparison to those with high ATPmax and faster walk times. Since a significantly higher proportion of those with higher ATPmax and slower walk times reported being in discomfort at the end of the 400m walk, we examined models stratified by discomfort status. In LIFE participants not reporting discomfort at the end of the walk, ATPmax was not statistically significantly associated with walk-time. However, interestingly, the direction of the relationship between ATPmax and time to walk 400m was reversed, indicating higher ATPmax was associated with faster walk-times in these individuals. This is the opposite relationship that was observed in the entire LIFE cohort and those in LIFE experiencing discomfort. In the SEA and LIFE combined population without discomfort, higher levels of ATPmax were significantly related to shorter walk-times. After adjusting for age, race, sex, BMI and study, higher levels of ATPmax remained borderline significantly related to shorter walk-times in the combined population not experiencing discomfort.

Interestingly, the relationship between ATPmax and time to walk 400m was similar in those SEA participants both with and without discomfort at the end of the walk. This is in obvious contrast to the LIFE population. The combination of being lower functioning, sedentary and obese may affect the relationship between ATPmax and walk-time in those experiencing discomfort at the end of the walk. That is, these characteristics, along with the discomfort, are resulting in slower walking speeds than ATPmax would predict; meaning that the LIFE participants are not physically robust enough to overcome their discomfort in order to continue to

ambulate at their preferred pace. In contrast, the reason ATPmax is related to time to walk 400m in SEA participants with discomfort and not LIFE may have to do with the SEA participants being physically robust enough to overcome their discomfort and walk at their preferred pace. Thus, oxidative capacity of the mitochondria present in the quadriceps (ATPmax) is a limiting factor in these SEA participants, but not in LIFE participants with discomfort. We then compared the characteristics of those experiencing discomfort in SEA to those experiencing discomfort in LIFE and the data presented seem to support this hypothesis, as participants in LIFE with discomfort had slower 4m gait-speeds, slower 400m walk-times, higher BMI (not statistically significant, but clinically significant) lower SPPB score and lower levels of moderate PA.

To our knowledge, this is the only study to have examined mitochondrial function in older adults with and without discomfort during an objective walking test. This is a novel and important finding. The current study supports the hypothesis that mitochondrial function is related to physical function in older adults and expands upon previous work in this area[312-314]. Specifically, this study suggests that declines in mitochondrial function may be an early marker of functional decline in high functioning older adults or older adults able to walk a longer distance without discomfort. Thus, preserving mitochondrial may be particularly effective in preventing declines in physical function and physical or mobility disability in older adults who are still functionally adept, or beginning to decline functionally, but are still able to walk a quarter of a mile (400m) without experiencing discomfort.

We have several possible explanations for why ATPmax was significantly related to time to walk 400m in those without discomfort after the walk, but in neither those experiencing discomfort in LIFE, nor in the entire SEA and LIFE cohort. First, the presence of discomfort during the walk may have caused participants to slow down for reasons unrelated to oxidative

capacity of skeletal muscle. Higher mitochondrial energy production may not have been enough to overcome physical discomfort during the test. Joint impairments[374], arthritis[65,375], knee pain[376], back pain[377] and bodily pain[378] are associated with slower walking speed and/or physical disability in older adults. Likewise, those reporting dizziness or lightheadedness likely slowed down during the walk for reasons unrelated to lower mitochondrial function. Again, these pain symptoms could have resulted in slower walk-times, "overriding" any benefit of higher oxidative capacity or mitochondrial energy production of the quadriceps.

Secondly, we were lacking mitochondrial function data from biopsy and a measure of oxygen delivery to the quadriceps. In the study conducted by Coen et al, from the SEA study, mitochondrial efficiency was calculated as the ratio of functional, in vivo mitochondrial energy production from ^{31}P MRS, over maximal mitochondrial function measured in mitochondria isolated from muscle fibers extracted from the quadriceps via muscle biopsy. Coen et al. also showed that mitochondrial efficiency (ATPmax/state 3 respiration) was related usual walking-speed independent of aerobic capacity (VO2peak). The LIFE ancillary study did not take muscle biopsies. Lower mitochondrial efficiency, due inner mitochondrial membrane or electron transport chain impairments, may be related to walking speed in lower functioning older adults (LIFE) or older adults with a wide-range of function (LIFE and SEA combined). Oxygen delivery to skeletal muscle is also crucial for mitochondrial energy production. We did not obtain a measure of oxygen delivery and it is possible that having a measure of O2 delivery and calculating the ratio of oxygen delivered to energy produced may be more related to walking speed than ATPmax. However, in the Coen et al. study state 3 respiration multiplied by quad volume explained about 33% of the variance in VO2 peak, which is the about the amount of

oxygen consumption the quadriceps has been shown to contribute; this suggests a lack of 0_2 delivery impairment[379].

Finally, it is plausible that those with both higher levels of ATPmax and slower walk times are less biomechanically efficient than compared to other participants. Meaning they are producing more energy (ATP) to do less work; for example, producing more energy but walking more slowly. This hypothesis seems to be supported by the data presented in this study. First, in the LIFE population, those in the slowest quartile of walk-time also had the second high levels of ATPmax. Additionally, when we examined the relationship between ATPmax and walk-time in those reporting no discomfort at the end of the walk in the combined SEA and LIFE cohort, ATPmax was significantly related to walk-time and was not in the entire cohort. Additionally, higher levels of ATPmax were actually associated with slower walk-times in LIFE participants experiencing discomfort. These findings seem to support the hypothesis that the LIFE participants, due to being functionally limited, more obese, more sedentary and experiencing more discomfort at the end of the walking test were walking less biomechanically efficient. Therefore higher ATPmax levels may not have been associated with shorter walk-times because these participants were producing similar or larger amounts of ATP to do less work. We were able to account for some of this by eliminating those reporting discomfort after the walk.

Discomfort during walk the walk resulting in slower walk-times than ATPmax would predict may, in part, explain why ATPmax was related to time to walk 400m in higher functioning, active SEA participants, but not in the lower functioning, sedentary LIFE participants nor the combined LIFE and SEA cohort. The two main theories of human ambulating, the six determinants of gait[380] and the inverted pendulum theories both center around the idea that human gait is designed to move the body, or center of mass, in the most energy

efficient manner possible[381,382]. Alterations to normal gait, in terms of step-length, frequency and width may result in a less biomechanically efficient gait[382]. These alterations may be caused by a number of factors including joint pain, obesity and unsteadiness. Further studies designed to measure gait characteristics and efficiency, and how they affect and/or modify the relationship between ATPmax and walking-speed are needed.

This study has numerous strengths. This study is that this is the largest study, to our knowledge, to examine the relationship between skeletal muscle mitochondrial energetics and walking performance in older adults. This study also examined this relationship in an older adult population with a wide-range of functional abilities, and also in a large number of both men and women and white and African Americans. The noninvasive and direct measure of ^{31}P MRS was used to measure mitochondrial energy production in vivo. This technology involves little participant burden, whereas the traditional method of muscle needle biopsy is highly invasive, induces considerable participant burden and must be performed by a physician. Additionally, mitochondrial measurements using biopsy may not accurately reflect actual mitochondrial energy production in the living human body whereas MRS is a direct measure of in-vivo MEP. Also, physical function was measured objectively using the performance measure of time to walk 400m. This study also has several limitations. The participants come from two separate studies; however, the primary outcome and predictor were identical. Also, study cohort was adjusted for in the combined multivariable regression models. Several of the limitations have been discussed previously, such as the lack of biopsy measures to calculate P/O and the lack of muscle mass quantification. Additionally, we were lacking a measure of muscle mass. Muscle mass may be a key “missing piece” from these analyses. For example, the LIFE participants have similar mitochondrial function to the SEA participants but this may not be resulting in faster walking

speeds due to lack of muscle mass to produce and utilize the ATP. Conley et al. have shown that ATPmax*muscle volume is closely associated with VO_2peak[379]. Therefore, the reason ATPmax was related to time to walk 400m in higher functioning, active SEA participants, and not in the lower functioning, sedentary LIFE participants nor in the combined LIFE and SEA cohort may be due to differences in muscle mass between the SEA and LIFE participants. Finally, although this is the largest study of its kind to our knowledge, there were still a relatively small number of participants included.

In summary, ATPmax (functional oxidative capacity of the quadriceps) was related to time to walk 400m in higher functioning, active SEA participants, but not in the lower functioning, sedentary LIFE participants, nor the combined LIFE and SEA cohort. However, when those reporting no discomfort at the end of the walk were examined separately, ATPmax was a significant predictor of walk-time the combined SEA and LIFE cohort. This suggests that oxidative capacity of skeletal muscle mitochondria limit walking speed in higher function older adults and functionally impaired older adults able to walk 400m without experiencing discomfort. Additionally, since both cohorts had similar mitochondrial function, this may suggest that lower functioning sedentary older adults, are less biomechanically efficient and production more energy to do less work, than higher functioning, active older adults.

In conclusion, this study provides important novel insight into the effects of skeletal muscle mitochondrial function on walking performance in older adults. Specifically, mitochondrial dysfunction (impaired energy production) may be a marker of early functional decline in higher functioning older adults or older adults able to walker a longer distance without discomfort. Interventions, such as resistance and/or aerobic training, specifically designed to prevent mitochondrial dysfunction or promote mitochondrial energy production in aged skeletal

muscle may be particularly effective in preventing early declines in physical function in older adults. Larger studies examining mitochondrial energetics in older adults with wide ages (e.g. aged 50-85+), mitochondrial function and physical abilities are needed. These studies should be adequately powered to measure subgroups, such as high and low functioning, normal and abnormal gait and high and low muscle mass.

4.0 MITOCHONDRIAL FUNCTION IS RELATED TO FATIGABILITY IN OLDER ADULTS

Fatigue is primarily considered an energy disorder and the capacity for oxidative phosphorylation in skeletal muscle and mitochondrial function has been shown to decrease significantly with age. Thus it has been hypothesized that age-related decreases in mitochondrial function may contribute to higher levels of fatigability in older adults. The relationship between fatigability, fatigue referenced to activities of specific intensities and durations, and mitochondrial function was examined in the Study of Energy and Aging-Pilot (N=30). Fatigability was defined as Rating of Perceived Exertion (RPE) at the end of a 5-min treadmill walk at 0.67m/s at 0% grade. Phosphocreatine recovery in the quadriceps was measured following an exercise-bout using ^{31}P magnetic resonance spectroscopy and images of the quadriceps were captured and used to calculate quadriceps volume. ATPmax (mM ATP/s) and oxidative capacity of the quadriceps (ATPmax*Quadriceps Volume) were calculated. Maximal aerobic capacity (VO_2 peak) was measured during a modified Balke protocol. Participants were 78.5 ±5.0 years of age, 46.7% female, 93.3% white, BMI of 25.9±2.7kg/m^2 and usual gait-speed of 1.2±0.2. ATPmax*quadriceps volume was 162.61mM ATP/s lower (p=0.03) in those with high (RPE $\geq$10) vs. low (RPE $\leq$9) fatigability. ATPmax 0.08mM ATP/s lower in those with high vs. low fatigability, p=0.09. Participants with high fatigability reached a significantly higher proportion of their VO_2 peak during the treadmill walk compared to those with low fatigability

(58.7±19.4 vs. 44.9±13.2%, $p<0.05$). After adjustment for age and sex, higher ATPmax was associated with lower odds (OR: 0.34, 95% CI: 0.11-1.01, p=0.05) of having high fatigability. Similarly, ATPmax*quadriceps volume was associated with lower odds (OR:0.39, 95% CI:0.16-0.96, p=0.04) of having high fatigability. Adjusting for age and sex attenuates this to borderline significance (OR:0.37, 95% CI:0.13-1.10, p=0.07). These relationships were attenuated when adjusting for either physical activity or VO_2 peak. Decreased physical activity likely contributes to both lower mitochondrial function and higher fatigability. Mitochondrial function is one of the two primary components comprising peak VO_2 peak. Therefore mitochondrial dysfunction, perhaps by contributing to lower VO_2 peak, may lead to the onset of higher levels of fatigability in older adults.

