Tools and Technologies for Assessing, and Exercise Strategies for Promoting, Neuromuscular Function and Mobility in Aging

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Abstract

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Tools and Technologies for Assessing, and Exercise Strategies for Promoting, Neuromuscular Function and Mobility in Aging

Directors of Dissertation: Brian C. Clark and David W. Russ

Age-related declines in physical function and mobility can be attenuated by routine physical activity, however, less than 50% of adults over the age of 65 exercise regularly. As such, time-efficient exercise strategies are being investigated as a means of improving and/or maintaining physical function. If these strategies are to be systematically evaluated for effectiveness, it is essential that the techniques employed to quantify exercise-induced changes have been validated to assess the outcome of interest. The widespread use of standardized measurement techniques allows for more accurate cross-investigation analyses (i.e., meta-analysis), while simultaneously making it easier to assess reproducibility. However, our search for knowledge is hampered when inappropriate techniques are employed, or when appropriate techniques are employed in a manner for which they have not been validated (e.g., cross-sectional vs. longitudinal). These potentially erroneous reports result in inconsistent messages, making it difficult to determine optimal treatment strategies for unique clinical populations (e.g., older adults). Thus, the global aim of this dissertation was to **1)** systematically examine tools for assessing physiological, morphological, and functional adaptations where exercise served as a stimulus for change, and **2)** employ said tools in the development and execution of a proof-of-concept, proof-of-mechanism clinical trial investigating the effectiveness of a novel exercise strategy against existing strategies.

Experiment 1 challenged the recommendation that dual-energy X-ray absorptiometry (DXA) should be considered the reference standard for the assessment of muscle mass, particularly as it relates to exercise- and/or disease-induced changes in muscle mass. Thigh muscle size was quantified with DXA and magnetic resonance imaging (MRI) in 26 adults (29.2 ± 9.5 years) before and after 10 weeks of low-load resistance exercise, and relationships between the two measures were examined cross-sectionally and longitudinally. We found that DXA- and MRI-derived measures of muscle size were strongly associated both at baseline (r = 0.89) and post-intervention (r = 0.90), but the relationship between percent change in DXA and MRI was much weaker (r = 0.49). This led us to conclude that DXA is not an accurate tool for the assessment of change in muscle size (see Chapter 3 for full results).

Experiment 2 was designed to evaluate and standardize a technique for detecting neural deficits in voluntary rate of force development (RFD) in 20 young (22.0 ± 1.7 years) and 16 older adults (72.3 ± 7.5 years). Voluntary RFD during an isometric ballistic effort was calculated at 50 ms intervals from force onset to 200 ms, and expressed relative to electrically evoked peak RFD, resulting in a new variable that we termed the Central Activation Ballistic (CAB) force ratio. Older adults demonstrated a 10% neural deficit in RFD from 100-150 ms, as detected by the CAB force ratio. In contrast, electromyography measures were not significantly different between young and older adults, suggesting that the CAB force ratio is a more precise method of assessing neural deficits during isometric ballistic efforts (see Chapter 4 for full results).

Experiment 3 consisted of a proof-of-concept, proof-of-mechanism clinical trial designed to assess the effectiveness of high-intensity interval training (HIIT) to induce

muscular, cardiorespiratory, and physical function adaptations in older adults, relative to adaptations seen in response to moderate-intensity continuous training (MICT) or resistance training (RT). Contrary to our initial hypothesis, HIIT did not have a substantial effect on muscular measures. However, the effects of HIIT on cardiorespiratory and physical function measures were moderate-to-large. MICT had equally small effects on muscular measures, and similar, but muted effects on cardiorespiratory and physical function measures. Surprisingly, RT had moderate-to-large effects on cardiorespiratory measures, in addition to the expected muscular adaptations. These findings indicate that RT may be an effective standalone exercise strategy for older adults (see Chapter 5 for full results).

These three experiments represent unique approaches to improving rigor and reproducibility of biomedical research. The first of these approaches was to challenge the assumptions that support the recommendation of a specific measurement technique in order to strengthen the recommendation through evidence-based corroboration of the assumptions, or to provide evidence that the recommendation should be re-evaluated. The second of these approaches involved the development and standardization of a novel method of measurement with the goal of providing future investigators with a simple and theoretically sound research tool. The final approach involved using previously standardized research tools to assess the effectiveness of a novel intervention. Having a strong understanding of each of these approaches is an invaluable skillset for a successful biomedical researcher.

Dedication

To my partner, Hailee, and our daughters, Gryffin and Virginia

Thank you for keeping me grounded

Acknowledgments

I would like to express my sincere gratitude to Dr. Brian Clark. You are the perfect example of maximizing one's potential, and your commitment to excellence infects anyone lucky enough to be near you. Thank you for taking me on as a mentee; your unwavering support and faith in my abilities gave me the opportunity to reach past what I thought was possible and has had a profound effect on my life. I am also grateful to Dr. David Russ for stepping in as co-advisor. Your ability to anticipate potential problems and force me to think through solutions has strengthened my critical analysis skills and prepared me to be an independent and open-minded researcher. Thank you.

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Abbreviations

Chapter 1

ACSM: American College of Sports Medicine
CAB: central activation ballistic force ratio
CSA: cross-sectional area
CVD: cardiovascular disease
DART: dual-benefits of aerobic and resistance training
DXA: dual-energy X-ray absorptiometry
EMG: electromyography
HIIT: high-intensity interval training
MICT: moderate-intensity continuous training
MRI: magnetic resonance imaging
NIA: National Institute on Aging
NIH: National Institutes of Health
RFD: rate of force development
ROI: region of interest
RT: resistance training

Chapter 2

ACh: acetylcholine
ADL: activity of daily living
AET: aerobic exercise training
AP: antagonistic pleiotropy
ATP: adenosine triphosphate
Ca^{2+}: calcium
CMAP: compound muscle action potential
CRF: cardiorespiratory fitness
CSA: cross-sectional area
CT: computed tomography
CVD: cardiovascular disease
DHPR: dihydropyridine receptor
DXA: dual-energy X-ray absorptiometry
E-CC: excitation contraction coupling
EMG: electromyographic
FF: fast fatigable
FoxO: Forkhead box O
FR: fast fatigue-resistant
GXT: graded exercise test
HDsEMG: high-density surface electromyography
HIIT: high-intensity interval training
IADL: instrumental activity of daily living
IGF-1: insulin-like growth factor 1
MA: mutation accumulation
MHC: myosin heavy chain
MN: motor neuron
MRI: magnetic resonance imaging
MSTN: myostatin
mTOR: mammalian targe of rapamycin
mTORC1: mTOR complex 1
MU: motor unit
MUNE: motor unit number estimation
NA^{+}: sodium
NMJ: neuromuscular junction
PAP: postactivation potentiation
PAPE: postactivation performance enhancement
PTP: posttetanic potentiation
RFD: rate of force development
ROS: reactive oxygen species
RT: resistance training
RYR: ryanodine receptor
S: slow
SC: satellite cell
SMUP: single motor unit potential
SR: sarcoplasmic reticulum
T2D: type 2 diabetes
VO_2max: maximal oxygen uptake

Chapter 3

A: area
CSA: cross-sectional area
CT: computed tomography
CV: coefficient of variation
DXA: dual-energy X-ray absorptiometry
LOA: limits of agreement
MRI: magnetic resonance imaging
MV: muscle volume
MVC: maximal voluntary contraction
ROI: region of interest
T: known distance between MRI slides

Chapter 4

BMI: body mass index
CAB: central activation ballistic force ratio
DXA: dual-energy X-ray absorptiometry
EMD: electromechanical delay
EMG: electromyography
EMG_{AVG}: average amplitude of the electromyographic signal
EMG_{MAX}: average amplitude of the electromyograph signal at peak force of maximal voluntary contractions
EMG_{RR}: rate of rise of the electromyographic signal
MVC: isometric maximal voluntary contraction
NRFD: normalized rate of force development
$NYank_{VOLUNTARY}$: normalized voluntary peak rate of force development
RFD: rate of force development
$RFD_{VOLUNTARY}$: voluntary rate of force development
RMS: root mean square
SPPB: short physical performance battery
VA: voluntary activation
$Yank_{EVOKED}$: peak rate of force development during the doublet-evoked contraction
$Yank_{VOLUNTARY}$: voluntary peak rate of force development.

Chapter 5

4SST: four-square step test
A: area
AE: adverse event
ACSM: American College of Sports Medicine
CSA: cross-sectional area
CVD: cardiovascular disease
DART: dual-benefits of aerobic and resistance training
DXA: dual-energy X-ray absorptiometry
ECG: electrocardiograph
GXT: graded exercise test
HIIT: high-intensity interval training
HRR: heart rate reserve
MICT: moderate-intensity continuous training
MV: muscle volume
MVC: maximal voluntary contraction
MRI: magnetic resonance imaging
RPE: rating of perceived exertion
RT: resistance training
SAE: serious adverse event

SPPB: short physical performance battery
T: known distance between magnetic resonance imaging slices
VO_2max: maximal oxygen uptake
W: watts

Chapter 6

BMD: bone mineral density
CAB: central activation ballistic force ratio
CRF: cardiorespiratory fitness
CT: computed tomography
CVD: cardiovascular disease
DXA: dual-energy X-ray absorptiometry
EMG: electromyography
HIIT: high-intensity interval training
MICT: moderate-intensity continuous training
MRI: magnetic resonance imaging
RFD: rate of force development
RT: resistance training
SIT: sprint interval training
T2D: type 2 diabetes

Chapter 1: Introduction

Background

Nearly half of US adults over age 60 report difficulty performing one or more activities of daily living essential to maintaining independence, a fraction that has remained stable over the last twenty years despite an overall increase in the mean age of the US population [1]. Increasing numbers of older adults with disabilities will continue to drive up healthcare costs, making maintenance of health and independence a top priority for both middle-aged and older adults [2]. As the population of older adults has continued to increase there has also been increased interest in, and effort put toward, understanding age-related reductions in health, physical function, and mobility, with the goal of improving quality of life and healthspan in later decades. For example, the annual budget appropriation for the National Institute on Aging (NIA) has nearly doubled in the last ten years, from $1.1 billion in 2009 to $2 billion in 2019 [3]. This makes up ~10% of the increase in funding over the same period for the National Institutes of Health ([NIH] the parent organization of the NIA that provides funding for biomedical research across several disciplines, currently exceeding $41 billion annually [4]). Unfortunately, a significant proportion of this funding is unproductive due to improper methodological and reporting practices (e.g., use of clinically unachievable concentrations, distorted presentation of results) [5,6]. For example, recent reports indicate that upwards of 90% of preclinical studies have irreproducible results [6], and that up to 80% of phase II and 50% of phase III clinical trials fail to meet their expected outcomes [7]. As a result there has been an increased emphasis on improving rigor and reproducibility of biomedical

research in the last decade [5–7]. In regard to aging research, this is exemplified by the standardization of techniques for assessing physiological, morphological, and functional changes in response to an intervention [8–10].