4.1 INTRODUCTION

Fatigue is primarily considered an energy disorder, thus it has been hypothesized that age-related decreases in mitochondrial function may contribute to higher levels of fatigability in older adults[11,12]. The capacity for oxidative phosphorylation in skeletal muscle and mitochondrial function has been shown to decrease significantly with age, mainly due to mutations and/or deletions to mtDNA from prolonged exposure to reactive oxygen species[236,241]. Diseases involving mutations/deletions to mtDNA result in increased levels of fatigability and exercise intolerance[311]. Similarly, age induced mitochondrial dysfunction may contribute to age-related increases in fatigability. Additionally, VO_2 peak has also been shown to decrease significantly with age independent of muscle loss and decreases in physical activity levels[231]. The ability of skeletal muscle mitochondrial to produce ATP with the oxygen delivered to them via the

cardiorespiratory system is one of two major components comprising $V0_2$ peak. Decreased VO_2 peak is one of the hallmark manifestations of mitochondrial disorders[345,346]. Thus, mitochondrial dysfunction may contribute to higher levels of fatigability via lower aerobic capacity due partly to decreases in mitochondrial energy production. A schematic illustration of this relationship can be found in Figure 9.

Fatigue is a common complaint among older adults and a large proportion cannot be attributed to underlying diseases[9,10]. Research has also shown that fatigue is significantly related to physical function and disability, independent of disease status[8]. Therefore fatigue is an independent risk factor for functional decline and physical disability and is present in the disablement pathway. Recently, it has been suggested that fatigability, in lieu of global fatigue, should be measured in older adults[12]. Fatigability is fatigue referenced to a specific activity of a specific intensity and duration. Measuring fatigability provides better insight into the degree to which an individual may be limited functionally due to fatigue. Preventing or lowering fatigue levels in older adults may aid in preventing age-related declines in physical function and physical disability. In order to decrease age-related increases in fatigability, it is important for researchers to better understand its etiology. A recent focused on idiopathic fatigue and fatigue and fatigability in older adults have called for research aimed to reveal underlying causes of unexplained and increased fatigability[11].

Higher levels of fatigability may cause older individuals to significantly decrease their activity levels[330], increasing the risk of functional impairment and physical disability. In order to design interventions and treatments to lower fatigability levels, it is imperative to gain insight to its underlying causes. The research described in this paper aimed to determine if oxidative capacity of mitochondria present in the quadriceps (ATPmax) and oxidative capacity of the

quadriceps (ATPmax*muscle volume), measured by ^{31}P MRS, is related to higher levels of fatigability, following a standardized physical performance test (Simonsick, et al., in press). Additionally, it has recently been shown by our group that mitochondrial efficiency (P/O, ATPmax/ State 3 respiration), which is a ratio of the amount of ATP produced in vivo over the maximal amount of ATP production determined by respiromerty, was related to walking speed in older adults[314]. This is a marker of energy conversion of O_2 into ATP generation. The relationship between mitochondrial efficiency and fatigability was also examined.

The primary hypothesis was that lower ATPmax and ATPmax*quadriceps volume would be associated with high levels of fatigability and that VO_2 peak would mediate this relationship (Figure 9). Additionally, we hypothesized that objectively measured physical activity levels would likely explain a large proportion of the variance in the relationship between fatigability and mitochondrial function. The impact of physical activity levels on the relationship between age and mitochondrial function has been described elsewhere[247]. However, mitochondrial dysfunction may be a contributor as well as a consequence of age-related decline in physical activity. To our knowledge, this is the first study to examine the relationship between mitochondrial energy production and fatigability in older adults.

4.2 METHODS

4.2.1 Participants

Participants were community-dwelling (n=37) men and women aged 70–89 years from the Pittsburgh, PA area. They were recruited from the Pittsburgh Claude D. Pepper Participant

Registry Eligibility was determined via a standardized telephone screening interview. The inclusion criteria were age 70–89 years; body weight less than or equal to 285 lbs. for men and less than or equal to 250 lbs. for women; body mass index 20–32 kg/m2; ability to walk without the assistance of a device or another person and free of basic activities of daily living disability. To be included, participants also had to have no symptomatic cardiovascular or pulmonary disease; no regular pain, aching, or stiffness in the legs, hips, knees, feet, or ankles when walking; no bilateral difficulty bending or straightening fully the knees. Finally, to be eligible, participants could not regularly taking Coumadin, Plavix, Aggrenox, Ticlid, or Agrylin/Xagrid. Exclusion criteria included a heart attack, angioplasty, or heart surgery within the past 3 months, or a cerebral hemorrhage within the past 6 months, stroke within the past 12 months, or chest pain during walking in the past 30 days. Participants also had to be able to have a magnetic resonance scan (able to lie still on back for 1 hour; and no metal or other implants, artificial joint replacements, or tattoos) They had to be able to wear the armband activity monitor (no disability in right arm; no swimming or water aerobics ≥ 3 times/week; and no supplemental oxygen use in household); and have ability to understand and sign an informed consent. The previous inclusion and exclusion criteria were assessed over the phone.

At the first clinic visit, to continue as a study enrollee, participants had to provide written informed consent and complete clinic visit, including the clinic questionnaire, height and weight, blood pressure and pulse, leg extensor strength (1-repetition maximum) and power, physical exam, short physical performance battery (SPPB), and the 400 meter walk. All participants provided written informed consent. The study was approved by the University of Pittsburgh Institutional Review Board. Additionally,

4.2.2 Clinical Examination and Measurements

Participants were examined at the Health Studies Research Clinic at University of Pittsburgh, Center for Aging and Population Health. Body height (cm) was measured using a wall-mounted stadiometer and body weight (kg)with a standard certified calibrated scale and were used to calculate BMI (weight (kg)/height (m^2)). Participants completed questionnaires concerning demographic information and self-reported medical history. History of diseases were self-report and phrased in the following manor: "Has a doctor ever told you that you had_____?".

Object lower extremity function was measured by the Short Physical Performance Battery, which includes a 4m walk, chair stands and a balance. More details concerning the SPPB can be found elsewhere [23]. Usual gait-speed was derived from the SPPB's 4m walk. 7-day free living physical activity was measured objectively by the multi-sensor Sensewear™ (Bodymedia Pittsburgh, PA) armband. The manufacturer devolved proprietary algorithm was used to measure minute's per-day of moderate intensity (≥3METs) physical activity.

4.2.3 VO_2 Peak Test

Maximal oxygen consumption (VO_2 peak) was determined by a graded treadmill exercise test[383]. A resting 12-lead electrocardiogram was conducted prior to the VO_2 peak test to screen for cardiac arrhythmias. To ensure participant safety, continuous electrocardiogram monitoring was also performed during the VO_2 peak test. During the test, the participant's self-selected usual walking speed was used and the treadmill grade was increased by 2% every 2 minutes until attainment of peak volitional exhaustion. The test was terminated as per the criteria outlined in the American College of Sports Medicine guidelines[383].

4.2.4 Determination of ATPmax by ^{31}P MRS

^{31}P MRS measures the regeneration of phosphocreatine (PCr) after a short bout of exercise to characterize mitochondrial ATP production. PCr is a high energy phosphate that can be readily converted to ATP and vice-versa via the creatine kinase reaction (PCr + ADP H+ ↔ ATP + Cr). PCr is the initial full-source of skeletal muscles. It has been shown that a linear relationship links change in PCr with exercise to mitochondrial oxidative phosphorylation rate[275,276]. Therefore, measuring the dynamics of the PCr shuttle provides a method to estimate muscle oxidative capacity in vivo[156,277]. Furthermore, this method has been confirmed to be a good measure of oxidative capacity in rodent and human studies showing that ATPmax varies in direct proportion to the activity of oxidative enzymes in skeletal muscle [278,279]. It has also been shown to reflect mitochondrial content in human skeletal muscle[280]. The amount of ATP production from glycolysis has been shown to be minimal in resting skeletal muscle (<8%)[237]. Regardless, this method can tease out the amount of ATP production by glycolysis by determining the pH from the chemical shift of the free phosphorous peak relative to the PCr peak[281]. H+ ions, which would lower the pH, are a byproduct of glycolysis.

Briefly, the exercise protocol took place in a typical MRI magnet (3T TIM Trio magnetic resonance scanner, Siemens' Medical System). Participants laid flat on their backs with the knee of the right leg (unless contraindicated) supported so that the leg was slightly elevated at a ~30 degree angle. Straps were place over the legs and a 2.5" surface RF coil tuned to ^{31}P was placed over the right quadriceps. At two different points during the scan, they were asked to kick repeatedly as hard and as fast as they can, producing fast contractions at the highest rate possible. The first bout was for 30 seconds while the second was for 36 seconds. Both exercise bouts were followed by a 6 minute rest period. The protocol was designed to deplete PCr stores by at least

33-66% without lowering pH below 6.80 during the recovery period. PCr recovery rate was measured during this rest period.

Phosphorus spectra was collected using a 3T TIM Trio magnetic resonance scanner (Siemen's Medical System, Erlanger, Germany), this has also been described elsewhere[314]. A standard one pulse experiment was used to determine the levels of PCr, ATP, Pi, and pH throughout exercise and recovery. PCr, Pi, and ATP peak areas in the fully relaxed spectra were measured by integration using Varian VNMR 6.1C software (Varian Medical Systems, Palo Alto, CA). Areas of the PCr and Pi peaks were expressed relative to the ATP peak and quantified using a resting PCr value of 27mM as determined from biopsies of human vastus lateralis muscle[156]. Changes in PCr and Pi peak areas during the tests were analyzed as previously described[372,373].

Seven total participants are missing ATPmax data. Four are missing ATPmax data due to either inadequate PCr breakdown (<33%) or pH levels dropping too low (<6.80) during the recovery period. Additionally, three other participants are missing ATPmax data, one had a possible metal implant in their ear, one had possible metal in their eye, and one reported claustrophobia at their MR visit. Therefore, 30 of the 37 SEA-Pilot participants comprise the analytic sample used for these analyses.

4.2.5 Determination of Muscle Size and Oxidative Capacity of the Quadriceps

MRI was used to determine quadriceps cross-sectional area and volume according to a previously described method[314,384]. Using a 3T TIM Trio magnetic resonance scanner (Siemen's Medical System), images were collected every 3cm from the hip to the thigh (15–25 slices per participant). The patient lay supine for imaging. The images were performed following the

execution of the MRS protocol. Standard stereologic techniques were used to determine the largest muscle cross-sectional area for the quadriceps[384]. Subcutaneous and intramuscular fat and other noncontractile tissues were excluded from the calculation of muscle contractile cross-sectional area. Oxidative capacity of the quadriceps was calculated by multiplying the ATPmax (mM ATP/s) parameter by the quadriceps volume (mM ATP/s*mL).

4.2.6 Muscle Biopsy Procedure and Preparation of Permeablized Muscle Fiber Bundles

Percutaneous biopsies were obtained at the University of Pittsburgh's Clinical Translational Research Center on a morning after an overnight fast. Participants were instructed not to perform physical exercise 48 hours prior to the muscle biopsy procedure. Muscle biopsy samples were obtained from the middle region of the musculus vastus lateralis as described previously[385]. Following the procedure, the biopsy specimen was immediately blotted dry of blood and interstitial fluid and dissected free of any connective tissue and intermuscular fat. A portion of the biopsy specimen (~10mg) was immediately placed in ice-cold BIOPS solution (10mM Ca–EGTA buffer, 0.1M free calcium, 20mM imidazole, 20mM taurine, 50mM potassium 2-[N-morpholino]-ethanesulfonic acid, 0.5mM dithiothreitol, 6.56mM $MgCl_2$, 5.77mM ATP, and 15mM phosphocreatine [PCr], pH 7.1). The individual muscle fibers in the sample were then gently teased apart in a petri dish containing ice-cold BIOPS solution using fine-nosed forceps and a dissecting microscope (Leica Microsystems, Heerbrugg, Switzerland). The fiber bundles were then permeabilized with saponin (2mL of 50 ug/mL saponin in BIOPS solution) for 20 minutes at 4°C on an orbital shaker, and then washed twice for 10 minutes at 4°C with Mir05 respiration medium (0.5mM EGTA, 3mM $MgCl_2 \cdot 6H_2O$, 60mM K-lactobionate, 20mM taurine, 10mM KH_2PO_4, 20mM HEPES, 110mM sucrose, and 1g/L BSA, pH 7.1) on an orbital shaker[386].

The permeabilized muscle fiber bundles were then placed into the respiration chambers of an Oxygraph 2K (Oroboros Inc., Innsbruck, Austria). This procedure has been described elsewhere[314].

4.2.7 Mitochondrial Respiration Protocol

Measurement of oxygen consumption in permeabilized fibers was conducted over a period of approximately 1 hour 40 minutes, at 37°C and in the oxygen concentration range 220–150 nmol O_2/mL. Following the assay, the fiber bundles were recovered and dried. A dry weight was then determined with an analytical balance (Mettler Toledo, XS105). Steady state O_2 flux for each respiratory state was determined and normalized to fiber bundle weight using Datlab 4 software (Oroboros Inc.). Further details regarding this procedure can be found elsewhere[314].

4.2.8 Determination of Fatigability and Measurement of VO_2 during Test

Fatigability level was determined following a 5-minute treadmill walking bout (steady state walk) at 1.5mph (.67 m/s) and at 0% grade. At the completion of the 5-minute walking bout, participants were asked to rate their level of perceived exertion (RPE), using the BORG scale (6-18)[341]. Participants were categorized as having high fatigability if they reported an RPE ≥10 and as having low fatigability if they reported an RPE <10. This cut point was establish using empirical evidence in Baltimore Longitudinal Study of Aging showing that this threshold is associated with an elevated risk for functional decline (Simonsick, in press). Oxygen consumption (VO_2), using the same equipment that was used to measure VO_2 peak during, was

measured for the duration of the 5-minute treadmill walk. Average oxygen consumption for the duration of the test was calculated.

4.2.9 Statistical Analyses

The final analytic sample included 30 of the 37 original participants. Seven participants were missing the primary predictor of ATPmax for reasons discussed above. Univariate statistics, means and standard deviations or counts and percents where appropriate, were calculated for the entire cohort and for the high and low fatigability groups separately. Univariate statistics for predictors, covariates and other variables were compared between the high and low fatigability groups using t-tests, chi-squared tests and nonparametric tests where appropriate. Least squared means and standard errors, adjusted for age and sex, for ATPmax and ATPmax*quadriceps volume were also generated and compared between high and low fatigability groups. Multivariate logistic regression models were generated to determine the odds of having high fatigability associated with increases in the primary predictors of ATPmax and ATPmax*Quadriceps volume and Mitochondrial efficiency. Separate models for each primary predictor were generated. Standard deviation increases in ATPmax and ATPmax*Quadriceps Volume were used to generate odds ratios for ease of interpretation, as odds ratios using original units results in an upper limit that approached infinity. Several models for each predictor were examined. Model 1 was unadjusted, model 2 was adjusted for age and sex, model 3 was adjusted for age, sex and VO_2 peak and model 4 was adjusted for age, sex and physical activity levels. In order to determine the relationship between quadriceps volume and fatigability level, models for quadriceps volume were also examined. All analyses were performed using SAS v9.3.

4.3 RESULTS

4.3.1 Participant Characteristics

Baseline demographic characteristics, medical history, physical function and mitochondrial function, both stratified by fatigue group and for the entire analytic sample, are described in Table 16. Participants were 78.5 ±5.0 years of age, 46.7% female, 93.3% white and BMI of 25.9±2.7kg/m^2. Participants had a range of physical function but were higher functioning on average with SPPB scores of 10.9±1.4 (range: 7.0 - 12) and usual gait-speeds of 1.2±0.2 (range: 0.7-1.6). Similarly, participants had a wide range of aerobic capacities (fitness) with meanVO_2 peak 22.3±5.9 (range: 7.8-33.40 ml/kg/min (Table 16). Participants were also relatively healthy, with few having histories of PAD, CHF, MI, COPD or arthritis (Table 16); however 53.3% (n=16) reported a history of cancer (skin cancer included). Data concerning history of specific cancers were not obtained. Additionally, 27.7% (n=8) participants were current or former smokers and 27.7% (n=8) reported drinking 6 or more alcoholic beverages per week. Participants had a fairly wide range of oxidative capacity of mitochondria present in the quadriceps (ATPmax) and oxidative capacity of the quadriceps (ATPmax*quadriceps volume) with values of 0.52±0.13 mM ATP/s (range: 0.30 - 0.83) and 591.25±203.93 mM ATP/s*mL (range: 222.50 - 965.04).

4.3.2 Participant Characteristics by Fatigability Level

A comparison of demographic characteristics, medical history, physical function and mitochondrial function between those determined to have high and low fatigability are presented

in Table 16. Participants with high and low fatigability were fairly similar, except those with high fatigability had significantly lower moderate physical activity levels (36.8±24.5 vs. 100.6±83.7min/day, $p<0.05$), lower VO_2 peak's (18.9±4.4 vs. 24.4±5.8ml/kg/min, $p<0.05$) and lower oxidative capacity of the quadriceps (493.69±203.95 vs. 656.30±181.19 mM ATP/s*mL, $p<0.05$) (Table 17) compared to those with low fatigability. ATPmax was borderline significantly lower in those with high fatigability compared to low fatigability (0.47± 0.12 vs. 0.55±0.14, $p=0.09$, Table 17). Age and sex least squared adjusted mean ATPmax was significantly lower in those with high compared to low fatigability (0.46±0.03 vs. 0.56±0.03 $p=0.04$). Average oxygen consumption during the steady state treadmill bout was nearly identical between those with high and low fatigability (10.4±1.8 vs. 10.4±1.0 ml/kg/min, $p>0.99$). However, those with high fatigability reached a significantly higher proportion of their VO_2 peak during the steady state walk compared to those with low fatigability (58.7±19.4 vs. 44.9±13.2%, $p<0.05$, Table 17).

A much higher proportion of those with low fatigability (44.4%, n=8) reported being a current or former smoker compared to those with high fatigability (16.7, n=2), but this did not reach statistical significance ($p=0.23$). Similarly, a much higher proportion of those with low fatigability (38.9%, n=7) reported consuming 6 or more alcoholic beverages per week compared to those with high fatigability (8.3%, n=1), but this also did not reach statistical significance ($p=0.11$). Finally, those with low compared to high fatigability reported significantly lower levels of perceived exertion following the 5 minute steady-state treadmill bout (7.9±0.9 vs. 11.0±1.0 RPE, $p<0.05$). This was expected, as rating of perceived exertion (RPE) following the 5 min steady state treadmill bout was used to categorize those as having low or high fatigability (Table 17).

4.3.3 Relationship between Fatigability and Mitochondrial Function

Logistic regression was used to determine the odds of having high fatigability associated with mitochondrial function. High fatigability was 2.2 times lower (OR: 0.45, 95% CI: 0.20-1.17, p=0.11, Table 18) per standard deviation increase in ATPmax. After adjustment for age and sex, high fatigability was 2.94 times lower (OR: 0.34, 95% CI: 0.11-1.01, p=0.05, Table 18) per standard deviation increase in ATPmax. This relationship was completely attenuated when adjusting for either physical activity level or VO_2 peak (Table 18). As mentioned previously, ATPmax was borderline significantly lower in those with high fatigability compared to low fatigability (0.47± 0.12 vs. 0.55±0.14, p=0.09, Table 17). A graphic comparison of ATPmax values between those with high and low fatigability is depicted in Figure 10.

No relationship existed between mitochondrial efficiency and fatigability, neither univariately (OR: 0.67, 95% CI: 0.32-1.41, p=0.29, Table 19) nor when adjusted for age and sex (OR: 0.69, 95% CI: 0.31-1.53, p=0.36, Table 19).

The odds of having high fatigability was 2.56 times (OR: 0.39, 95% CI: 0.16-0.96, p=0.04, Table 19) lower per standard deviation increase in oxidative capacity of the quadriceps. Adjusting for age and sex slightly attenuates this relationship to borderline significance (OR: 0.37, 95% CI: 0.13-1.10, p=0.07, Table 20). Adjusting for physical activity or VO_2 peak completely attenuates the relationship between fatigability and ATPmax*Quadriceps volume (Table 20). Oxidative capacity of the quadriceps by fatigability level is depicted graphically in Figure 11 and shows that levels of oxidative capacity of the quadriceps were significantly lower in those with high compared to low fatigability (p=0.04, Table 2 and Figure 11).

Quadriceps volume alone was not a significant predictor of fatigability, either univariately (OR: 0.69, 95% CI: 0.32-1.50, p=0.35, Table 21) or after adjustment for race and sex (OR: 1.02, 95% CI: 0.25-4.08, p=0.98, Table 21).