One area of intervention research that has received considerable attention is the use of exercise to slow and/or prevent the age-related decline in physical function and mobility. This field of study is not immune to the irreproducibility issues facing the scientific community, wherein the lack of technique standardization hampers the assessment of exercise effects. Further complicating the problem is the fact that common tools for assessing intervention-induced changes are often used to answer questions for which they have not been validated (e.g., for longitudinal study designs when they have only been validated cross-sectionally), resulting in potentially erroneous reports [8]. Consequentially, the optimization of exercise strategies for delaying or reversing physical function decline remains problematic. Age-related physical function decline is characterized by poor cardiorespiratory fitness and skeletal muscle impairments [11–13], which can partially be attributed to reduced physical activity with age [14–16]. Exercise is clearly effective at maintaining physical function in older adults, as evidenced by data indicating those who take part in supervised exercise programs demonstrate improvements in physical function outcomes [17–19]. The fact that adaptations have been seen in the oldest adults (90+ years) is particularly encouraging [20–22]. Unfortunately, less than 13% of older adults meet the exercise recommendations provided by the American College of Sports Medicine (ACSM) that include muscle strengthening and aerobic activities [1,23]. Consequently, the majority of older adults

who do exercise are either not getting the muscular adaptations necessary for maintaining independence, or they are not getting the necessary cardiorespiratory adaptations. With the vast majority of older adults not exercising regularly or only performing one type of exercise [1], a more pragmatic approach of emphasizing a single exercise type may be warranted. However, it is unclear whether a single established strategy (i.e., resistance training, aerobic training) is capable of inducing both cardiorespiratory and muscular adaptations in older adults, or if a novel strategy would be more effective. Central to this lack of understanding is the perception that adaptations to resistance and aerobic training are independent of one another [24]. As such, the global aim of this dissertation was to **1)** systematically examine tools for assessing physiological, morphological, and functional adaptations where exercise served as a stimulus for change, and **2)** employ said tools in the development and execution of a proof-of-concept, proof-of-mechanism clinical trial investigating the effectiveness of a novel exercise strategy against existing strategies. This goal was achieved by leveraging data from three separate intervention studies, detailed below.

Specific Aims

Aim 1. To determine the accuracy of dual-energy X-ray absorptiometry (DXA) at detecting changes in muscle size in response to exercise.

Approach. We examine the relationship between magnetic resonance imaging (MRI) and DXA-derived changes in thigh muscle size in response to a 10-week, low-load resistance training exercise intervention in young adults. MRI is considered the "gold standard" method, and any differences that exist between MRI and DXA-derived

measures would be considered to be related to DXA measurement error. Agreement between the two methods will be determined with a Bland-Altman analysis.

Hypothesis. The relationship between changes in MRI and DXA-derived measures of muscle size will be weaker than the relationship between cross-sectional measures.

Aim 2. To develop and standardize a technique capable of detecting neural deficits in ballistic rate of force development (RFD).

Approach. We describe and empirically examine a novel approach to quantify the neural contribution to ballistic RFD in both young and older adults, which we dubbed the Central Activation Ballistic (CAB) Force Ratio. We first examine the relationship between electromyography (EMG), CAB, and RFD cross-sectionally (older vs. young adults), and then in response to a 12-week resistance training intervention in older adults.

Hypothesis. We will observe **1)** a robust relationship between EMG measures, CAB, and RFD among healthy young adults, but that the relationship between EMG and RFD will be substantially weaker among community dwelling older adults, while the relationship between CAB and RFD would remain robust; and **2)** a modest relationship between the exercise-induced changes in surface EMG measures, CAB, and RFD among community-dwelling older adults.

Aim 3. To determine the effect of bicycle high-intensity interval training (HIIT) on cardiorespiratory/endurance, muscle strength/power and size, and physical function measures.

Approach. We quantify the effect of 12 weeks of bicycle HIIT on cardiorespiratory/endurance, muscle strength/power and size, and physical functional measures, compared to moderate-intensity continuous training (MICT) or resistance training (RT) in generally healthy but insufficiently active older adults. Primary outcomes include knee extensor power, maximal oxygen consumption, and quadriceps muscle volume. Secondary outcomes include knee extensor strength, knee extensor fatigue resistance, and body composition. Functional outcomes include six-minute walk distance, four-square step test, and grip strength.

Hypotheses. the HIIT group will see improvements in **1)** cardiorespiratory/ endurance measures equal to the MICT group and greater than the RT group; **2)** muscular strength/power and size measures equal to the RT group and greater than the MICT group; and **3)** functional measures greater than either the MICT or RT groups.

Significance

In recent years there has been greater emphasis placed on rigor and reproducibility of biomedical research in order to reduce waste and improve comparability between studies through standardization of techniques. The three experiments detailed in this dissertation were designed with this in mind. Our first experiment examined the relationship between MRI and DXA-derived measures of thigh muscle size in response to an intervention. DXA is by far the most common method of assessing muscle size in clinical trials [25] and has recently been recommended as the "reference standard" for the measurement of muscle mass [26]. However, DXA has only been validated cross-sectionally [27,28], and it is unclear if DXA is a reliable method of

detecting changes in muscle mass longitudinally. Consequently, if our hypothesis is correct this experiment will demonstrate the inability of DXA to detect changes in muscle size using MRI as a reference, which will provide researchers who are developing future studies more context for their choice of modality for measuring changes in muscle size. Our second experiment is designed to develop and standardize a novel technique to detect neural deficits in ballistic RFD. Surface EMG is commonly used to describe the neural determinants of RFD, however, there are several limitations inherent to the surface EMG signal that call into question its capability to detect altered neural drive to the muscle. If our hypotheses are correct it would suggest that our novel CAB Force Ratio measurement may be a simple and innovative approach for assessing neural deficits in ballistic RFD. Our final experiment was designed to quantify the effect of HIIT on cardiorespiratory/endurance, muscle strength/power and size, and physical function in older adults in order to determine if it is a more efficient standalone exercise strategy than either MICT or RT. The age-related loss of mobility and physical function is largely a result of reduced cardiorespiratory and neuromuscular function that worsens with increasing age, and HIIT is an exercise strategy that has the potential to produce both cardiorespiratory and neuromuscular improvements simultaneously. If our hypotheses are correct it would provide evidence that HIIT could allow individuals to realize the benefits of aerobic and resistance training through a single exercise strategy and decrease the risk of physical function decline and cardiovascular disease.

Innovation

The proposed work is innovative for three reasons. First, Aim 1 compares the effects of exercise on changes in muscle size using two common techniques and directly challenges the assumption that DXA-derived change in muscle size is accurate because of its strong association with MRI in cross-sectional investigations. Second, Aim 2 leverages the relationship between the neural and muscular systems to develop a novel method of quantifying neural deficits in RFD by defining the muscular contributions, which in turn allows for the neural contributions to be defined as well. Finally, Aim 3 **1)** directly compares the physiological adaptations of the experimental exercise strategy (HIIT) against established exercise strategies (MICT or RT) to determine feasibility and effectiveness of the new program, and **2)** measures cardiorespiratory/endurance outcomes and muscular strength/power measures for all exercise groups, allowing us to better describe the effects of HIIT in relation to exercise strategies that are commonly employed in an older adult population.

Assumptions, Limitations, and Delimitations

Assumptions.

- Participants provided accurate self-report data for all questionnaires.
- Participants gave maximal effort during all voluntary assessments that required maximal exertion.
- Equipment employed (e.g., MRI scanner, Biodex) was in proper working condition, and all data collected from such equipment was accurate.
- Any changes in outcome measures were induced by the exercise intervention.

- Participants were honest regarding compliance with pre-testing instructions

Limitations.

- All Aims
    - Participants were recruited from the Ohio University campus and surrounding community through flyers, email, and word of mouth, and as such the sample is not truly random
    - Findings may not be applicable to individuals outside of the age ranges tested in each of the individual experiments.
- Aim 1
    - The MRI scanner uses a 0.25-tesla magnet and may produce lower quality scans than high-field magnets. Scan time was increased to reduce the signal-to-noise ratio.
    - MRI-derived muscle size was quantified from a five-slice section (approximately 4 inches in length) of the mid-thigh and may not truly represent the total thigh muscle size.
    - The data from this experiment was derived from a previous randomized controlled trial that was not specifically designed to answer the scientific question.
- Aim 2
    - The surface EMG signal was only recorded from the vastus lateralis, while knee extension relies on multiple muscles.

  - The stimulation duration and frequency were not sufficiently high enough to elicit a true maximal RFD *per se*.
  - Participants who were enrolled in the exercise portion of the study volunteered and were not randomly assigned.

- Aim 3
  - The final sample was not adequately powered to compare differences between groups due to the COVID-19 pandemic.
  - Due to the nature of the intervention it was impossible to blind participants to their allocation group.
  - Neither work output nor heart rate where tracked in the RT group.

Delimitations.

- Aim 1
  - To determine whether differences in the region of interest (ROI) between MRI and DXA-derived measures of muscle size influenced our findings we created a customized ROI to analyze the middle third of the thigh using the DXA software, making MRI and DXA scans directly comparable. We found that there was even less agreement between the two measures when using the customized ROI.
- Aim 2
  - Data for this experiment was generated from the best of three voluntary trials instead of taking an average value of the three trials. The goal of the

study was to determine maximal capacity of the muscle to produce rapid force, and for this reason we analyzed a single trial.

- We chose to analyze RFD in the first 200 ms using sequential time points (e.g., 0-50 ms, 50-100ms), instead of overlapping time points (e.g., 0-50 ms, 0-100 ms) in order to reduce the influence of early RFD contributors at later time points.

- Aim 3
  - We chose to test low-volume HIIT (i.e., less than 15 minutes of high-intensity work per session) instead of high-volume HIIT in our older adult population in order to make the HIIT sessions half the duration of the MICT sessions. It is recommended that older adults perform moderate-intensity endurance-type exercise for at least 150 minutes per week, or vigorous-intensity exercise for at least 75 minutes per week [23]. In consideration of this, we classified HIIT as "vigorous-intensity" and MICT as "moderate-intensity", and calculated exercise duration for the HIIT group to be half the duration of the MICT, which most closely matched a low-volume HIIT protocol.
  - We utilized a Peloton stationary bicycle with pre-recorded workout sessions for both the HIIT and MICT cycling groups to 1) easily track and store participant data, and 2) improve adherence to the MICT sessions by reducing the monotony associated with long-duration, continuous endurance exercise.

- We recruited older adults who were insufficiently active, which we defined as either not meeting the aerobic or muscle strengthening guidelines, instead of those who were already exercising regularly in order to determine the effectiveness of starting a new exercise program at improving overall health.

Funding

NIH R21AR063909 **PI: Brian Clark** **07/01/2014-08/31/2017**

National Institute of Health: NIAMS $408,375

Novel exercise interventions to improve trunk muscle function: A Pilot Study

This application proposed to conduct an exploratory trial to determine if a novel exercise protocol—blood flow restricted exercise— increases erector spinae muscle morphometry and function in patients with recurrent LBP. Data from this study was used to determine if DXA was able to accurately detect changes in thigh muscle size, using MRI as a reference standard (Aim 1).

Role: Data Analyst

NIH R01AG044424 **PI: Brian Clark** **09/01/2014-01/31/2021**

National Institutes of Health: NIA $1,376,867

Neural mechanisms of dynapenia

This translational physiology study seeks to determine the neurological mechanisms (or contributors) to muscle weakness (i.e., Dynapenia) classically observed in older adults. Data from this study was used to develop a standardized technique capable of assessing neural deficits in ballistic RFD (Aim 2).

Role: Data Analyst

AHA 19PRE34380496 **PI: Dallin Tavoian** **07/01/2019-06/30/2021**

American Heart Association $53,688

Cycle high-intensity interval training to improve cardiorespiratory and muscular function in older adults: The DART Study

The goal of this work is to determine the effects of twelve weeks of high-intensity interval training on cardiorespiratory, muscular, and physical function in older adults, compared to adaptations seen in response to traditional aerobic or resistance training.

Role: Principal Investigator

Ohio University - Internal **PI: Dallin Tavoian** **03/17/2020-03/16/2021**

Student Enhancement Award $6,000

Cycle high-intensity interval training to improve cardiorespiratory and muscular function in older adults: The DART Study

This award supplemented AHA 19PRE34380496, covering direct project costs (Aim 3).