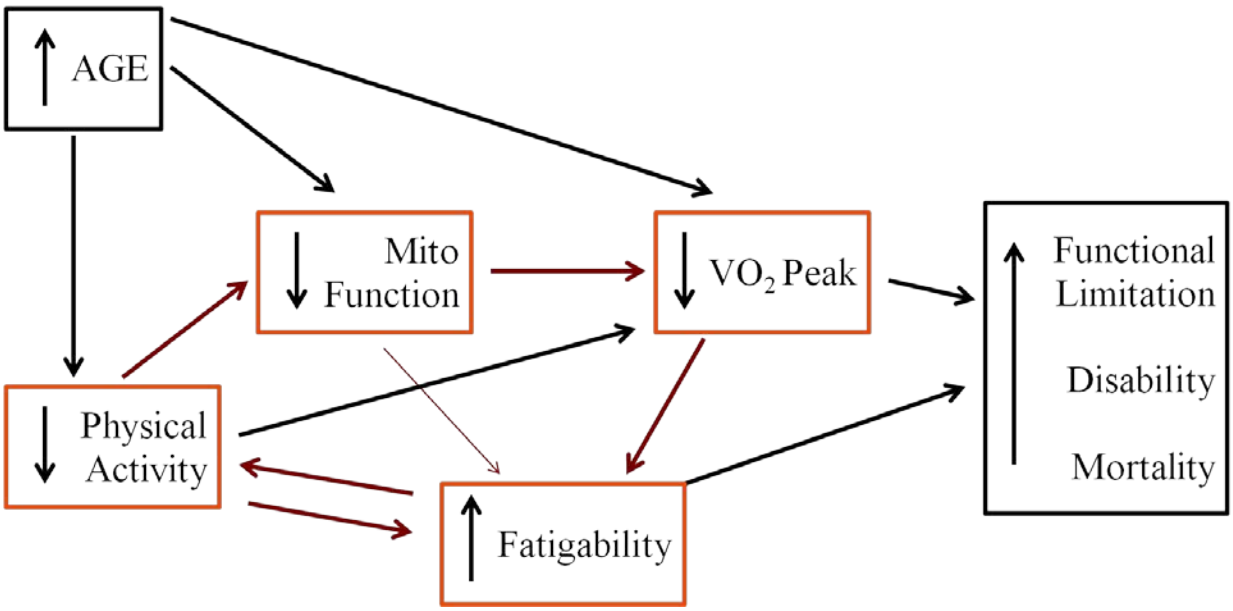


Figure 9. Conceptual Model of the Relationship between Physical activity, Mitochondrial Function, Fitness and Fatigability in Older Adults

Table 16. Demographic Characteristic by High and Low Fatigability

	Low Fatigability Group (RPE ≤ 9, n=18) Mean (SD) or N (%)	High Fatigability Group (RPE ≥ 10, n=12) Mean (SD) or N (%)	Entire Cohort (n = 30)
Age, years	78.2 (5.3)	79.0 (4.6)	78.5 (5.0)
Gender, % female	38.9 (7)	58.3 (7)	14 (46.7)
Race, % white	88.9 (16)	100 (11)	16 (93.3)
BMI	25.7 (2.6)	26.2 (3.0)	25.9 (2.7)
Moderate Physical Activity, min/day	100.6 (83.7)*	36.8 (24.5)	71.2 (66.5)
Smoker Current/Former	44.4 (8)	16.7 (2)	27.7 (8)
Alcohol Intake, 6+ drinks•week^{-1}	38.9 (7)	8.3 (1)	23.3 (7)
Diabetes, yes	4.8 (1)	0 (0.0)	3.3 (1)
History of PAD	0.0 (0)	0 (0.0)	0.0 (0)
History of CHF	0.0 (0)	8.3 (1)	3.3 (1)
History of MI	5.46 (1)	16.7 (2)	10.0 (3)
History of COPD	0.0 (0)	8.3 (1)	3.3 (1)
History of Arthritis	16.7 (3)	50.0 (6)	28.9 (11)
History of Cancer	61.1 (11)	41.7 (5)	53.3 (16)
Depressed, CESD Score >10	11.1 (2)	25.0 (3)	16.7 (5)
Health Rating, Fair or worse	0.0 (0)	16.7 (2)	6.7 (2)
CESD Score	6.8 (2.8)	7.7 (3.3)	7.2 (3.0)
Usual Gait Speed, m/s	1.3 (0.2)	1.1 (0.2)	1.2 (0.2)
SPPB Score, 0-12	10.9 (1.3)	10.8 (1.5)	10.9 (1.4)

*significant difference between fatigability groups, $p<0.05$

Table 17. Perceived Exertion, Aerobic Capacity, Muscle Size and Mitochondrial Function by High and Low Fatigability

	Low Fatigability Group (RPE ≤ 9, n=18) Mean (SD)	High Fatigability Group (RPE ≥ 10, n=12) Mean (SD)	Entire Cohort (n = 30)
RPE at end of Steady State Walk, 6-20	7.9 (0.9)*	11.0 (1.0)	9.2 (1.8)
VO_2 peak, ml/kg/min	24.4 (5.8)*	18.9 (4.4)	22.3 (5.9)
VO2 peak, ml/min	1739.0 (447.1)*	1310.8 (358.0)	1577.0 (460.2)
Avg VO_2 during Steady State Walk, ml/kg/min	10.4 (1.8)	10.4 (1.0)	10.4 (1.5)
Avg VO2 during Steady State Walk, ml/min	738.8 (156.9)	744.3 (150.4)	741.0 (151.8)
% of Peak VO_2 Reached during Steady State Walk	44.9 (13.2)*	58.7 (19.4)	50.2 (16.9)
ATPmax, mM ATP/s	0.55 (0.14)	0.47 (0.12)	0.52 (0.13)
Quadriceps Volume, mL	1204.05 (307.91)	1081.16 (427.09)	1154.9 (358.5)
Oxidative Capacity of the Quadriceps (ATPmax*Quad Volume), mM ATP/s*mL	656.30 (181.19)*	493.69 (203.95)	591.3 (203.9)
State 3 Respiration, (pmol/s*mg DW)	177.16 (79.46)	171.49 (56.10)	174.6 (68.3)
Mitochondrial Efficiency, (mM ATP/s/pmol O2/s*mg DW)*1000	3.92 (2.21)	2.96 (0.88)	3.5 (1.8)

*significant difference between fatigability groups, $p<0.05$

Table 18. Odds of having High Fatigability as Predicted by Mitochondrial Function

Model	Odds Ratio	95% Confidence Intervals	Wald X^2 P-value	Likelihood Ratio X^2 (p-value)
Model 1, unadjusted	0.45	0.20 – 1.17	0.11	3.06 (0.08)
Model 2, age and sex				6.10 (0.11)
ATPmax*	0.34	0.11 – 1.01	0.05	
Age	0.97	0.81 – 1.17	0.77	
Sex, male	0.22	0.04 – 1.36	0.10	
Model 3, Age, sex and VO_2 peak				9.93 (0.04)
ATPmax*	0.57	0.18 – 1.84	0.35	
Age	0.88	0.7 1– 1.09	0.25	
Sex, male	0.40	0.06 – 2.91	0.36	
VO_2 peak, ml/kg/min	0.78	0.61 – 1.01	0.06	
Model 4, Age, sex and Physical Activity*				16.17 (0.002)
ATPmax*	0.41	0.09 – 1.83	0.24	
Age, 5 yr	083	0.63 – 1.10	0.19	
Sex, male	0.15	0.02 – 1.37	0.09	
Physical Activity, min/day >3METs	0.94	0.89 – 1.00	0.04	

*per std. increase

Table 19. Odds of having High Fatigability as Predicted by Mitochondrial Efficiency

Model	Odds Ratio	95% Confidence Intervals	Wald X^2 P-value	Likelihood Ratio X^2 (p-value)
Model 1, unadjusted	0.67	0.32 – 1.41	0.29	1.54 (0.22)
Model 2, age and sex				1.93 (0.59)
Mitochondrial Efficiency	0.69	0.31 – 1.53	0.36	
Age	1.04	0.84 – 1.28	0.72	
Sex, male	0.57	0.07 – 4.41	0.59	
Model 3, Age, sex and VO_2 peak				12.01 (0.02)
Mitochondrial Efficiency	0.33	0.08 – 1.39	0.13	
Age	0.69	0.42 – 1.13	0.13	
Sex, male	0.75	0.03 – 16.66	0.85	
VO_2 peak, ml/kg/min	0.50	0.24 – 1.05	0.07	
Model 4, Age, sex and Physical Activity*				12.30 (0.02)
Mitochondrial Efficiency	1.24	0.49 – 3.16	0.65	
Age, 5 yr	0.82	0.51 – 1.32	0.41	
Sex, male	0.05	<0.001 – 3.04	0.15	
Physical Activity, min/day >3METs	0.92	0.83 – 1.02	0.10	

Table 20. Odds of having High Fatigability as Predicted by ATPmax*Quadriceps Volume

Model	Odds Ratio	95% Confidence Intervals	Wald X^2 P-value	Likelihood Ratio X^2 (p-value)
Model 1, unadjusted	0.39	0.16 – 0.96	0.04	5.02 (0.03)
Model 2, age and sex				5.10 (0.16)
ATPmax*Quad Volume*	0.37	0.13 – 1.10	0.07	
Age	0.98	0.82 – 1.16	0.77	
Sex, male	1.08	0.17 – 6.89	0.94	
Model 3, Age, sex and VO_2 peak				10.64 (0.03)
ATPmax*Quad Volume*	0.46	0.13 – 1.59	0.22	
Age	0.87	0.70 – 1.08	0.20	
Sex, male	1.28	0.14 – 11.86	0.83	
VO_2 peak, ml/kg/min	0.77	0.59 – 0.99	0.04	
Model 4, Age, sex and Physical Activity*				15.00 (0.005)
ATPmax*Quad Volume*	0.62	0.14 – 2.71	0.53	
Age, 5 yr	0.85	0.66 – 1.11	0.23	
Sex, male	0.448	0.03 – 6.74	0.56	
Physical Activity, min/day >3METs	0.947	0.902 – 0.995	0.03	

*per std. increase

Table 21. Odds of having High Fatigability as Predicted by Quad Volume

Model	Odds Ratio	95% Confidence Intervals	Wald X^2 P-value	Likelihood Ratio X^2 (p-value)
Model 1, unadjusted	**0.69**	**0.32 – 1.50**	**0.35**	0.89 (0.35)
Model 2, age and sex				1.36 (0.71)
Quad Volume*	**1.02**	**0.25 – 4.08**	**0.98**	
Age	1.04	0.88 – 1.23	0.63	
Sex, male	0.43	0.03 – 6.37	0.54	
Model 3, Age, sex and VO_2 peak				9.28 (0.05)
Quad Volume*	**0.63**	0.12 – 3.40	0.59	
Age	0.87	0.69 – 1.09	0.22	
Sex, male	1.25	0.04 – 37.68	0.90	
VO_2 peak, ml/kg/min	0.74	0.56 – 0.97	0.03	
Model 4, Age, sex and Physical Activity				15.38 (0.004)
Quad Volume*	2.57	0.31 – 21.34	0.38	
Age, 5 yr	0.88	0.67 – 1.15	0.34	
Sex, male	0.04	<0.001 – 4.37	0.18	
Physical Activity, min/day >3METs	0.94	0.89 – 0.992	0.03	

*per std increase

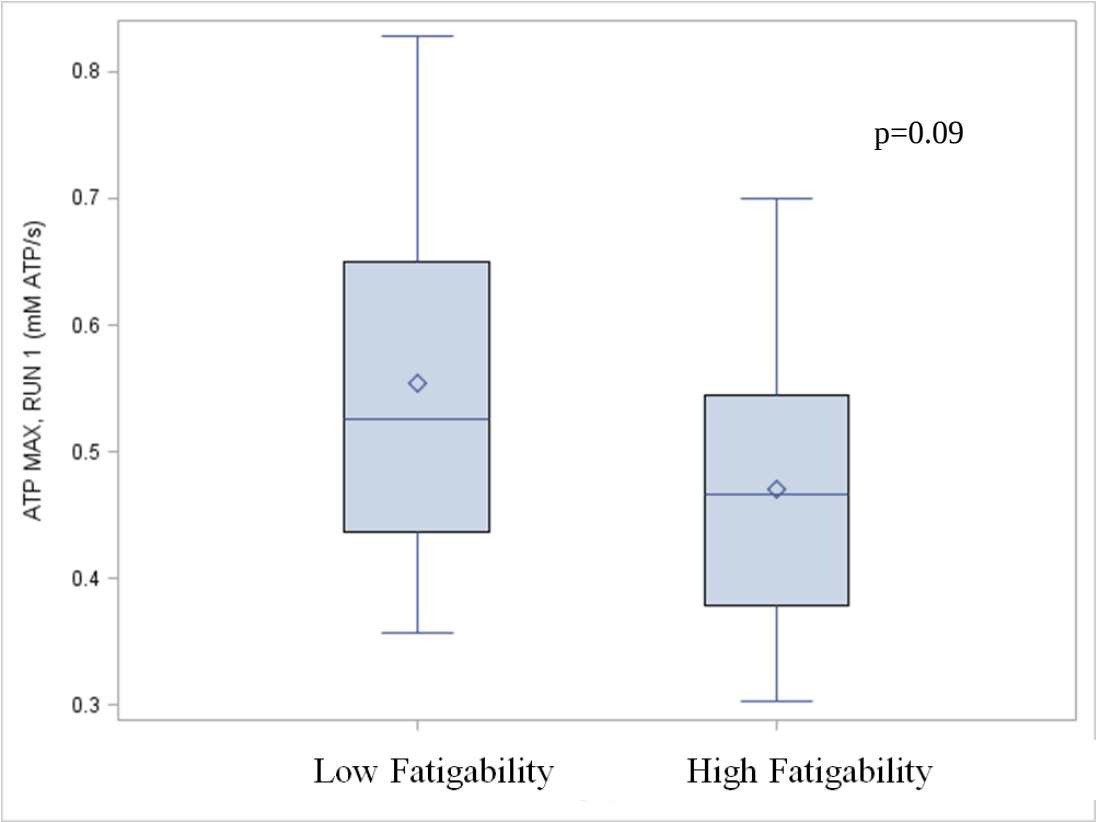


Figure 10. ATPmax by Low and High Fatigability

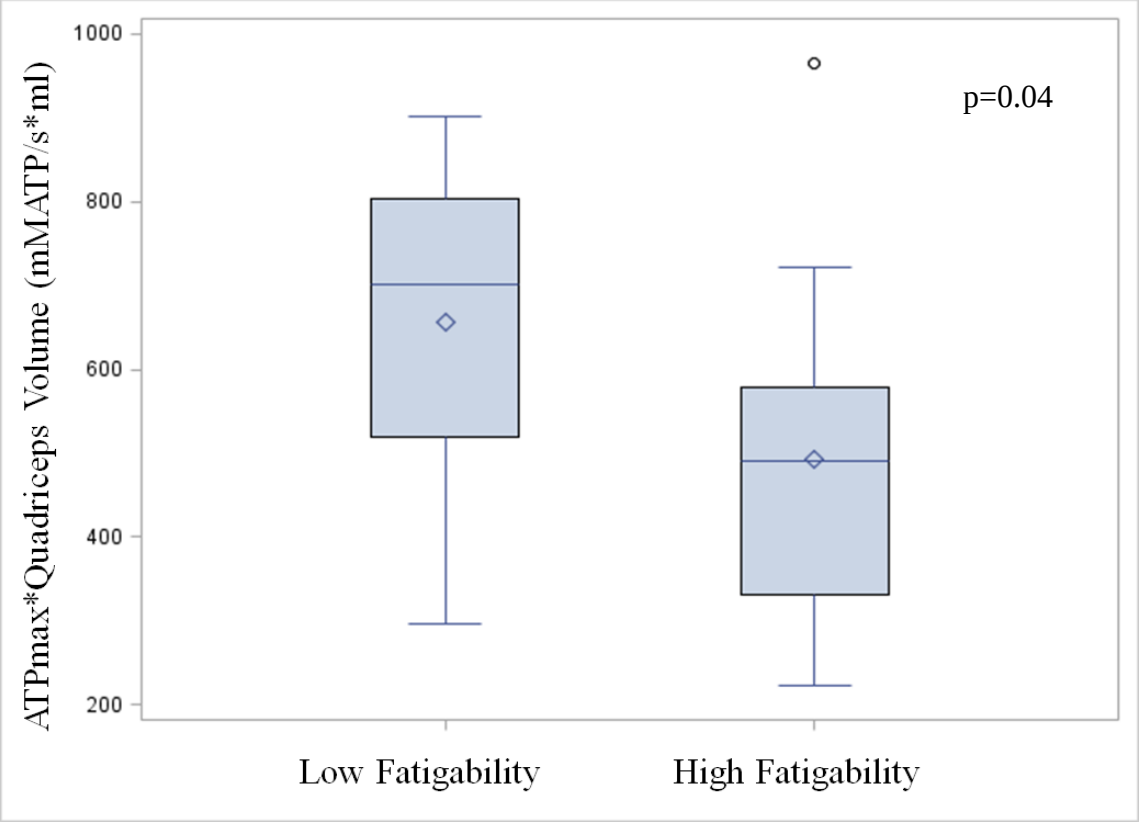


Figure 11. Oxidative Capacity of the Quadriceps by Low and High Fatigability

4.4 DISCUSSION

To our knowledge, this is the first study to examine the relationship between in vivo mitochondrial energetics and fatigability in older adults. Both functional oxidative capacity of mitochondria in the quadriceps (ATPmax) and oxidative capacity of the quadriceps (ATPmax*muscle volume) were significantly lower in older adults with higher levels of fatigability. This is consistent with mitochondrial disorder patients possessing higher levels of exercise intolerance[311,344],[311]. This is also consistent with animal models, specifically, mitochondrial gene ANT1-knockout mice which are a model for chronic ATP deficiency, display lower exercise tolerance and higher fatigability compared to wild type[342,343].

Another important finding of this research was the fact that those with high and low fatigability consumed nearly identical amounts of oxygen on average during the steady state treadmill test. This suggests that the energy cost of walking, per kilogram of body weight, was similar throughout the test in both those with low and high fatigability. However, those with high fatigability were working significantly closer to their maximum aerobic capacities. Therefore, they were likely reaching the threshold where energy supply, in the form of ATP, was starting to diminish, and thus the body began to generate a sensation of fatigue. This phenomenon is described in greater detail by Alexander et al.[11]. Briefly, Alexander et al. state that in some older adults, certain activities may approach maximum aerobic capacity ($V0_2$ peak) or energy, and then while performing such an activity, the brain senses this and elicits a feeling of fatigue which causes the individual to decrease their activity levels which then leads to decreases in fitness and ultimately physical function[11]. This hypothesis is discussed in more detail elsewhere[11]. The ability of skeletal muscle mitochondrial to produce ATP, from oxygen delivered via the cardiorespiratory system, is one of two major components comprising aerobic capacity ($V0_2$

peak). It has been shown that that mitochondrial function, measured by ^{31}P magnetic resonance spectroscopy (MRS), and oxidative capacity of the quadriceps are highly related to aerobic capacity in older adults[314,379]. Additionally, decreased aerobic capacity is one of the hallmark symptoms exhibited by patients with mitochondrial disorders[311,344] and has been shown to decrease with age independent of activity level and decreases in skeletal muscle mass[231]. Thus, mitochondrial dysfunction may contribute to higher levels of fatigability via lower aerobic capacity due partly to decreases in mitochondrial energy production. Longitudinal studies are needed to establish causality.

Adjusting for physical activity completely attenuated the relationship between mitochondrial function and fatigability. However, this was expected, as both mitochondrial function and fatigability levels are closely associated with physical activity[236,247]. Mitochondrial dysfunction may be a contributor, as well as a consequence of age-related declines in physical activity. For example, mitochondrial dysfunction may lead to an increase in fatigability, which may in turn lead to a decrease in activity levels due to increased exercise intolerance. Evidence exists that skeletal muscle mitochondrial dysfunction activate apoptotic pathways; activation of these pathways may lead to nuerodegeneration and could contribute to declines in muscle mass with age[240,248]. These changes may also result in a decrease in physical activity level. Increased physical activity leads to an increase in mitochondrial function and energy production, even in aged skeletal muscle[304,30]. Similarly, decreasing fatigability may result in a prolonged increase in physical activity levels due to an increased tolerance to more activity. Longitudinal and intervention studies are needed to better understand how changes to mitochondrial function and physical activity impact each other throughout the lifespan. Interventions aimed at improving fatigability via improving mitochondrial function, such as specially tailored exercise programs or

other treatments, such as lipid replacement and antioxidant therapy, which has been shown to improve mitochondrial function and fatigue levels in those with CFS[387] or similar treatments to used utilized by patients with mitochondrial myopathies[311], may be affective at preventing or reversing increased fatigability in older adults.

No association was observed between mitochondrial efficiency, a marker of the conversion of oxygen to ATP at the mitochondrial level, and fatigability. This suggests that inefficiency in ATP production at the mitochondrial level, possibly caused by a mitochondrial inner membrane impairment or electron leak in the electron transport chain, is not contributing to higher fatigability. Additionally, mitochondrial efficiency was not associated with VO_2 peak, so it was unlikely to affect fatigability via our main hypothesized pathway. Thus, impairments in absolute mitochondrial energy production, rather than proportion of maximal mitochondrial energy production reached, seem to be associated with fatigability in older adults. However, Coen et al showed that lower mitochondrial efficiency was related to slower usual walking speed in the SEA cohort, independent of VO_2 peak[314]. Additionally, lower mitochondrial efficiency would impair absolute energy production. Perhaps no relationship was observed due to this study including a small number (n=30) of higher functioning older adults. The effect of mitochondrial efficiency on fatigability warrants further study is a larger population of older adults with a wide range of physical function and fatigability levels.

This study has numerous strengths. Mitochondrial function was measured in vivo, which reflects actual mitochondrial energy production in the living skeletal muscle as opposed to energy production measured in isolated mitochondria obtained from muscle biopsy. Muscle volume was measured via the gold standard method of MRI and we were able to calculate a measure of oxidative capacity of the quadriceps (ATPmax*Quad Volume). Fatigability was

measured following a standardized performance test, eliminating any contextual and recall biases associated with simply asking participants how fatigued they feel after engaging in certain activities. Physical activity was measured objectively using a valid and reliable multisensory device. This study also has several limitations. Although fatigability was assessed following a standardized performance test, a certain degree of subjectivity and perception remained, as participants still had to rate their perceived exertion, as opposed to observing a deterioration in performance, such as slowing down due to fatigue. This population of older adults were both quite healthy and high functioning, thus it remains unclear whether or not mitochondrial function contributes to fatigability in lower functioning, more frail older adults. Finally, this study included a small sample (n=30) of older adults.