Role: Principle Investigator

Ohio University - Internal **PI: Dallin Tavoian** **11/08/2018-10/31/2019**

John J. Kopchick MCB/TBS Research Fellowship $10,000

Cycle high-intensity interval training to improve cardiorespiratory and muscular function in older adults: The DART Study

This award supplemented AHA 19PRE34380496, covering direct project costs (Aim 3).

Role: Principle Investigator

Chapter 2: Review of the Literature

Portions of this chapter have been published in *Scientific Reports* [29] and *Frontiers in Physiology* [30], or are under review for publication in *Journal of Applied Physiology* and *Journal of Frailty and Aging*.

Introduction

The following sections in the chapter provide an overview of available literature related to the scope of this dissertation work regarding the different aspects of aging and exercise strategies that slow or reverse age-related declines in physical and physiological function, as well as tools and technologies used to assess these age-related changes. Part I discusses theories that explain why we age, as well as current and future aging demographics. Part II covers the physiological mechanisms underlying neuromuscular adaptations that occur during the aging process. Part III focuses on the factors that lead to age-related declines in physical function and mobility. Part IV discusses the effects of recommended exercise strategies on physiological and physical function in older adults. Finally, Part V summarizes the strengths and weaknesses of tools and technologies that are used to assess physiological and morphological adaptations to aging and exercise.

Part I: Human Aging

The vast majority of animals never reach a point in their lives where body systems shut down of their own accord, or in other words, they do not die of old age. A large portion of wild animals die at birth or shortly after, and animals who survive infancy are exposed to harsh environments, disease, predators, accidents, and potential starvation [31]. In contrast, the infant mortality rate in the United States has dropped to less than

0.06%, and nearly half of all deaths in the US are a result of cardiovascular disease or cancer [32]. Aging is defined, for the purpose of this dissertation, as the age-progressive decline in physiological function and adaptation, leading to an increase in the age-specific mortality rate [33]. Instrumental to this decline is senescence (i.e., the age-related loss of function at the cellular, tissue, or organ level) [33]. It should be noted that at some point in the aging process, if an individual lives long enough, there appears to be a cessation in physiological decline [34], though total physiological failure and death are eventual certainties. Theories attempting to explain the reason for aging fall into two main camps: programmed senescence and damage/error senescence [35]. Regardless of the reason, the human body slowly stops being able to adapt and overcome tissue damage until a point where organs or entire systems fail, ultimately resulting in death. The relatively recent explosion of medical advances, along with other improvements (e.g., enhanced nutrition) has extended life expectancy, though maximal lifespan remains unchanged [36]. Hand in hand with increasing age come higher rates of chronic disease, and ultimately, higher rates of disability [37]. The result is that people are living longer, but the quality of those extra years is not necessarily high. As life expectancy continues to increase it is important that health and quality of life be maintained during the later years. As the proportion of the global population of young and old shifts, plans are already in motion to address the needs of future generations.

Aging Theories. There are over 300 published theories attempting to explain and define aging, with no consensus [38]. However, nearly all contemporary theories agree that aging is a multifaceted and complex process [39], resulting in heterogeneous

responses within the human population [38]. These theories explain aging through one of two lenses: programmed senescence or damage/error senescence [34,35,38–41]. Programmed senescence is rooted in the idea that aging follows a biological timetable, similar to that of puberty [35]. On the other hand, damage/error theories claim that the human body accumulates damage through environmental stressors, and that over time enough cells become dysfunctional to result in organs or entire systems to also become dysfunctional [35]. Human aging undoubtedly consists of both programmed senescence and damage accumulation, but first it is important to understand why we age from an evolutionary perspective.

Early biologists believed that aging, or a "death mechanism", was an adaptation ensuring species survival by leaving room and resources for future generations [42]. However, recent work rejects this hypothesis, instead proposing that aging is due either to mutation accumulation (MA) [43] or antagonistic pleiotropy (AP) [33], or both. The MA theory describes the effectiveness of natural selection at weeding out individuals with deleterious genetic mutations. Individuals with negative mutations that result in symptoms that reduce survival likelihood at young ages are less likely to reproduce and pass along the mutation [43]. However, if symptoms are not expressed until after the reproduction, therefore not affecting reproductive success, then the effects cannot be selected for or against [42,43]. Further, if environmental mortality is high, as it was for most of human evolution, then the effects of deleterious genetic mutations that manifest later in life may never be seen. Over time these mutations would accumulate, and eventually as humans evolved and environmental mortality was reduced, humans would

live to an age where they would experience this accumulation of negative effects (i.e., aging) [43]. The AP theory built on this idea, arguing that some genetic variants may be beneficial at younger ages, but have deleterious effects later in life [33]. If true, these genetic variants would be favored via natural selection as they would improve survival and reproduction, but would also result in the evolution of aging [33]. These theories are graphically displayed in Figure 2.1. With the evolutionary reasons for aging established, contemporary theories of biological aging will now be addressed.

Programmed senescence can most easily by visualized by comparing it to childhood growth and development. In general, human maturation takes place sometime between 9 and 20 years of age, with different tempos of changes and growth rates within and between sexes [44]. It can be postponed or accelerated due to environmental changes, but maturation is ultimately programmed into our DNA to start and stop [44]. Programmed aging theories are similar in that they are based on the premise that age-related changes in cellular function, hormone regulation, and immune function are programmed to start at a specific point in the life cycle [35,41]. These changes lead to increased vulnerability to infectious disease, environmental disturbances, and comorbidities, eventually ending in death [35,38,40,41]. One obvious example of programmed change is the onset of menopause in women. However, programmed aging cannot fully explain why or how we age. It is clear that our environment and lifestyle choices can affect our rate of aging [45,46], which strongly suggests that damage accumulation moderates the aging process.

One of the earliest damage accumulation theories consisted of the idea of "wear and tear" [35]. This theory posits that the human body is like a machine, and that repeated use results in damage to certain components until failure is inevitable. The theory has been criticized, at least in its original form, as higher rates of use (i.e., regular exercise) result in improved health and longevity, not earlier failure as would be predicted [41]. It has been adapted so that positive lifestyle behaviors like diet and exercise are considered "machine maintenance" [41], though it still leaves many aspects of aging unexplained. The Rate of Living theory is based on the relatively strong association between maximal lifespan and body size, a stand-in for metabolic rate [47,48]. The defining aspect of this theory is the belief that all animals are born with a finite amount of energy, and the rate of aging increases if that energy is used quickly. Larger animals generally live longer and have relatively slower metabolic rates than smaller animals, as well as slower rates of growth, heart rate, respiration, and reproduction (although within a species the opposite is often the case) [47]. Exceptions to this rule are intriguing. For example, naked mole rats regularly survive past 25 years, nine times longer than mice of the same body size [49]. Additionally, cancer appears to be nonexistent in these animals, nor are there impairments in insulin sensitivity or reproduction with increasing age, hallmarks of aging in most mammals [49]. Research into such long-lived animals will likely result in novel insights into the aging process.

The prevailing theory on aging is that of free radicals, or reactive oxygen species (ROS) [50]. This theory proposes that certain chemical compounds, classified as ROS, are produced by the body during normal metabolic processes, as well as by exposure to

external sources like X-rays or cigarette smoke [50–52]. Free radicals are unstable, highly reactive, and often attack macromolecules, leading to cell damage and cell death (oxidative stress) [52]. Antioxidants are the body's defense against ROS, however, oxidative stress occurs when ROS formation exceeds antioxidant protection capabilities [53]. ROS have been implicated in cardiovascular disease [54], cancer [55], diabetes [56], aging [57], and Parkinson's disease [58]. In aging specifically, it is believed that as ROS damage accumulates, cells, organs, and entire systems become dysfunctional [59]. Overall the oxidative stress theory is only intermittently supported, despite, or possibly due to, increased interest from an ever-expanding number of fields [59]. For example, longer-lived species generally exhibit lower ROS production than shorter lived species [60]. However, the naked mole rat has relatively high levels of ROS, yet has an extraordinary lifespan and no apparent detrimental health effects of high ROS [47]. Further, results from transgenic animals with antioxidant knock-out or overexpression modulations only show inconsistent support for the theory [60]. In general, it appears that ROS plays a detrimental role in cellular function, but may play a beneficial role in other pathways [61].

Lifespan and Healthspan. The variability in maximal lifespan among species is dramatic. The *pygmy goby* fish is the shortest-lived vertebrate on record, with sexual maturity occurring at 10 days and a maximal recorded lifespan of 56 days [62]. In contrast, the longest-lived vertebrate (*Somniosus microcephalus*, or Greenland shark) reaches sexual maturity around 150 years of age, and has a maximal estimated lifespan of 400 years [63]. Humans have a maximal lifespan of ~120 years, and, despite recent

increases in average lifespan, it is not expected to increase [64]. Life expectancy at birth, a forecast that half of those born in a given year will reach a certain age, has increased considerably over the last century. In 1900 life expectancy at birth in the US was 47.3 years; it has increased continuously until peaking in 2014 at 78.9 years before declining modestly [65,66]. Despite being the world leader in health spending by a significant margin [67], the US is currently ranked 28th in life expectancy at 78.6 years, well behind Japan, the current world leader at 84.2 years [68]. Life expectancy is predicted to increase over the next 10 years in most industrialized countries, though the US will only see a relatively small increase [69]. The recent dip and low projected increase in life expectancy in the US are driven by higher rates of drug overdose, alcoholic liver disease, and suicide in younger adults, as well as poor health behaviors (i.e., high calorie intake per person, rate of traffic accidents involving alcohol, and firearm-related violence), large proportion of uninsured citizens, and greater socioeconomic inequality in the general population [66,70–72]. However, while reduced life expectancy at birth is concerning, it is not necessarily an indicator of the health of older adults.

As age increases so does the likelihood of living past your predicted longevity. Life expectancy at age 65 provides an average number of years remaining prior to death for adults of that age. While an infant born in 2018 has a life expectancy of ~79 years, 50% of adults aged 65 in 2018 can expect to live until they are 85 [73]. In fact, 33% of 65-year-olds are expected to reach at least 90 years of age, while nearly 15% should reach 95 [74] (although Covid-19 will likely have a substantial negative effect on these projections). This is particularly significant as the life expectancy of a 65-year-old the

year of their birth (1955) was 69 years [65]. Death rates at older ages are lower than they were 60 years ago, though the pattern of exponential increases in age-dependent death rates starting at middle age are similar (Figure 2.2) [75]. There are many factors that can affect life expectancy, including genetic predisposition [76], biological sex [77], income inequality [78], geographic location [71], educational attainment [79,80], and even optimism [81]. Despite these factors, adherence to positive lifestyle behaviors can result in substantial improvements. Li et. al., [46] reported that adherence to five low-risk lifestyle behaviors (never smoking, healthy body weight, healthy diet, regular physical activity, and moderate alcohol consumption) could prolong life expectancy at age 50 by 12.2 and 14.0 years for men and women, respectively, compared to individuals who do not adhere to any of said behaviors. Further, the more low-risk behaviors that are adopted, and the earlier in old age that an individual begins adhering to these low-risk behaviors, the greater the increases in life expectancy (Figure 2.3) [46].

Women continue to have a higher life expectancy than men at birth and at age 65, by five and 2.5 years, respectively [65]. Currently there are 79 men aged 65 for every 100 women aged 65, and only 54 men aged 85 for every 100 women of the same age [82]. The reasons for this disparity are unclear, though men die at a higher rate than women from virtually all of the most frequent causes of death [77]. Several theories attempting to explain this disparity have been presented, including more active female immune functioning, the protective effect of estrogen, lower growth hormone and insulin-like growth factor 1 (IGF-1) in females, and lower oxidative stress in females [77]. Counter-intuitively, women have higher reported rates of disability and illness than men [83].