In conclusion, this research provides novel evidence showing that mitochondrial dysfunction may be implicated in the etiologic pathway of age-related fatigability. Fatigue is a common complaint among older adults and has been shown to be related to functional decline[8-10]. Increased levels of fatigability may cause older adults to decrease their activity levels, which will in turn lead to decreased fitness and ultimately functional impairments and disability. Understanding its etiology is vital for preventing its onset. This research suggests that mitochondrial dysfunction may lead to higher levels of fatigability by contributing to lower aerobic capacity. Physical inactivity may this initiate the process. However, improving mitochondrial function may improve fatigability, which may then increase physical activity due to an increased exercise tolerance. The causal role of mitochondrial dysfunction and lower aerobic capacity in age-related fatigability needs to be studied longitudinally and in a larger population of older adults with a variety of physical function.

5.0 SUMMARY AND CONCLUSIONS AND FUTURE RESEARCH

The overall objective of this dissertation was to examine novel aspects of the disablement pathway related to skeletal muscle and to investigate their relationships with physical function and fatigability in older adults. Specifically, two aspects of skeletal muscle aging were studied, skeletal muscle adiposity and skeletal muscle mitochondrial function. First, I found that decreases to specific fat depots present in skeletal muscle and VAT following a PA+WL or PA+SA intervention were related to improvements in function. Second, I found that mitochondrial function is associated with walking performance. This relationship was complex in lower functioning older adults. Finally, I found that mitochondrial function is associated with higher fatigability levels. This work contributes to the knowledge base by filling important gaps regarding the effects of skeletal muscle adiposity and mitochondrial function on physical performance in older adults and has also generated new important questions.

The work regarding decreases in the fat depots of IMAT, HU and VAT provides empirical evidence concerning the possible mechanisms by which intentional weight-loss, in combination with physical activity, improves physical function in older adults. To our knowledge this is the first study showing a direct relationship between decreases in IMAT, VAT and HU, following a PA+WL or PA+SA intervention, and improvements in physical function. Previous research has shown that weight loss in combination with physical activity improves function to a greater degree than either weight loss or physical activity alone. These findings

extend this work by showing specific mechanisms related to body composition remodeling for why intentional weight loss in combination with physical activity improves function. This study is consistent with and expands upon previous cross-sectional work showing that IMAT, VAT and HU are associated with worse muscle or physical performance and adverse health conditions that may negatively affect muscle or physical performance[52,161,347,348,363]. Additionally, this work is also consistent with and expands upon previous research showing intentional weight loss in adults 50-60 years of age results in a preferential loss of IMAT and VAT compared to SUBQ fat[177]. This study extends this research in two important ways. First, by showing a preferential loss of IMAT and VAT following intentional weight-loss and physical activity in adults aged 60 and older; and secondly, by showing these decreases are directly associated with improvements in function. The direct relationship between decreases in skeletal muscle fat depots, VAT and improved function detected by this work unveils important information regarding the mechanisms by which intentional weight-loss and PA improve function in older adults. To advance this work, future research should examine the specific mechanisms by which decreasing these fat depots improve function. This will be discussed in more detail in the next section.

The study examining the relationship between walking performance and mitochondrial function in older adults contributes to the field of muscle aging in a few important ways. This is one of two studies to have examined the relationship between walking performance and mitochondrial function in older adults. It is the first to illustrate the heterogeneity of age-related disability in regard to skeletal muscle energetics, as mitochondrial energy production was a limiting factor only in those higher function older adults and older adults able to walk 400m without discomfort and not in lower functioning older adults. This work has also led to new hypotheses and research questions which will be discussed below.

First, to summarize the results of this study, contrary to our hypotheses, oxidative capacity of mitochondria present in the quadriceps (ATPmax) was not associated with time to walk 400m in either the low functioning LIFE population or the combined SEA and LIFE population. This was in stark contrast to the work of Coen et al.[314], who showed a significant relationship between ATPmax and gait-speed during the 400m walk in the higher functioning SEA cohort. The only other study to examine this relationship also showed a positive relationship between ATPmax and a modified get-up and go test[312]. Since identical protocols, MR magnets, technicians and MR spectroscopy analysts were used for the LIFE and SEA studies, we hypothesized that confounding characteristics were present in the LIFE cohort. Discomfort following the walk was identified as a possible confounding factor. This is biologically plausible, as discomfort during the walk could lead to slowing for reasons unrelated to oxidative capacity of skeletal muscle mitochondrial function. In LIFE participants without discomfort, ATPmax was not significantly associated with walk-time; however, interestingly, the direction of the relationship between ATPmax and time to walk 400m was reversed in these individuals (higher ATPmax associated with faster walk-times) compared to the entire LIFE population and those experiencing discomfort. In the SEA and LIFE combined population without discomfort, higher levels of ATPmax were significantly related to shorter walk-times.

This research poses several new questions, as research regarding the effects of skeletal muscle mitochondrial function on physical performance in older adults is really in its infancy. These questions include: is skeletal muscle mitochondrial function a limiting factor in higher but not lower functioning older adults? If so, why? Does an interaction exist between mitochondrial function and gait-efficiency? The results of this study beg this question, for two reasons. First, those in the LIFE study, despite being lower functioning and more sedentary, had similar

ATPmax values compared to the higher functioning more active SEA participants. Secondly, a drastically different relationship between ATPmax and walking performance was observed between the two cohorts. We were able to explore this hypothesis to some degree by examining those who were experiencing discomfort at the end of the walk, and possibly walking less efficiently due to this discomfort separately. Another important question generated as a result of this research is the role of muscle mass. It is possible that those in the LIFE cohort, despite having similar oxidative capacity of skeletal muscle mitochondria, may have lower oxidative capacity of the whole quadriceps muscle due to lower muscle mass. However the LIFE participants had larger overall body sizes (BMI); therefore, they are likely to possess higher levels of leans mass. Finally, do those lower functioning individuals have too many other adverse health conditions unrelated to oxidative capacity of skeletal muscle, such as musculoskeletal or joint pain, that limit walking speed? This could very well be the case and larger studies are needed to answer these questions. The specific design of a future study aimed to examine these questions is discussed in more detail in the following section.

The study examining the relationship between walking performance and mitochondrial function also illustrates the importance of selecting methods for measuring physical function in older adults. In general, performance based measures of function are superior to self-report, as they are objective measures of function and eliminate recall and perception biases. However, choosing the most appropriate objective measures of function in epidemiologic research is crucial and can have a large impact on the results and conclusions of research studies. For example, a usual paced 400m walk test was employed in the SEA and LIFE study. Perhaps this was not the best test to evaluate the physical performance of older adults with a wide-range of function, because the lower functioning older adults were likely working closer to their

maximum capacitates, where as the higher functioning older adults would not have been. We were unable to evaluate this directly; as a higher or maximal level test was not employed. Additionally, the SPPB is often used to measure physical function in older adults; however this test may not be appropriate if the primary predictor is more related to aerobic function, like mitochondrial function, as the SPPB is a more anaerobic test. Similarly, gait-speed is often measured over a short distance, e.g. 3 or 4 meters, perhaps gait-speed should be measured over a longer distance, e.g. 20m, or a longer walking test, such as the 6 minute walking test or 400m walk should be used if the primary predictor is more closely related to aerobic function, such as mitochondrial function or fatigability. Future research enrolling older adults with a wide range of functional abilities should employ multiple performance based tests, such as a fast and usual paced 400m walk. Conducting both a usual and fast paced 400m walk would allow for better differentiation, in terms of physical ability, specifically in higher functioning participants.

The research described in the third paper was aimed at determining if oxidative capacity of mitochondria present in the quadriceps (ATPmax) and oxidative capacity of the quadriceps (ATPmax*muscle volume), measured by ^{31}P MRS, is related to higher levels of fatigability, following a standardized physical performance test. This study showed that both functional oxidative capacity of mitochondria in the quadriceps (ATPmax) and oxidative capacity of the quadriceps (ATPmax*muscle volume) were significantly lower in older adults with higher levels of fatigability. To our knowledge, this is the first study to examine the relationship between in skeletal muscle mitochondrial energetics and fatigability in older adults. The findings are consistent with studies showing that patients with mitochondrial disorders have significantly higher levels of exercise intolerance[311,344]. Another important finding shown by this research was the fact that those with high and low fatigability consumed nearly identical amounts of oxygen

on average during the steady state treadmill test. This suggests that the energy cost of walking, per kilogram of body weight, was similar throughout the test in both those with low and high fatigability. However, those with high fatigability were working significantly closer to their maximum aerobic capacities. The ability of skeletal muscle mitochondrial to produce ATP, from oxygen delivered via the cardiorespiratory system, is one of two major components comprising aerobic capacity (VO_2 peak). Mitochondrial function, measured by ^{31}P magnetic resonance spectroscopy (MRS), and oxidative capacity of the quadriceps are highly related to aerobic capacity in older adults[314,379]. Additionally, decreased aerobic capacity is one of the hallmark symptoms exhibited by patients with mitochondrial disorders[311,344] and has been shown to decrease with age independent of activity level and decreases in skeletal muscle mass[231]. Thus, mitochondrial dysfunction may contribute to higher levels of fatigability via lower aerobic capacity due partly to decreases in mitochondrial energy production. In other words, VO_2 peak may be a mediator in the relationship between mitochondrial function and fatigability. This is depicted schematically in Figure 9. The data presented in this study suggest this, as including VO_2 peak in a model predicting fatigability with ATPmax largely attenuates this relationship.

The results of this study can also be taken into the account with the previous study which examined the relationship between mitochondrial function and walking performance. Lower mitochondrial function may contribute to lower gait-speed due not only due the direct effect of lower availability of energy to skeletal muscles, but also by contributing to lower levels of aerobic capacity and higher levels of fatigability. A vicious cycle may exist - lower physical activity leading to lower VO_2 peak and mitochondrial function[247], then mitochondrial dysfunction exacerbating the declines in VO_2 peak[311], which leads to increased fatigability, which then leads to a further decrease in physical activity levels[330], all of which may contribute

to slower walking speeds (Figure 9). Slower walking speed is associated with increased risk for disability[25], institutionalization[40] and death[41], so understanding the potential mechanisms and etiology of age-related slowing and why some decline more than others is vital for its prevention.

Additionally, the findings from the study examining mitochondrial function and fatigability can be discussed in the context of the first study examining skeletal muscle adiposity. This study showed that physical activity seems to target IMAT and that decreases in IMAT were directly associated with improvements in physical function. Thus, mitochondrial dysfunction, by contributing to lower levels of peak aerobic capacity, resulting in increased fatigability which may then cause one to decrease their activity levels, may result in an increase in IMAT. Cross-sectional research has shown that those with higher levels of fatigability perform less physical activity[330], but longitudinal and intervention studies are needed to determine if lowering fatigability increases physical activity levels in older adults. The effect of lower PA on IMAT has also been illustrated by a study showing that 4-weeks of single leg immobilization results in a significant gain in IMAT in both the calf and thigh, despite a statistically significant decrease in total body mass[363].

Few intervention studies have actually measured changes to fatigability in older adults. However, there have been some agents that show promise in lowering fatigue such as L-carnatine[332,333], lipid replacement antioxidant therapy (specifically a dietary supplement called NTFactor®)[334] and Yoga[335]. Moderate physical activity consisting of over-ground walking and lower extremity resistance training was ineffective at lowering fatigue; however this study did not measure fatigability[12]. Future intervention studies examining the effective of pharmaceutical agents, such as antioxidants and physical activity trials should measure fatigability as opposed to global fatigue. Intervention trials are needed to establish effective ways to improve fatigability

because lowering may result in a prolonged increase in activity levels due to an increase in exercise tolerance. The beneficial effects of increasing physical activity are abundant, well documented and include improvements in body composition, mitochondrial function, muscle performance and ultimately physical function in older adults[48,175,194,301,304].

In conclusion, the research presented in this dissertation contributes to the field of preventing age-related physical disability by providing evidence implicating novel potential underlying causes and mechanisms of age-related declines in physical performance, namely skeletal muscle adiposity and skeletal muscle mitochondrial function. Sarcopenia, the loss of muscle mass with age, was thought to be the primary neuromuscular cause of decreasing mobility and physical function with age; however, longitudinal evidence suggests that decreasing muscle mass only modestly predicts declines in muscle performance and mobility in older adults[83,124,369]. Understanding the etiology of age-related declines in physical function and mobility is important, as lower function and slower gait-speed are related to increased health care costs, institutionalization and an increased risk for mortality[6,22,25,41,44]. The neuromuscular system is a critical component for maintaining function into old-age. This research contributes to the field by first, showing for the first time that decreases in skeletal muscle fat depots are directly associated with improvements in physical function. Secondly, this work shows that mitochondrial function may contribute to declines in physical performance in higher functioning older adults and functionally limited older adults able to walk 400m without discomfort. Finally, for the first time, mitochondrial dysfunction was found to contribute to increased levels of fatigability.

The findings presented in this dissertation mainly pertain to higher functioning older adults, as those in the WELL study, despite being overweight to moderately obese, and the SEA

study, were high functioning. Additionally, ATPmax seemed to only be a potential limiting factor in the lower functioning LIFE participants able to walk without discomfort. These findings are still important and may be potential subclinical markers of early declining function. That is, those with higher levels of skeletal muscle adipose tissue and lower levels of mitochondrial function, although still functioning at a relatively high level, may be at greater risk for future functional decline compared to those with lower levels of IMAT and HU and higher mitochondrial function. Therefore, these may be new therapeutic targets to treat and prevent declining physical function in older adults who have yet to manifest functional limitations, or who have begun to decline physically, but can still walk longer distances without discomfort. Preventing declines in function in those who are still higher functioning may have large public health implications, as preserving function in those who are still high functioning will prevent the adverse effects that declining function precipitates, including lower quality of life, higher health care cost and risk for future disability and institutionalization.

Finally, the most effective ways to prevent disability in older adults are unknown and it is likely, due to the heterogeneity of age-related disability, that different interventions will be more effective in certain subsets of older adults compared to others. For example, the work presented in this dissertation highlights this, as the second paper showed that mitochondrial function may be a limiting factor in high but not lowering functioning older adults. Therefore not all older adults will benefit from interventions, such as antioxidant therapy, specifically designed at promoting mitochondrial function. Similarly, weight-loss, resistance, power and endurance training may be more effective in certain subsets of older adults compared to others. Research examining the most effective dose and type of training in specific subsets of older adults is needed. However, it does seem clear that avoiding obesity and doing physical activity will

benefit most older adults, as the benefits of avoiding obesity and doing physical activity are widespread[48,194,199,350].

5.1.1 Directions for Future Research

Opportunities for future research regarding skeletal muscle adiposity and mitochondrial function are evident as a result of this work. The biological mechanisms by which decreasing specific fat depots may improve physical performance remain unclear. VAT is strongly associated with insulin resistance independent of overall adiposity and with higher levels of proinflammatory cytokines[52,347,348], which can negatively affect muscle performance. Therefore, decreases in VAT may result in improvements in metabolic and inflammatory states, which may then positively affect muscle and physical performance. Similarly, higher levels of IMAT and HU have been shown to be related to insulin resistance and the metabolic syndrome[52,146,161,351]; therefore, decreasing IMAT and HU levels may result in a more optimal metabolic state, resulting in improved physical performance. However, fitness level has been shown to be an important mediator in the relationship between mitochondrial function, insulin resistance and HU content. Specifically, those with the highest levels of fitness also have the highest levels of HU content, as it is a readily available and high energy fuel source; however, in more sedentary individuals, high HU content has been shown to be related to insulin resistance. Furthermore, following participation in a progressive exercise intervention consisting of mostly walking and stationary cycling, older adults showed an increase in intramyocellular fat content measured via muscle biopsy and a decrease in insulin resistance[388]. Therefore, fitness level and changes to fitness would be important to measure in future studies. A few recent trials conducted in younger adult men (age 30-65) and women (age 20–41) have shown that decreases in VAT are associated with

improvements in insulin resistance[364] and decreases in inflammatory markers[365]. These studies did not examine how these changes affected muscle or physical performance. To our knowledge, the relationship between decreases in the fat depots present in skeletal muscle, IMAT and HU, and metabolic and inflammatory markers has not been examined. Further intervention trials are needed examining the specific relationships between decreases in IMAT, HU and VAT with changes in inflammatory and metabolic markers and how they impact physical function in older adults.

Although it is an observational study, the Health ABC study would be an ideal setting to examine how these fat depots may affect skeletal muscle and physical performance, as CT images were obtained at multiple time points in a subset of participants. However, future intervention studies, or even completed ones with stored biospecimens, should examine changes in inflammatory and metabolic markers and how these changes impact physical function. Research is also needed regarding specific modes (e.g. aerobic vs. strength training) and doses of physical activity, in combination with weight-loss, to elucidate the most effective methods for optimizing body composition and thus improving function in adults. Again, it is likely that certain subsets of older adults, e.g. high and low functioning, obese and not obese, arthritic and not arthritic etc..., will benefit differently from specific interventions. For example, those who are obese would likely benefit from weight-loss to a greater degree than those who are normal weight. Similarly, those who are arthritic may benefit more from power training, which emphasizes speed at low levels of resistance, which is easier on joints than traditional resistance training, compared to those who are not arthritic or experiencing joint pain. Future interventions should examine subgroups of older adults to determine the best methods for improving function,

as age-related declines in function can be precipitated by a wide-variety of adverse health conditions.

Further research regarding the etiology of fat depots present in skeletal muscle is also needed and mitochondrial dysfunction may be in the etiology pathway. Particularly, if mitochondria are unable to metabolize fuel, e.g. fat via beta oxidation, efficiently this may result in an accumulation of adipose tissue both in and surrounding skeletal muscle. Although longitudinal research is needed to establishing causal relationships, a logical next step would be to design a cross-sectional study aimed to examine the relationships between IMAT and intramyocellular lipid content (IMCL), mitochondrial function and insulin resistance, or fasting glucose levels in those with diabetes. Although CT was used to indirectly measure IMCL content by using muscle density in HU as a surrogate, it would be ideal to measure IMCL content by H^1 MRS, as this non-invasive technique can directly distinguish between and quantify intra and extracellular adipose tissue. Physical activity and fitness would be important to measure in such a study, due to the previously mentioned mediating effects of fitness level on the relationships between IMCL content, mitochondrial function and insulin resistance. In mostly sedentary individuals, IMCL content would be more likely to be related to lower mitochondrial function and higher levels of insulin resistance. Conversely, in more active individuals, higher IMCL levels would be more likely to be related to higher mitochondrial function and lower insulin resistance. Finally, higher IMAT levels would be more likely to be related to lower mitochondrial function and higher insulin resistance regardless of activity level as IMAT is not a fuel source. Additionally, higher IMAT levels have been shown to be correlated (unadjusted) with lower mitochondrial function in a study consisting of both older (75.7±1.0 years) and younger (27.2±1.0) adults[162]. However, the results of this study could have been an affect of age,

decreased physical activity or another confounding factor and not higher IMAT per se. The proposed study would provide important information regarding the relationship between IMCL and IMAT content, mitochondrial function and insulin resistance and could provide preliminary data to propose a longitudinal study to establish causal relationships.

Further illustrating the importance of metabolic health to muscle aging is the finding in the Health ABC study that older adults with type 2 diabetes, especially those undiagnosed, experienced significantly greater decreases in muscle mass and strength compared to non diabetic older adults[389,390]. Future work should also examine changes in muscle mass and performance in those with impaired fasting glucose. The biological mechanisms for why older adult diabetics experienced significantly greater decreases in muscle mass and strength compared to non diabetic older adults, but the authors suggested it may have to do with impairments to protein synthesis. Mitochondrial dysfunction, as mentioned above, may also be a partial driver this process, as a vicious cycle precipitated by mitochondrial dysfunction has been suggested[241,391]. Specifically, mtDNA mutations, leading to mitochondrial dysfunction and apoptosis of muscle fibers, which in turn causes further ROS production and exposure acerbating mitochondrial dysfunction, may be causal to the process of sarcopenia and insulin resistance[241,290]. A longitudinal study, similar to the cross-sectional study mentioned above would be needed to establish empirical evidence supporting this vicious cycle hypothesis.

The study of skeletal muscle energetics and physical performance into old age is really in its formative years. It is important to determine how changes in mitochondrial function (decreases in energy production), regardless of the underlying mechanisms, impacts physical function in older age needs. However, the underlying causes of mitochondrial function are important, especially when it comes to designing treatments and interventions to improve

mitochondrial function. Ultimately though, from a public health stand point, how mitochondrial dysfunction affects the physical abilities of older adults, differs by physical ability and the best methods to prevent or reverse mitochondrial dysfunction are of interest. Specifically, a larger study similar to the one conducted in the SEA and LIFE cohorts is needed because the SEA and LIFE combined study was not powered to examine subgroups, for example those experiencing or not experiencing discomfort following the 400m walk. Instead of being cross-sectional, a prospective cohort design should be employed in order to collect data at multiple time points, which would allow for the assessment of longitudinal relationships, temporality and causal associations. There would be two primary hypotheses of the aforementioned study. First, that mitochondrial function will be associated with walking performance in those individuals who are higher functioning or just beginning to decline, but lack adverse health conditions affecting their ability to walk normally and without pain or discomfort. Secondly, that longitudinal decline in mitochondrial function will be more closely associated with functional decline in those who are higher functioning at baseline. Secondary hypotheses would include: more mtDNA damage will be associated with lower mitochondrial function, higher levels of oxidative stress would be associated with more mtDNA damage and that lower mitochondrial function will contribute to declining VO_2 peak.

The population should include older ages with both a wide age range (e.g. 50 and older) and functional capacities in order to increase both the variability and range of ATPmax. The primary outcomes would be lower extremity function measured by time to complete both a fast and usual paced 400m walk. Incorporating both a fast and usual paced 400m walk would greatly eliminate any floor or ceiling effect. A portable oxygen consumption device should be utilized during each of the walking tests, if possible. A secondary outcome would be fitness measured by

a VO_2 max test conducted on a cycle. Utilizing a cycle as opposed to treadmill test would allow participants with joint and musculoskeletal pain to complete it. Fatigability, measured using the method described in the third paper, would be another secondary outcome. Oxygen consumption should also be measured during the 5-minute treadmill bout used to determine fatigability.