However, higher disability and illness rates in women may be more of a reflection of gender behaviors than actual morbidity, due specifically to differences in reporting behaviors, response to illness, utilization of health services, and/or physician behaviors [83].

As global life expectancy continues to increase it raises the question of quality of life of those extra years. Are today's older adults healthier than the older adults of previous generations, or are we simply able to keep people alive longer through improved interventional strategies? Healthspan, also referred to as disability-free life expectancy, is the portion of a person's life during which they are generally in good health [36]. Compressing morbidity (i.e., increasing healthspan) to a shorter timeframe prior to death (Figure 2.4) has become a major research focus in recent years. From 1970-2010 in the US there was an increase in disability-free life expectancy of 2.7 and 2.4 years for 65-year-old men and women, respectively [84]. However, during that time there was also an increase in life with disability of 2.1 and 1.2 years for men and women, respectively [84]. While the proportion of disability-free life after age 65 has increased slightly, the absolute increase in the amount of time living with disability is discouraging. Further, those with lower socioeconomic status and education are more likely to have more years of life with disability [85], and more years with disability leads to higher healthcare expenditures and reduced independence [37]. With the projected global increase in the number of adults reaching old age, significant changes to scientific, societal, and public policy will need to occur.

Demography of Aging. Over the next 40 years in the US the number of adults over 65 years of age will more than double from 46 million in 2014 to 98 million by 2060, while the number of adults aged 100+ will see a 10-fold increase over that time to more than 600,000 [86]. A similar rate of change is being seen worldwide, and is even higher in less developed countries [87]. Driving this increase upward is the "baby boomer" generation; Americans born between 1945 and 1964 when there was a dramatic increase in the rate of live births. Over the next 15 years this group will move into the 65+ age category, which will account for 25% of the US population [86]. This fact, paired with a global decline in fertility, will be the first time in human history when adults age 65 and over will outnumber children under age 5, and the gap is only expected to increase (Figure 2.5) [75,87]. The effects of our aging population are far-reaching, including greater financial burdens on individuals and families from medical and nursing home costs, shifting patterns of work and retirement, changing family structures, and evolving social insurance systems [86,87].

With increasing age comes an accelerated decline in physical function and a concomitant increase in comorbidities [41], as does the utilization of life support and other extreme treatments engaged with the hope of extending life [88]. In fact, half of a person's lifetime health care expenditures occur after the age of 65 [89]. Health care spending in the United States will increase exponentially for the foreseeable future, largely driven by accelerated Medicare spending [90]. Medicare and Social Security are the two major programs that rely on working adults (typically aged 18-64) to continue to be successful, and ones that are exclusively utilized by adults over the age of 65. In 2014

there were ~4.3 working-age adults for every person over 65, but this is expected to decline to 2.4 by 2060 [86], potentially crippling both programs. However, older adults are retiring later in life than previous generations as many countries are incrementally increasing the age at which workers are eligible for full public pension benefits [87], and as many as 15% of older adults are returning to work after retirement [91]. This is partially due to extended life expectancy. Greater life expectancy translates into an increase in the number of years lived after retirement, requiring that older adults have more money in savings prior to retirement in order to maintain their lifestyle for those additional years. The change in retirement age is just one example of how policy makers are preparing for this tumultuous demographic change. However, it is impossible to predict all of the adaptations that need to be made as we confront this new series of societal challenges.

Part II: Neuromuscular Morphology and Physiology

The neuromuscular system is a complex organization of machinery tasked with converting electrical signals from the brain into purposeful movement [92]. Humans have multiple types of muscle fibers with unique characteristics (e.g., innervation ratios) that allow a wide variety of movement capabilities, ranging from small and precise to large and powerful, that can be performed in short bursts or for long durations [93]. Muscular control is directed by the nervous system, wherein force production is increased through recruiting a larger number of muscle fibers and increasing the rate of firing [94,95]. Further, the neuromuscular system has the ability to adapt to meet new demands placed on it. The human body has evolved to reserve resources when possible, and skeletal

muscle has high metabolic need [96]. As a result, when the demands placed on muscle are consistently lower than the capacity of that muscle, atrophy occurs, while consistent overload results in hypertrophy [97]. Aging has a significant effect on several aspects of neuromuscular health, with apparent reductions in fiber size, contractile velocity, force production, firing rates, and signal transmission, among others [98–101], eventually leading to losses in physical function. While there has been substantial work aimed at determining the relative contribution of neural versus muscular changes to age-related deficits, much remains unknown.

Characteristics of a healthy neuromuscular system.

Skeletal muscle morphology. Humans have three distinct fiber types in skeletal muscle: type I (slow twitch), type IIa (intermediate), and type IIx (fast twitch) [93]. These fiber types are characterized by their myosin heavy chain (MHC) isoform composition, or capacity to utilize adenosine triphosphate (ATP) (i.e., level of myosin ATPase activity). Briefly, type I fibers have low ATPase activity and are therefore oxidative, with more mitochondria and capillaries, higher levels of fatigue resistance, and slower contraction velocity and force production than type II fibers [93]. Type IIx fibers have high ATPase activity and are therefore glycolytic, meaning they fatigue quickly but can produce high levels of force at higher speeds than type I fibers. Type IIa fibers are also glycolytic, but with moderate ATPase activity and thus they have some characteristics similar to both type I and IIx fibers, with modest fatigue resistance and force production capabilities [93]. All skeletal muscles consist of each type of fiber, though the proportion of type I and type II fibers is different across muscle groups depending on if the primary function

is endurance- or power-related [93]. However, it seems that within individuals there is a cross-muscle phenotype, with the proportion of type I and type II fibers being relatively similar among muscles [102]. Additionally, there is high variability in the proportion of fiber types between individuals [103], indicating that some people generally have "faster" muscles while others have "slower" muscles [102].

Contractile properties of skeletal muscle. The sarcomere is the basic contractile unit of a muscle, comprised of thick (myosin) and thin (actin) filaments [92]. Myosin consists of two globular heads and a single tail, with each head being a heavy chain having two light chains attached to it [94]). Actin filaments are strings of actin molecules linked by strands of tropomyosin, which in turn house a complex of three polypeptides, troponin T, I, and C. Troponin T attaches the complex to tropomyosin, troponin I binds to actin and prevents actin-myosin interaction, and troponin C has the ability to bind to calcium (Ca^{2+}), resulting in a conformational change of the complex that allows actin-myosin cross-bridge interactions [94]. Actin and myosin filaments are organized in series, and activation of the myosin cross-bridge results in temporary attachment to an actin molecule and subsequent propulsion of the actin filament to a new position (i.e., a power stroke), ultimately shortening the muscle. ATP then combines with the myosin head to release myosin from actin, allowing another power stroke to take place [92]. A single power stroke of all cross-bridges in a muscle would shorten the muscle by 1%. Therefore, in order for a maximal contraction to happen several concurrent, but asynchronous, power strokes must occur until individual sarcomeres have shortened to a point where the actin filament can no longer be propelled forward [93]. The driving force

behind muscle contraction is a sudden increase in Ca^{2+} concentration around actin and myosin, initiated by a muscle fiber action potential. The process of converting a muscle fiber action potential into force production is called excitation contraction coupling (E-CC) [92].

The steps involved in E-CC include **1)** depolarization of transverse tubules and activation of dihydropyridine receptors (DHPRs), and **2)** diffusion of Ca^{2+} ions from the sarcoplasmic reticulum (SR) into the myoplasm through ryanodine receptors (RYRs) [94]. The quantity of RYRs is 2-3 times greater in type II fibers than type I fibers, resulting in greater Ca^{2+} release in type II fibers [92]. In resting state, most of the Ca^{2+} is stored in the SR. DHPRs act as a voltage sensor [94], and when threshold is reached a signal is transmitted to the RYRs to release Ca^{2+}, which in turn binds to troponin C to allow cross-bridge interaction [92]. When the action potential has passed, Ca^{2+} is pumped back into the SR by Ca^{2+} ATPase, allowing actin and myosin to return to their original positions [92]. The cycling of cross-bridges continues as long as there is sufficient Ca^{2+} and ATP in the muscle cell [92].

An intriguing aspect of muscle physiology is the phenomenon of postactivation potentiation (PAP). PAP is characterized by an increase in twitch force for a short period of time after voluntary activation (or posttetanic potentiation [PTP] when the conditioning contraction is electrically stimulated) of that muscle [104]. First described in animal muscle [105], PAP is considered a property of muscle fibers unrelated to the nervous system [106]. The mechanisms underlying PAP are thought to be related to phosphorylation of the myosin regulatory light chains during the conditioning

contraction, which increases the sensitivity of the contractile proteins to activation by Ca^{2+} and enhances twitch force [107]. Maximal potentiation typically requires a maximal-effort conditioning contraction of 5-10 seconds, followed by stimulation of the muscle within a few seconds of relaxation [108]. PAP declines relatively quickly, with a half-life of ~28 seconds [109], but effects can be seen up to 10 minutes afterward [108]. The strength of PAP is affected by intensity and duration of the conditioning contraction, as well as length of the muscle [108]. This potentiation effect may also extend to voluntary efforts after the conditioning contraction (postactivation performance enhancement [PAPE]), but inconsistencies between the theoretical underpinnings of PAP and methodological approaches to assessing PAPE have resulted in equivocal results [109].

Motor unit morphology. The nervous system, as it relates to skeletal muscle control, can be divided into three distinct regions, **1)** supraspinal/cortical, **2)** spinal, and **3)** peripheral nerves and the neuromuscular junction. Neurons in the primary motor cortex project to the spinal cord via axons to the corticospinal tract [92], primarily made up of white matter, or myelinated axons, which synapse directly onto alpha motor neurons (MNs) for direct muscle control. Alpha MNs are located in the ventral horn of the spinal cord and represent the final common pathway of signal integration from the nervous system prior to sending an impulse along a motor (efferent) axon to each of the muscle fibers that it innervates [110]. A motor unit (MU) consists of a single MN and all of the muscle fibers that it innervates, and innervation occurs where the branched end of a motor axon terminates at the motor endplate where interaction with the muscle fiber can

take place [92]. This neuromuscular junction (NMJ) is where the action potential begins to be translated into contraction of the muscle fiber. The axon terminal has several vesicles filled with acetylcholine (ACh) and ATP that can release into the synaptic cleft between the axon and the muscle fiber in response to a descending action potential [92]. ACh in the synaptic cleft then binds to ACh receptors on the motor endplate, opening voltage-gated sodium (Na^+) channels and allowing Na^+ to rush into the cell, creating an electrical charge that becomes a muscle action potential [92].

There are anywhere from tens to thousands of MUs innervating individual human muscles [94,111,112], with each MU occupying a territory that can range from 8-76% of a given muscle [92]. The number of muscle fibers innervated by a single MN varies, ranging from as few as five fibers per neuron, to nearly 2,000 fibers per neuron [92,111,112]. The muscle fibers in a MU are usually confined to a restricted volume of muscle, wherein most fibers are not contiguous, but are randomly assorted within a dense region and interspersed with fibers from other MUs [113]. In healthy muscle, all of the fibers within a given MU are of the same fiber type [113], suggesting that MUs follow a developmental construct similar to muscle fiber types, with some exceptions. The three MU types are slow (S), fast fatigue-resistant (FR), and fast fatigable (FF) [114], and are associated with innervation of type I, IIa, and IIx muscle fibers, respectively [115]. S MUs have small neuronal bodies and axonal diameters [116], innervate relatively fewer muscle fibers per unit [116], have longer contraction and relaxation times [94,117], are fatigue resistant [115], and have lower stimulation thresholds than faster motor units [116]. As a result, S MUs generally develop less tension and are activated sooner than FR

or FF MUs [116]. This phenomenon is called the size principle of motor unit recruitment and will be discussed later in this section. FF MUs are larger and faster than slow MUs, whereas FR MUs have intermediate properties [115,117]. Of significance are the differences in the proportion muscle fiber types and MU types in a given muscle. Muscles are generally dominated by S MUs, even when the proportion of type I muscle fibers in a muscle are low. For example, the triceps brachii muscle consists of ~33% type I fibers, but 75% of the MUs that innervate the triceps brachii are S [118]. While determining the exact innervation number for each individual MU in a muscle is currently impossible in living humans, we know that S MUs have a relatively low average innervation number and fast MUs have a relatively high average innervation number [118].

Neural control of skeletal muscle. Skeletal muscle contraction can be initiated in three ways, **1)** spinal reflexes, **2)** automatic behaviors, and **3)** voluntary actions [92]. In general, spinal reflexes result in either greater excitation or greater inhibition of muscular contractions, and are designed to evoke rapid responses to external stimuli [93]. Briefly, activation of certain sensory receptors transmits a signal to the spinal cord, initiating a response at the spinal cord level that activates the motor neuron and results in efferent activation and contraction (or inhibition and relaxation) of a muscle or muscle group without the need for a voluntary reaction [92]. The Hoffmann reflex is a spinal reflex that is commonly exploited in research and clinical settings [92,119,120]. It is evoked by stimulating a peripheral nerve and measuring the electromyographic (EMG) signal or twitch force. Stimulation of the peripheral nerve results in both efferent and 1a afferent

activation, culminating in an immediate muscular response from efferent axon stimulation (M-wave), followed by a delayed muscular response from 1a afferent stimulation and subsequent reflexive motor neuron activation [92]. Reflex latency is the primary outcome variable, and higher latency can be indicative of neuropathy [120]. Automatic behaviors are actions that are not consciously controlled, but can be overridden through conscious effort [92]. Examples of this are postural orientation, walking, and balance. Voluntary actions include all conscious control of human movement.

Initiation of voluntary movement begins in the cerebral cortex in response to incoming sensory signals. These signals are integrated and a movement plan is formulated by the motor cortex, at which time a motor command is sent to the spinal cord [93]. This motor command is modified by the cerebellum, brain stem, and/or spinal cord prior to reaching the target motor neuron [92,93]. When the input reaches the neuromuscular junction, ACh is released from into the synaptic cleft to bind with receptors on the motor endplate, initiating an action potential that is transmitted through the transverse tubules and begins the E-CC process [93]. When a MN is activated, the resulting action potential initiates a contraction in all of the muscle fibers that it innervates [92]. Force output of a muscle is moderated either through increasing the number of active MNs, increasing the firing frequency of MNs, or both [94,95]. When low force is required, S MUs are preferentially activated as they typically have lower activation thresholds than FR or FF MUs [116]. Because S MUs innervate type I muscle fibers, and smaller number of fibers per unit than fast MUs, maximal tension is low

[93,121]. As force requirements increase, a greater number MUs of increasing strength capacity, and therefore activation threshold, are recruited in a graded manner (i.e., the size principle) [116,122]. Eventually FR and then FF MUs are recruited until all MUs have been recruited [123]. However, after all MUs in a given muscle are recruited force continues to increase [123], indicating an increase in firing rate of involved MUs. In fact, MUs can increase their mean force up to 10-fold by increasing firing rates [94]. S MUs have longer refractory periods, or intervals of time where they cannot be activated, than FR or FF MUs, and as such have lower maximal firing rates [92]. As a result of these morphological and functional differences (i.e., tendency for FF MUs to innervate more muscle fibers, those fibers being higher force-producing type IIx fibers, and having shorter refractory periods), FF MUs can produce up to 100 times more force than slow MUs [94]. Maximal force production relies on both increased recruitment and firing rate, though the extent to which each are employed are different from one muscle to another [124]. It has been suggested that small distal muscles rely more on firing rate to achieve maximal force, while large, proximal muscles rely on enhanced MU recruitment [94,124]. It is worth noting that some forms of voluntary activation do not adhere to the size principle. For example, joint position, intended movement about a joint, and type of contraction (e.g., eccentric or concentric) can change the order of MU recruitment [125,126]. While it was initially reported that FF MUs were selectively activated during ballistic contractions and corrective movements [127,128], the current consensus is that recruitment order is the same for slow ramp and ballistic contractions [110].

Molecular underpinnings of skeletal muscle hypertrophy and atrophy.

Regulation of skeletal muscle mass in adults depends on protein turnover [97]. Hyperplasia, an increase in the number of muscle fibers, has been reported in some animals [129], but has not been substantiated in humans [130]. Therefore, muscle fiber hypertrophy, or an increase in the size of existing fibers through accumulation of proteins, organelles, and cytoplasm, is the means by which humans increase muscle mass [131,132]. Hypertrophy is induced through stimulation of anabolic pathways and/or depression of catabolic pathways, while atrophy is induced in the opposite manner [132,133], though the mechanisms driving these changes are not well understood [134]. Specific hypertrophic regulators include IGF-1 and, to some extent, insulin, with Akt and ultimately mammalian target of rapamycin (mTOR) as the downstream targets [97]. IGF-1 is synthesized by the liver and skeletal muscle, inducing hypertrophy and preventing atrophy through activation of Akt, which in turn upregulates mTOR activity, thereby increasing protein synthesis [97]. Myostatin (MSTN) is a negative regulator of muscle mass, and blocking MSTN activity also results in hypertrophy [135]. However, MSTN-induced hypertrophy does not maintain the size of the myonuclear domain, as it increases the amount of cytoplasm in muscle cells but not myonuclei [136]. The significance of the size of the myonuclear domain is that a single nucleus can only function effectively for a limited area of cell volume [93]. Therefore, increasing the size of the myonuclear domain without a concomitant increase in myonuclei would result in portions of the muscle cell being dysfunctional. This is exemplified in MSTN-induced hypertrophy, where absolute force increases modestly, but specific force (single fiber *ex vivo* force normalized to

CSA) decreases [136]. In contrast, overexpression of IGF-1 maintains the size of the myonuclear domain through increasing the number of myonuclei, and specific force is preserved [136]. Additionally, IGF-1 overexpression combined with MSTN depletion increases hypertrophy to a greater extent than either intervention alone, indicating different mechanisms of action [137].

Atrophy is induced by disuse, aging, starvation (i.e., low resource availability), and certain disease states [138]. The pathways involved interact with those involved in hypertrophy, but have their own distinct signaling events [97,138]. Most of what we know about muscle atrophy has come from animal models. For example, glucocorticoids in rat skeletal muscle have been shown to induce atrophy by both reducing protein synthesis and increasing degradation [138]. Additionally, increased glucocorticoid levels are associated with increased Forkhead box O (FoxO) activation [139], which in turn enhances activation of two distinct E3 ubiquitin ligases, muscle atrophy F-box (MAFbx) and muscle RING finger 1 (M*u*RF1) [140]. Ubiquitin ligases are known to induce protein degradation, and are implicated in atrophic events following denervation, disuse, and immobilization [141]. Interestingly, it was recently reported that pharmacological inhibition of the Notch signaling pathway prevents glucocorticoid-induced atrophy and MAFbx/M*u*RF1 expression in mice [141], identifying it as a potential target to prevent skeletal muscle atrophy. As mentioned previously, MSTN is also a negative regulator of muscle mass, acting through activation of SMAD2 and SMAD3 phosphorylation [135,142]. There is undoubtedly some crosstalk between the mTOR, FoxO, and SMAD pathways [97], however, the extent to which they interact remains unclear.

Satellite cells (SCs) are undifferentiated cells that play an invaluable role in muscle growth and repair [93]. They are named based on their anatomical location, outside the sarcolemma and below the basal lamina [143]. SCs are unique as muscle cells in that they can replicate, in contrast to myonuclei which are irreversibly postmitotic [144]. These undifferentiated cells are quiescent until stimulated by damage to the muscle (e.g., from acute injury or microdamage from resistance training), and then proliferate to fuse with existing muscle fibers, replacing damaged myonuclei [145]. Additionally, there appears to be some heterogeneity of SC function, as some SC populations only refill the SC pool and do not become myonuclei [146]. Others can undergo asymmetrical division, wherein the parent satellite cells divides and returns to quiescence, while the newly created daughter cell begins symmetrical division and differentiation [144,147]. The end result is the creation of multiple daughter cells without depleting the SC pool. However, MSTN can inhibit SC activation, signal quiescence, and negatively regulate SC renewal [148]. The distribution of satellite cells among different muscle groups appears to be driven by the fiber type proportion of those groups. Type I fibers have up to five times greater SC density than type II fibers [149], suggesting higher regenerative capacity of type I fibers. This higher concentration is likely linked to the tendency for SCs to be located near capillaries [149] and motor neuron junctions [150], structures that are also more plentiful in type I fibers [149,150].

Age-related changes in neuromuscular function.

Fiber type shifts, atrophy, and anabolic resistance. There are several characteristics that distinguish young, healthy muscle from aging muscle. First among

these differences is the arrangement of the fiber types within a muscle. Fiber types in older adults tend to be grouped together, in contrast to the random arrangement seen in young adults (Figure 2.6) [98]. This is likely due to a loss of MNs and subsequent reinnervation of muscle fibers by existing MUs, though fibers may only partially reinnervated, and by random MN types [151]. The implications of this will be discussed in the next section. Regardless of the cause, it appears that with age there is a general fiber type shift to type 1/IIa [98,152]. Andersen and colleagues [152] dissected single fibers from muscle biopsies to determine MHC isoform composition through gelelectrophoresis. Compared to young muscle, older muscle had a lower proportion (by half) of MHC I fibers, and a similar proportion of MHC IIa, MHC IIx, and hybrid MHC IIa/x fibers [152]. However, they also reported that some fibers contained all three isoforms, a phenotype that has not been reported in young muscle [98]. Further, the most common fiber type was the hybrid MHC I/IIa, a much higher proportion than is found in young muscle [152]. The reduction in type IIx fibers may be related to disuse as these fibers are used primarily for activities that require high speed, high force contractions; activities that are seldom performed in older adults [153]. It is unclear why the proportion of type I fibers is also reduced, as it would be logical to assume that the proportion would increase when more time is spent performing low-intensity activities.

Another characteristic of aging muscle is the tendency for atrophy to occur. It is estimated that muscle mass decreases from maximal by 10% by age 50, and nearly half of muscle mass is lost by age 80 [154]. Compared to young muscle, aging muscle fibers are smaller in size and often present with a squashed or banana-like shape [98]. The

reduction in type II fiber size in particular appears to be the greatest contributor to whole-muscle loss of mass [154,155]. Type II fibers are reported to be up to 30% smaller in aged muscle than young muscle, with type I fiber size largely unaffected [156]. However, these adaptations are not universal throughout the entire fiber pool, as there are often normal-looking fibers next to severely atrophied fibers [98]. A reduction in the total number of muscle fibers (hypoplasia) with age also appears to contribute to whole-muscle atrophy [154,157], though this is contested in the literature [155].