The primary predictors would be mitochondrial function, ideally from both in vivo and in vitro measures. Employing both in vivo and in vitro measures would allow for the quantification and study of a variety of mitochondrial function parameters including energy production, enzyme activity, protein content, mtDNA content and ROS production. Ideally, mtDNA sequencing would be conducted in order to measure point mutations and deletions. This would allow investigators to examine the role of mtDNA damage in mitochondrial dysfunction and whether or not this damage is due to ROS production. Other measures should include a walking test performed on a gait-mat or similar device able to measure specific gait-characteristics, muscle mass and muscle adiposity by CT or MRI and a measure of both muscle strength and power. These additional measures would allow investigators to answer some of the question posed above concerning the effects of skeletal muscle energetic on walking performance in subgroups of older adults with normal vs. abnormal gait and high vs. low muscle mass. A proposed longitudinal study similar to the one described above would be invaluable, as it would provide empirical evidence regarding the causal role of mitochondrial dysfunction in sarcopenia, insulin resistance, skeletal muscle adiposity and declining function. The role of accumulated damage to mtDNA and whether or not this is caused primarily by ROS exposure would also be able to be determined.

It is also important to determine if improving mitochondrial function is related to improvements in function. Both resistance and endurance training improve mitochondrial

function (Table 4), however; one or the other may be more effective, or possibly a combination of both would be most beneficial and if so, perhaps the order of training matters. Perhaps initial resistance training is necessary to promote mitochondrial biogenesis and turnover and should be followed up with an aerobic exercise regimen to train mitochondria to produce energy more efficiently and rapidly. Epidemiologists, exercise physiologists, and basic science researchers need to collaborate to design studies that can uncover information regarding both the mechanisms and impacts of age-related mitochondrial function and how to reverse, prevent or delay its onset. Specifically, a randomized controlled trial could be implemented examine the effects four different interventions on mitochondrial and physical function. These four interventions would include a group that conducts resistance training for a period of time (e.g. 6 weeks) followed by 6-weeks of aerobic training, another that conducts the aerobic training first followed by a period of only resistance training and finally, the two remaining groups would conduct either only aerobic or resistance training for the entire duration of 12-weeks. Power training, which emphasizes speed at low levels of resistance, could also be utilized in older adults with joint or other pain issues preventing their participation in a traditional resistance training regimen. The outcomes of such a proposed study would be mitochondrial function, muscle performance, aerobic capacity (VO_2 peak) and physical function measured using the 400m walk test.

Longitudinal studies are also needed to examine the possible causal role of mitochondrial function in increased levels of age-related fatigability. Fatigue is a common complaint among older adults and has been shown to be related to functional decline independent of disease status. Thus, it is important to study this relationship in longitudinally and in a larger population of older adults. Interventions aimed at improving fatigability via improving mitochondrial function,

such as specially tailored exercise programs or lipid replacement and antioxidant therapy, which has been shown to improve mitochondrial function and fatigue levels in those with CFS[387] or similar treatments to those utilized by patients with mitochondrial myopathies[311], may be effective at preventing or reversing increased fatigability in older adults. Theoretically, lowering fatigability may result in an increase in physical activity levels due an individual being able to tolerate both more frequent and intense activities. Thus, improving fatigability may result in the prevention of functional decline or improved function by promoting physical activity levels.

5.2 PUBLIC HEALTH IMPACT OF THIS WORK

In the United States, the absolute and relative number of older adults (age ≥65 years) is starting to rise rapidly as the baby boomers (born 1946-1964) begin to turn 65. By 2030, one in every five Americans (72 million) will be aged 65 and older[1]. Additionally, the United States Census Bureau estimates that the number of US citizens age 85 and older will increase 3-fold from 5.7 million in 2008, to 19 million by 2050[1]. The risk for functional limitations and physical disability increases with age and is one of the main reasons for why the aging of the US population is a large public health issue[2-4]. In addition to increasing incidence, the prevalence of physical disability in older adults is also quite high. According to NHANES, 20% of those aged 60 and older have ADL disability, 23% have IADL disability, 30% have mobility disability and 48% experience functional limitations. Physical disability is also quite expensive, as physically disability attributable to sarcopenia alone, in the United States in 2000, was estimated to cost between $11.8 billion and $26.2 billion[6]. Furthermore, community-dwelling older adults who are functional disabled or transition to being functionally disabled spend $10,000 more on healthcare

over a 2-year span compared to those who remain functionally independent[5]. Therefore, research designed to study the etiology and risk factors of age-related physical disability is vital and could have large public health ramifications.

Obesity in older adults is also a large public health concern, as obesity has been shown to exacerbate physical disability in older adults[163,164]. Furthermore, in 2010, men and woman age 60 and older possessed the highest prevalence rates of obesity compared to any other age group, with rates of 42.3% and 36.6% respectively[349]. The first paper presented in this dissertation provides novel evidence concerning mechanisms, specifically decreased in skeletal muscle and visceral adiposity, by which weight-loss and weight-loss plus physical activity improves physical function in older adults. This research could prove to be very useful to clinicians and public health professionals. For physicians, this work provides further evidence that weight-loss in older adults is safe, and that targeting specific fat depots through lower extremity exercises and caloric restriction may be particular effective in improving physical function. More research is needed to determine subsets of participants that would benefit from having a detailed body composition exam and the cost/benefit of such exams. For public health professionals and researchers, this work provides information that can be used to design more effective interventions.

The next two papers presented in this dissertation focused on the role of skeletal muscle mitochondrial energetics and its effect on walking performance and fatigability in older adults. This research is really in its infancy, as epidemiologic studies are just now beginning to explore the effects of mitochondrial dysfunction at the whole-body (i.e. walking performance) and population levels. The role of the neuromuscular system in inducing age-related declines in physical function is still poorly understood. This research provides novel evidence that deficits in mitochondrial energy production may play a role in age-related declines in function and

increased fatigability levels. As previously stated, the prevalence and cost of physical disability in older adults is quite high. Thus, determining novel underlying causes of functional decline, such as mitochondrial dysfunction, is important to effectively prevent and treat physical disability in older adults. As mentioned previously, more research is needed to indentify subsets of older adults in which mitochondrial dysfunction may be particularly important and to determine the best methods to improve mitochondrial function in older adults.

The prevalence of fatigue in older adults has been shown to be as high as 68%. Fatigue has also been shown to be an independent risk factor for functional decline and disability in older adults. Therefore fatigue in older populations is a significant public health issue. Furthermore, a majority of fatigue can be attributed to underlying diseases; however a large portion cannot (~30%)[9]. The third paper presented in this dissertation provides novel evidence implicating mitochondrial dysfunction as a possible underlying cause of increase fatigability levels. Determining non-disease related causes of increase fatigability levels in older adult in order to better understand its etiology and prevent or treat its onset is important.

Treating or preventing fatigue in older adults may have large public health ramifications for two reasons. First, as previously stated, the prevalence of fatigue in older populations has been shown to be quite high. Secondly, decreasing fatigue or fatigability (exercise tolerance), may be very effective in increasing an individual's level of physical activity. That is, if one can tolerate more activity, it is logical to think that one may be more inclined to do more activity. Thus, improving fatigability may result in a lasting, long-term increase in physical activity, which would promote mitochondrial function and optimize body composition. Cross-sectional research indicates that older adults wither higher levels of fatigability are less physically active, however longitudinal and interventions studies are needed to establish causality and to determine

if decreasing fatigability results in increased levels of physical activity. Finally, individuals who are fatigued may benefit from a light physical activity program, consisting mainly of walking and supplemented with lower extremity resistance or power training. In a fatigued population of older adults, it would be important to begin with very low levels of exercise, and then progressively increase the intensity and duration of activity over the course of the trail. Starting with low levels of activity would aid in not overburdening participants, resulting in poor adherence and dropouts.

In conclusion, this research provides novel evidence regarding specific aspects of muscle aging, including skeletal muscle adiposity and mitochondrial dysfunction. Neuromuscular performance is an important component to maintain in order to preserve physical function into old age. In order to determine the most effective ways to prevent physical disability, understanding its etiology is vital. This research is important as the number of older adults is rising rapidly and the prevalence of physical disability in older adults is quite high and is related to high health care costs, lower quality of life and increase risk for mortality. The findings presented in this dissertation mainly pertain to higher functioning older adults. Preventing functional decline in higher function older adults would have a large public health impact, as it would keep these older adults functionally intact into older ages, avoiding the risks and complications that coincide with functional limitations, including further functional decline, higher health care costs, institutionalization and death. Mitochondrial function and skeletal muscle adiposity may be new therapeutic targets to treat and prevent declining physical function in older adults who have yet to manifest overt functional limitations, or who have begun to decline physically, but are still early in the disablement pathway.

APPENDIX: SUPPLEMENTAL TABLE

Table 22. Comparison between those in SEA and LIFE with Discomfort at the end of 400m Walk

	SEA (n=5) Mean (SD) or N (%)	LIFE (n=13) Mean (SD) or N (%)
Age, yrs	78.8 (7.2)	75.4 (5.6)
Sex , female	3 (60.0)*	13 (100.0)*
Race, white	5 (100.0)*	5 (38.5)*
BMI, kg/m^2	25.8 (3.1)	30.5 (6.1)
SPPB, 0-12	11.0 (1.7)*	7.7 (1.8)*
5 Chair-Stand Time, s	11.9 (5.5)**	18.4 (7.5)**
Gait Speed, m/s	1.3 (0.2)*	0.79 (0.18)*
Time to Walk 400m, s	344.6 (73.7)*	520.4 (133.6)*
ATPmax, mM ATP/s	0.44 (0.16, 0.30 - 0.70)**	0.52 (0.14, 0.36 - 0.90)**
Smoking Status, Current/Former	2 (40.0)	2 (15.4)
Alcohol Intake, 6+ drinks/week	0 (0.0)	1 (7.7)
How often Felt Tired, some or more	3 (60.0)	10 (76.9)
Diabetes	0 (0.0)	2 (15.4)
History of PAD	0 (0)	0 (0)
History of CHF	1 (20.0)	1 (7.7)
History of MI	0 (0.0)	1 (7.7)
History of COPD	0 (0.0)	1 (7.7)
History of Arthritis	3 (60.0)	1 (7.7)*
History of Cancer	3 (60.0)*	1 (7.7)*
Health Rating, Poor/V Poor/Fair	4 (80.0)	1 (7.7)
Minutes/Day Sedentary Activity, armband/actigraph	750.2 (126.3)	610.3 (84.8)
Minutes/Day Light Activity, armband/actigraph	228.2 (110.2)	229.3 (64.1)
Minutes/Day Mod Activity,	42.3 (27.2)*	1.2 (1.2)*
Minutes/Day All PA Mod	42.7 (27.5)*	1.2 (1.2)*

Table 22 Continued

and above		
Minutes/Day All Activity	270.9 (118.3)	230.5 (64.6)

***between group difference at p<0.05**
**** between group difference p=0.06**

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