Although the age-related morphological changes that occur in muscle are well established, the underlying mechanisms are still unclear. One potential mechanism is an imbalance in muscle protein synthesis and breakdown. If protein synthesis exceeds breakdown, the end result is an increase in the amount of skeletal muscle proteins. On the other hand, if breakdown were to exceed synthesis, there would be a net loss of skeletal muscle proteins [132]. There has been controversy whether or not there are differences in fasting rates of protein synthesis between young and old muscle [158–164], however, the current consensus is that there is little or no difference between young and old adults [165]. If basal skeletal muscle net protein balance is uncompromised with aging, what is the mechanism behind the inexorable loss of muscle mass with advancing age? One explanation centers around anabolic resistance. That is, the theory that aging muscle is less sensitive to anabolic stimuli (i.e., protein ingestion and resistance exercise). Older adults require higher levels of protein ingestion to stimulate protein synthesis, due to decreased sensitivity of the mTOR pathway [164,166], as well as impaired digestion and absorption of amino acids [167]. Other possible mechanisms include impaired delivery of

amino acids to peripheral tissues and/or impaired uptake of amino acids into muscle cells [168]. Aggravating the problem is the fact that a growing proportion of older adults consume less protein than is currently recommended [133], despite those recommendations being an underestimation of actual protein needs [169,170].

Anabolic resistance, paired with an overall decrease in physical activity with advancing age and atrophic episodes like muscle disuse or unloading (e.g., from hospitalization or illness), could largely explain incremental loss of muscle mass over time. Instead of the traditional view of sarcopenia, characterized by a consistent decline in muscle mass, whole-muscle atrophy from atrophic episodes would follow a terraced pattern, with a sharp decline in mass and a blunted rebound (Figure 2.7) [171]. Even lifelong exercisers lose muscle mass as they age, albeit at a slower rate [172], indicating that there are more mechanisms driving muscle atrophy than just disuse. Sandri et. al., [173] reported no difference in FoxO activation between young and old adults, suggesting that age-related atrophy is not due to increased proteolytic activity. However, dysregulation of the mTOR pathway, specifically mTOR complex 1 (mTORC1), is implicated as a contributor to anabolic resistance [173,174]. mTORC1 inhibits catabolism in muscle, and is paradoxically upregulated in older adults, as upregulation would suggest a smaller atrophic response [174,175]. However, higher mTORC1 activity leads to a buildup of excess ribosomes in muscle cells that impairs the structural integrity of the extracellular matrix which can reduce cellular function and induce insulin resistance [176]. Other potential contributors include altered SC, MSTN, and IGF-1 levels with increasing age. Renault et. al., [177] compared per-fiber myonuclei and SC number in

young and old men, finding no difference in the number of myonuclei, but a 60-70% reduction in the number of SCs. Similarly, Verdijk et. al., [156], reported a 45% reduction in SCs per fiber in older adults, but only in type II fibers, which may partially explain preferential atrophy of type II fibers with age. Older adults also experience blunted SC activation in response to a single bout of resistance exercise [178,179] and nine weeks of rehabilitation exercise after immobilization [180], specifically in type II fibers. Additionally, whole-muscle MSTN concentration has been reported to be two-fold higher in older adults than young adults [179], and is upregulated to a greater extent with immobilization and downregulated to a lesser extent during rehabilitation after immobilization [180]. Finally, lower circulating IGF-1 levels have been reported in frail older adults, compared to their non-frail, age-matched counterparts [181]. However, there is no downregulation of IGF-1 with increasing age [173], indicating that low IGF-1 levels in frail adults may be a result of lower muscle mass in that population, not the cause.

Characterization of the age-related changes in muscle fiber physiology and morphology is an ongoing process with many avenues still unexplored. Our current understanding of age-related muscle atrophy implicates fiber type shifts toward IIa and I/IIa hybrid fiber types, with some fibers expressing multiple types of MHC isoforms along their length. Additionally, dysregulation of protein pathways of anabolism and catabolism and impaired SC responses contribute to this decline, preferentially affecting type II fibers. Together, these changes result in an overall reduction in muscle mass and regenerative capacity.

Age-related changes in contractile and neural properties. While morphological changes to skeletal muscle are significant contributors to loss of strength with age, they do not fully explain the loss. *In vitro* studies of single fibers (effectively isolating the contribution of contractile from neural properties) show that specific force (force/fiber size) in humans is reduced by up to 30% in older adults [99], though there is still much unknown about the mechanisms leading to these impairments. Much of what we do know about age-related changes in contractile properties come from animal studies. Older muscle fibers from rats exhibit longer contraction and relaxation times in response to a twitch stimulus, functionally linked to reduced SR volume and function [182]. In addition, older mice have impaired Ca^{2+} release, a lower DHPR/RYR ratio, and a decrease in the number of functional DHPRs, culminating in impaired E-CC [183]. Both actin and myosin reportedly decline with age [184,185], whereas troponin and tropomyosin are upregulated [186–188]. This could be related to the lower proportion of myosin heads binding with actin in older muscle [189], which would result in fewer cross-bridges and a weaker power stroke. Further, cross-bridge attachment duration is longer in older muscle fibers [100], a characteristic associated with reduced shortening velocity [190]. Additional changes include impaired efficiency of ATPase activity under isometric conditions [191], as well as reduced ATPase activity under unloaded conditions [192]. Contractile properties assessed *in vivo* are impaired in older adults as demonstrated by prolonged contraction and half relaxation times in response to electrical stimulation, corresponding with lower PAP [106,193,194]. This slowing of evoked contractile properties is believed to be associated with a general slowing of muscle fiber contractile

properties [184], alterations in E-CC [183], reduced tendon stiffness [195,196], atrophy of type II fibers [154], and a general shift from type II to type I fibers [98,152]. Additionally, since the effects of PAP are stronger in type II than type I fibers [105], the age-related reduction in size and number of type II fibers likely limits the capacity for PAP in older adults [194]. Not only is twitch potentiation reduced with aging, but repeated stimulation potentiation is limited to a greater extent than in young adults, likely due to earlier Ca^{2+} saturation in aged muscle [106].

There has been a growing interest in parsing out the neural contributors to age-related declines in muscular function. Alterations anywhere along the path from cortical, spinal, MN, to the NMJ could negatively impact muscle action. Starting proximal to the muscle, the NMJ is the first potential site of impairment. It has been reported that both the motor end-plate and the NMJ remodel with age, preceding the loss of MNs and myofiber atrophy [197,198]. The age-related loss of MNs can be significant, with reported reductions as great as 50% in spinal cords of older adults [199], and in rats it has been reported that FF MUs suffer preferential denervation [200]. Longitudinal animal studies using *in vivo* MU number estimation (MUNE) methods report both a reduction of functioning MUs and an increase in MU size [201,202], indicating reinnervation of muscle fibers from deceased MUs by surviving MNs. In humans, cross-sectional investigations have also reported significant remodeling with age using MUNE [203–205], wherein it was reported that there was a 40% loss of MUs between 30 and 70 years, and an additional 33% reduction between 70 and 90 years of age, essentially doubling the rate of MU loss [206]. There is a limit to the number of muscle fibers that a single MN

can support and at a certain point the enlarged MUs become incapable of innervating new fibers [207]. Further, the innervation of more muscle fibers by existing MNs results in higher metabolic demand, which could ultimately lead to its death [208]. If a denervated fiber is innervated by an MU that is of a different type than its original MU, the surviving fiber is converted to the new MU fiber type, resulting in fiber type grouping [209]. This is in contrast to young muscles which have few contiguous fibers innervated by the same MU [113]. Reductions in the number of muscle fibers in older adults is evidence of failed reinnervation after MU loss, particularly in adults over the age of 80 [210]. In addition, MU maximal firing rates decrease and submaximal MU firing rates are more variable in older adults, resulting in lower maximal force production and impaired force steadiness [101,211]. Compared to young adults, it appears that age-related differences in firing rates are minimal for extensor muscles while they are substantial for flexor muscles [210]. Overall spinal excitability and excitability of spinal reflexes likely decline with age [212], and intracortical facilitation is reduced while inhibition is increased, impairing the ability of older adults to voluntarily activate skeletal muscle [213,214]. Additional supraspinal morphometric changes that occur with age and potentially disrupt the neural control of movement include reductions in white matter mass, myelinated nerve fiber length, and cell body size in the premotor cortex [212]. Abnormal levels of neurotransmitters and their receptors have also been reported [212].

Impairments in contractile and neural properties of skeletal muscle lead to overall reductions in strength, velocity, and steadiness at the whole-muscle level. The complexity of interactions occurring between the brain, spinal cord, MN, NMJ, and muscle makes it

difficult to isolate the relative contribution of specific adaptations to age-related muscle dysfunction. However, several targets have been identified for potential interventions in order to delay or reverse the loss of function seen with aging.

Part III: Age-Related Declines in Physical Function and Mobility

Sarcopenia was originally defined as the age-related decrease in muscle mass; literally from its Greek origin, "a poverty of flesh" [215]. However, this definition does not fully encapsulate the muscular changes that occur with aging, and complementary terms have been added to better describe said changes (e.g., "dynapenia" is the age-related loss of muscle strength, or "a poverty of strength, power, or force") [216]. To improve consistency and clarity, the definition of sarcopenia has changed from its literal translation to encompass more characteristics than just low muscle mass, and is now classified as a muscle disease defined by low levels of muscle strength, size, and quality, as well as poor physical performance, that accrue with age [217].

Defining physical function and mobility. Maintaining health and independence with age are the top priorities of middle-aged adults [2], and preserving physical function is essential for independence in old age. Loss of independence is a multidimensional process that can include impairments in cognitive, emotional, and physical function [218], and 10-15% of older adults suffer from chronic and disabling conditions that result in irreversible frailty [219]. In 1995, annual estimated medical and long-term care costs for older adults requiring in-home assistance or entering a nursing home were $18,000 and $36,000, respectively, compared to older adults who remained independent incurring only $4,800 in care costs annually [220]. Physical function is defined as one's ability to

carry out activities that require physical action, falling within four related subdomains: activities of daily living (ADLs), mobility, dexterity, and neck and back function [221]. ADLs are fundamental skills necessary to manage basic physical needs, including bathing, grooming, dressing, toileting, walking, climbing stairs, and eating [222]. Additionally, instrumental activities of daily living (IADLs) are activities related to independent living that include shopping, cooking, managing medications, performing housework, driving or using public transportation, and managing finances [222]. Both are necessary for maintaining independence, however, ADLs are more reliant on physical function capacity while IADLs rely more heavily on cognition [222]. Approximately 44% of adults over the age of 65 have difficulty with ADLs or IADLS, while 75% of adults over the age of 85 have difficulty [223]. Of the remaining domains, mobility is most commonly measured in regard to physical function in older adults [221]. Mobility impairment is one of the major causes of loss of independence in the US [220].

In research settings, mobility is commonly defined be one's ability (or inability) to complete a specific task (e.g., walking, stair climbing) for the purpose of classifying participants into distinct groups. For example, Clark et al., [224] defined major mobility disability as an inability to walk 400 meters in less than fifteen minutes without sitting, receiving help from another person, or using a walker. A broader definition of mobility that includes financial, psychosocial, environmental, physical, cognitive, gender, cultural, and biographical factors has been proposed to provide a more holistic view of mobility and aging [218]. Using this framework, it was reported that older adults with the lowest life-space (i.e., the extent, frequency, and independence of an individual's movement)

had 3.8 times greater risk of mortality than those with the highest life-space [225]. Both objective and self-reported measures of physical function and mobility have been reported to predict mortality in older adults [226–228]. As a result, there has been a concerted effort to characterize the underlying physiological mechanisms that contribute to physical function and mobility decline. Much of this work has targeted lower extremity muscles due to their clinical relevance to walking [229].

Contributors to physical function decline.

Muscle power. Muscle power, the product of the force and velocity of muscle contraction, declines earlier and more precipitously with advancing age compared to maximal muscle strength, and has emerged as an important predictor of functional limitations in older adults [229]. The capability to perform ballistic (explosive) movements is robustly related to survival across the animal kingdom, either through increasing hunting success of ambush predators or increasing chances of escape by prey [230]. For example, in adult garter snakes 'maximal burst speed' (the fastest speed recorded when a snake was being chased along a computerized race track) significantly predicts survival, whereas endurance (time crawled on a treadmill at a constant speed) does not [231]. In humans, success and optimization of certain time-constrained tasks (e.g., jumping, recovering from tripping) requires rapid force generation during the first 200 ms (or thereabout) of muscle action [232,233]. Knee extensor power is an independent predictor of functional dependency, and is more strongly associated with functional status than cardiorespiratory fitness or knee extensor strength in older adults [234]. For example, Clark et al., [235] demonstrated that mobility-limited older adults

produced lower isokinetic peak torque than age-matched healthy older adults, most pronounced at higher movement speeds (i.e., > 180°/s). As such, threshold levels of knee extensor power required to maintain independence and prevent major mobility limitation have been determined, and are regularly used to classify older adults into risk categories [236,237].

Rate of force development (RFD). Deficits in RFD are associated with fall risk [232,238] and reduced capability to perform functional tasks [239]. While RFD and power are closely associated with maximal strength (maximal force generation) in healthy adults [240–242], injury and aging can reduce this association [241–243]. This strongly suggests that the relative contribution of neural and muscular determinants of peak force/torque production and RFD are different. In light of these reports there has been increasing interest in understanding the physiological determinants of ballistic RFD, both for athletic performance and physical function assessment in response to injury, disease, and aging [9,244]. At present, however, the physiological determinants of ballistic RFD are poorly understood.

Absolute peak RFD declines by as much as 66% in the knee extensors by age 70 [245,246], though substantially smaller reductions (25-35%) have also been reported [247,248]. Additionally, significant reductions occur in the early phase (0-100 ms) and late phase (100-250 ms) of the force-time curve when time windows are used in place of maximal values [246–251]. The knee extensors are commonly used in the assessment of lower extremity muscle health for older adults, and RFD reportedly decreases at a greater rate than muscle strength [245]. RFD is strongly associated with maximal voluntary force

production, particularly in the later phases of the force-time curve [240]. Due to this association there is some controversy regarding whether RFD by itself is an effective predictor of function [247]. Several studies have reported that normalizing RFD to maximal muscle strength eliminates the differences between young and old adults [247,248,252], while others have reported that differences still exists after normalization [246,249,250,253]. This suggests that the current normalization techniques may not be sensitive enough to discriminate small, but significant, differences in RFD.

Muscle strength. Low muscular strength is also associated with mortality, chronic disease, and physical function [12,254,255], though not to the same extent as muscle power [234]. Grip strength is a simple method of strength assessment in older adults, and is also associated with cognitive function [256]. In the US, 10% of adults aged 60-79 have impaired grip strength, compared to the 53% of adults aged 80+ with strength impairments [257]. Grip strength is moderately associated with knee-extensor strength [258,259], and is commonly used as a proxy for lower extremity strength due to the relative ease of obtaining grip strength data [258]. However, lower extremity strength declines at a faster rate than upper extremity strength [260], and knee extensor strength is generally a better predictor of physical function impairment than grip strength [261], with some exceptions [262–264]. Older adults with low knee extensor strength relative to body weight (i.e., < 3.5 N-m/kg) have greater difficulty performing ambulatory functional tasks [265], although thresholds are task-specific. In addition, impaired lower extremity muscle strength is associated with balance impairments, reduced walking speed, and greater fear of falling [266,267]. While muscle strength was once considered

to be heavily dependent on muscle mass [215], it is now well accepted that changes in muscle mass and size can occur independently of one another [217,254,268]. For example, data from the Health ABC study, a 5-year longitudinal study in adults aged 70-79, indicated that change in quadriceps muscle only explained 5% of between-subject variability of changes in knee extensor muscle strength [268]. Further, even those who increased their muscle mass at the 5-year follow-up had, on average, a 10% reduction in strength from baseline [268].

Muscle mass. It is estimated that muscle mass decreases by 10% by age 50, and that nearly half of muscle mass is lost by age 80 [154]. The loss of muscle mass was once considered the major determinant of decreased strength and, consequently, increased disability prevalence in older adults [215]. However, reduced muscular force production can be attributed to several factors that are not mass-dependent (i.e., contractile and neural properties) [216]. In light of these and other similar reports, muscle mass has lost its prominent role as a predictor of disability, and has been replaced by measures of muscular strength and power [12,229]. However, muscle mass is still associated with strength and physical function [12], albeit weakly, and change in muscle mass is a primary outcome in a large number of clinical trials geared toward improving physical function in older adults [25]. In addition, low muscle mass is independently associated with risk of all-cause mortality [269], cancer-related mortality [270], type 2 diabetes [271], and cardiovascular disease [272].

Cardiorespiratory fitness. Just as with muscular measures, reduced cardiorespiratory functional capacity is a normal part of the aging process [273].

Maximal oxygen uptake (VO_2max) reportedly declines by about 12% per decade starting around age 50, though regular exercise can attenuate this decline by half [274,275]. This reduction is largely driven by age-related reductions in cardiac output, a function of both lower stroke volume and lower maximal heart rate [276]. Impaired aerobic capacity of skeletal muscle also contributes to declining VO_2max [277], though this is largely explained by age-related reductions in fitness and habitual physical activity [278]. VO_2max is one of the most commonly studied markers of physical fitness and is an independent predictor of mortality and capacity to live independently [237,275,279,280]. The minimum VO_2max required in order to maintain an independent lifestyle is estimated at 15-20 mL/kg/min [237,280], and the odds of becoming dependent over the next eight years decreases by 14% for every 1 mL/kg/min above this threshold [280]. These thresholds have clinical relevance in their relation to one's capacity for prolonged walking or standing. For example, the energy cost of walking is higher for very old adults (i.e., 80+ years) than for young adults, both absolutely and as a proportion of their VO_2max [281]. Further, for those with a VO_2max below 13 mL/kg/min, the simple act of static standing requires more than 50% of their maximal oxygen uptake capacity [281,282]. As such, maintaining cardiorespiratory fitness is essential for preventing the development of mobility limitations.

Part IV. Exercise Strategies for Improving and Maintaining Physical and Physiological Function in Older Adults

Aging is associated with both an increase in sedentary behavior and a decrease in structured physical activity [283]. As such, it can be difficult to separate the age-related

reductions in muscular and cardiorespiratory measures from those induced by disuse. Leisure time physical activity only minimally contributes to fitness in older adults [284], and regular, structured exercise is likely the most effective method of maintaining and/or improving physical function with increasing age [285]. Further, there is overwhelming evidence that lifelong exercise can delay the onset of at least 40 chronic conditions/diseases [286]. Older adults are more likely to suffer from multiple chronic conditions and poor health status than young (18-44 years) or middle-aged (45-64 years) adults [287], and these chronic conditions are accompanied by an increased rate of health care expenditures [89]. However, from a health economics perspective, older adults who participate in community exercise programs at least once per week have annual healthcare costs 21% lower than those who do not participate [288], due, at least in part, to the prevention or delay of chronic diseases [286].

Physical activity guidelines. In 2008 the first nationally-endorsed guidelines for physical activity were published based primarily on the Physical Activity Guidelines Advisory Committee's Scientific Report, with the intention to be updated every 10 years [289–291]. Physical activity guidelines are essentially the same for older adults and those under the age of 65, with the additional recommendation that older adults complete some sort of balance training as part of their weekly physical activity [290]. The 2018 national guidelines are intended as a resource for policy makers and health professionals, as well as the general public, for understanding the health benefits of different types, amounts, and intensities of physical activities for individual, community, and/or national implementation strategies [290]. The overarching goal is to increase the amount of

physical activity for all age groups, in turn reducing the burden of lifestyle-induced diseases and conditions that are largely preventable. Nationally endorsed physical activity guidelines recommend a minimum of 150 minutes per week of aerobic exercise training (AET), accompanied by muscle strengthening activities at least two days per week in order to maximize health benefits [290]. Unfortunately, only 13% of older adults achieve optimal health benefits by meeting both guidelines concurrently, whereas a third of older adults meet only AET or only muscle strengthening guidelines [292] (Figure 2.8). However, older adults may be overestimating their time spent in moderate-vigorous AET and/or misclassifying light-intensity activity as moderate-vigorous. For example, using accelerometer data it was reported that only 2.4% of older adults met the AET guidelines [293], while another study reported that only 35% of older adults who self-reported meeting AET guidelines actually met them [294]. Misclassification of intensity during muscle strengthening exercise is also a risk, however, this can be circumvented if exercises are performed to failure, even at low intensities (e.g., 30% of one repetition maximum) [295,296]. Current recommendations are based on the assumption that not all health benefits can be achieved through a single type of exercise, though greater emphasis is placed on the potential benefits of AET over muscle strengthening exercise [291]. Importantly, an individual can gain the health benefits of physical activity, regardless of age, as long as the threshold for irreversible frailty has not been reached [20,21]. Beginning regular exercise later in life results in lower incidence of physical and/or cognitive limitations, major chronic diseases, and poor mental health compared to

those who remain inactive [297], and improvements have even been seen in previously sedentary nonagenarians [20,21].

Aerobic Exercise Training. There are several types of activity that are classified as AET, including, but not restricted to, running, hiking or brisk walking, dancing, swimming, aerobic classes or water aerobics, bicycle riding, and yard work, such as raking or pushing a lawn mower [290]. AET is known to reduce all-cause and cardiovascular disease (CVD) mortality in a curvilinear dose-response manner, wherein the slope of the curve is steepest early in the relationship, but levels off with increased volume [298,299]. For example, Sadarangani et al. [300] reported decreased risk of all-cause and CVD mortality of 35% and 40%, respectively, in adults with diabetes who were meeting or exceeding the aerobic guidelines, compared to those who did not exercise. However, they also reported that those who performed some AET, but did not meet the AET guidelines, still had 26% and 32% lower all-cause and CVD mortality risk, respectively [300], implying that some AET is better than none, and nearly as good as meeting the guidelines. Other well-known benefits include lower incidence of CVD [301], reduced blood pressure [302], improved cardiorespiratory fitness (CRF) [303], and lower incidence of type 2 diabetes (T2D) and risk of cancer [301,304]. Attenuation of long-term weight gain has also been reported [305], though short-term weight loss is minimal without the addition of caloric restriction [306]. There is also moderate evidence that AET can reduce fall risk [307], increase bone mineral density [308], and delay onset and progression of physical disability in older adults [309].

While it is clear that AET can affect several aspects of human health, the inclusion of muscle strengthening activities in the guidelines implies that AET does not increase muscular strength. However, several investigations in older adults have reported increased strength in response to AET [310–314], though careful review of the methodology suggests these adaptations may depend on the mode of exercise employed. The rhythmic and continuous nature of AET requires that muscular contractions are of low enough intensity that they can be repeated for multiple cycles in order to reach adequate exercise duration (i.e., 30 minutes per day) [23], and low-intensity muscle contractions (i.e., <60% of maximal) are typically less effective at increasing muscle mass if not performed to failure [315]. Because AET has traditionally been conceived as exercise for the heart, measures of muscle mass, strength, power, and quality have largely been neglected. The few studies that have addressed muscular adaptations to AET report mixed results [316]. A recent meta-analysis comparing the hypertrophic response to either AET or resistance training (RT) found that while some AET protocols can result in knee extensor hypertrophy, RT is more effective at both the whole-muscle and myofiber level [316]. Of the included studies, none that utilized walking or running exercise resulted in hypertrophy, wherein half of those that utilized stationary bicycling resulted in hypertrophy, though not to the same extent as RT [316]. A number of other studies without an RT comparison group have reported hypertrophic effects using stationary bicycling in both young and older adults [312,317–320]. However, muscle size is, at best, a modest contributor to strength changes with exercise in older adults [321], and few studies have measured changes in strength in response to AET.

Increases in knee extensor isometric force and/or 1 repetition maximum squat have been reported in the range of 11-35% after 12-16 weeks of stationary bicycling in older adults [310–312], occasionally increasing to a greater extent than time-matched RT [314]. However, walking exercise does not appear to increase knee extensor strength [322–324]. Pooling different modes of AET results in large heterogeneity, exemplified by a meta-analysis in adults with coronary heart disease reporting changes in lower body strength ranging from -15.8% to +22.0% (median +6.3%) [313]. However, while the effects of AET on strength gains are inconsistent, long-term AET may at least protect muscular strength from age-related declines, as older adults who regularly participate in AET (10+ years) have higher knee extensor strength than sedentary older adults [325]. Measurement of muscle power and quality is rare in AET studies, but some improvements have been reported [312,318,326]. From the data summarized herein it appears that knee extensor muscle mass and strength can be improved with AET, particularly in older adults, but this has only consistently been demonstrated in response to stationary bicycling. While walking does not appear to improve strength of the knee extensors, strength improvements have been reported in the ankle plantar flexors and dorsiflexors, as well as the knee flexors [327] in response to six months of walking exercise. It should also be noted that AET is unlikely to have a global effect on muscle strength and mass, as improvements are specific to the muscles being used (i.e., lower extremities) [291].

Improvements in muscle strength and size can be traced back to adaptations at the myofiber, cellular, and molecular levels. Long-term (i.e., 12+ weeks) of stationary

bicycle AET results in a general fiber type shift from hybrid IIa/IIx to IIa, increased single fiber cross-sectional area of type I and type IIa fibers, and increased satellite cell content and myonuclear number in type I fibers [328]. Further, mitochondrial density is strongly correlated with VO_2max in older adults, and AET can increase mitochondrial content [329]. Stationary bicycling in particular has positive effects on mitochondria in older adults by increasing proteins involved with mitochondrial biogenesis, fusion, and fission [317]. In regard to protein metabolism, long-term AET reduces MSTN and FoxO signaling by 49% and 24%, respectively, resulting in a positive muscle protein balance [318]. Taken together, it is clear that AET has an effect on many mechanisms that have the potential to promote skeletal muscle hypertrophy and strength improvements [320]. However, to what extent the mode of AET affects each of these mechanisms is not well understood, though it appears that stationary bicycling has a greater overall effect on skeletal muscle.

Resistance training. It is widely accepted that RT promotes hypertrophy and strength gains at all ages [285,330–333], and that muscular power can be increased when a high-velocity component is included in the RT protocol [334]. Similar to AET, there appears to be a dose-response relationship regarding RT and health benefits, wherein higher intensities and higher volumes of RT result in greater improvements in strength and mass in older adults [285,330–333]. Whether the adaptive response to RT is equivalent in both young and old adults is still debatable, as several investigations have reported no difference between age groups [335–338], while others have reported a blunted response in older adults [339–342]. Regardless, RT is clearly beneficial for

musculoskeletal health, and is likely the most effective strategy for maintaining and/or increasing muscle mass and strength with age [285], in turn preventing and potentially reversing sarcopenia and delaying loss of independence [343]. Muscle quality, fatigue resistance, and physical function are also improved with RT [344]. Additionally, bone mineral density increases [345] and blood pressure is reduced to an equal or greater extent with RT compared to AET [346]. At the muscle fiber level, RT results in increased type II fiber CSA [337,347,348], increased satellite cell content in both type I and type II fibers [347,348], and a general fiber type shift from type IIx to type IIa [337,349,350]. Acute bouts of RT increase muscle protein synthesis, resulting in a positive muscle protein balance for up to 48 hours in young, untrained adults [351,352]. However, while the acute response to RT is similar in older adults, it is delayed by 3-6 hours [353]. Older adults tend to have lower baseline muscle protein synthesis than young adults, but synthesis rates can be increased to young adult levels with as little as two weeks of RT [354].

The benefits of RT, as summarized in the Physical Activity Guidelines Advisory Committee's 2018 Scientific Report, include 1) reductions in blood pressure equivalent to AET, 2) improved physical function, 3) reduced risk of falls and injury due to falls, and 4) maintenance of lean body mass during a program of weight [291]. The effects of muscle strengthening exercise on all-cause mortality, CVD mortality, CVD risk, T2D risk, and cancer risk were not addressed in the report [291], but not due to lack of available data. A recent analysis of nearly 400,000 Americans (age range 18-80) reported that individuals meeting muscle strengthening guidelines alone had lower prevalence of

hypertension, hypercholesterolemia, diabetes, myocardial infarction, and heart disease than those only meeting AET guidelines [355]. Additionally, regular RT is associated with reduced all-cause mortality [356,357], cancer incidence [358] and mortality [356], CVD morbidity and number of CVD events [357], and T2D risk and markers of metabolic dysregulation (i.e., glucose disequilibrium and insulin resistance) [359], independent of AET participation. A prospective cohort study from the Health Professionals Follow-up Study reported a comparable dose-response relationship between increased time spent on RT or AET and lower risk of T2D in men [359]. Causation can only be inferred from these reports, though there have been numerous randomized control trials and epidemiological studies that have attempted to better define these associations, which are discussed in the next paragraph [313,346,359–366].

As previously mentioned, RT significantly reduces blood pressure, particularly in hypertensive individuals [346]. The effect of RT on additional CVD and/or T2D risk factors has been reported as similar to those observed in response to AET. AET was once thought to be the lone type of exercise to reduce body fat and insulin resistance, however, research now supports RT as an effective treatment, especially if prescribed at moderate volumes and/or frequency [365,366]. For example, Ihalainen and colleagues [365] reported significant reductions in fat mass when RT was performed three days per week for six months, but not when performed one or two days per week. Additionally, RT appears to be more effective than AET at reducing fat mass (-7.3 kg vs. -6.3 kg) and attenuating loss of thigh muscle volume (-1.9% vs. -6.2%) when combined with caloric restriction [366]. Older adults with T2D can also improve skeletal muscle insulin action

in response to RT, independent of changes in skeletal muscle mass [367], highlighting the multifaceted benefits of RT in the skeletal muscle of older adults. Regarding CRF, RT produces similar improvements as AET (15.6% and 20.1%, respectively) in older adults with coronary heart disease [313]. Additionally, a review by Ozaki et al., [368] reported that CRF consistently increased in response to RT in older adults (6 of 9 included studies), while improvements were rare in young adults (3 of 17 included studies). The reason for a greater response in older adults is unclear, but may be related to the lower baseline levels of CRF associated with increasing age [369]. Indeed, it has been theorized that because older adults have lower muscle mitochondrial content than young adults, RT should have a greater effect on mitochondrial biogenesis, and ultimately, CRF, in older adults [370]. Additional risk factors of CVD and/or T2D that reportedly improve in response to RT in adults include insulin sensitivity [371], lipids and lipoproteins [362,372], triglycerides [362,372], and glycosylated hemoglobin [373]. Taken together, these results underline the critical role that RT may have in preventing and treating detrimental health conditions that target older adults.

High-intensity interval training (HIIT). HIIT has become a popular exercise strategy for young adults for the equivalent or greater health benefits to traditional aerobic training with only a fraction of the time commitment [374]. Additionally, in the last few years there has been a sharp rise in interest involving HIIT in an older adult population. For example, a search on PubMed.gov using the terms “HIIT” and “older adults” produces 30 results from 2008-2014, but 352 results from 2015-2020. Similar to what is seen in young adults, HIIT in older adults has substantial effects on

cardiorespiratory fitness, and a recent meta-analysis found greater improvements in cardiorespiratory fitness in older adults who performed HIIT compared to those who performed AET (3.76% greater increase in on average) [375]. Further, HIIT has effects similar to, or greater than, AET on cardiometabolic variables, including blood pressure, triglycerides, cholesterol, insulin sensitivity, and cardiac function [376,377]. Recently, a preliminary report from the Generation 100 study has indicated that older adults who participated in HIIT over a five-year period had a lower risk of all-cause mortality than those who only performed AET [378]. The Generation 100 study is the largest HIIT randomized clinical trial in older adults to date, wherein all inhabitants of Trondheim, Norway aged 70-77 years (N = 6,966) were invited to participate, of which 1,567 participated and completed the study [378]. In addition to mortality data, the authors also reported that HIIT resulted in higher peak oxygen uptake (+0.7 mL/kg/min) than AET, as well as higher physical and mental quality of life scores after five years of prescribed exercise [378].

As discussed previously, moderate-intensity continuous stationary bicycle training can increase muscular strength and size [311,318,319], and does not seem to interfere with the muscular adaptations to resistance training [379]. Bicycle HIIT in particular may be the ideal form of aerobic exercise able to elicit muscular adaptations. Young adults performing bicycle HIIT demonstrate improved muscle strength [380] and power [374,381], along with enhanced cardiorespiratory function [382], and at least one group has reported bicycle HIIT-induced strength gains in older adults [383]. In addition, satellite cell activation is greater after a single bout of HIIT than AET, though still less

than RT [384]. However, relatively few groups have assessed the effect of HIIT on muscular adaptations [383–387] or physical function measures [383,385–388]. The few that do indicate some beneficial effects. For example, improvements have been reported in 6-minute walk distance [385,387,388], the Timed Up and Go test (a measure of functional mobility) [383,386–388], chair rise [386–388], and walking speed [387,388] in response to HIIT.

Part V: Strengths, Weaknesses, and Considerations of Measurement Techniques for Assessing Changes in Neuromuscular Function

A variety of objectively measured physical performance tasks are commonly used in aging research (Table 2.1), ranging from walking speed or distance, to tests of speed and agility. While these tasks are clinically relevant and clearly related to physical function and mobility, they do not provide information regarding the physiological mechanisms underlying task performance. Measurement techniques that characterize physiological function in humans are abundant, some of which are simple and require little training to perform, while others are nuanced and require extensive technical training and a deep understanding of the underlying theoretical concepts. In order to determine the appropriate technique to assess an outcome of interest, it is essential to understand the limitations associated with said technique, as well as its reliability.

Assessment of human performance.

Muscular strength. Muscular strength is regularly assessed in one of three ways: isometric (no change in muscle length), isokinetic (constant velocity of contraction), or dynamic (variable velocity and force) measurements [92]. Isometric and isokinetic force

(torque) are assessed with an isokinetic dynamometer, an electromechanical device that measures and digitizes force production [389]. Both isokinetic and isometric strength testing using commercial dynamometers have been shown to be highly reliable (coefficient of variation = 3-5%) [390], and are useful during testing as a biofeedback tool, wherein participants can see their effort on a computer screen and attempt to improve their performance in the moment. In addition, because the force-time curve is digitally recorded it allows for the precise measurement of peak force attained during a trial and allows for in-depth offline data analysis (e.g., RFD). Isokinetic protocols provide objective measurements of muscle strength throughout the entire range of motion of the joint being tested [389]. In contrast, isometric protocols only assess muscle strength at a single joint angle, which is less functionally relevant than isokinetic protocols. However, isometric protocols are still commonplace in biomedical research because many of the tools used to assess the neural and/or musculoskeletal contributions to force production (e.g., electrical stimulation, EMG) are most readily and successfully applied under isometric conditions. While much of the within-subject variability in dynamometry-based strength measurement can be attributed to participant-specific factors (e.g., fatigue, neural drive), some is also due to the dynamometer itself. For example, de Ruiter et al. [391] reported small changes in knee angle of 3-7° during isometric testing using a dynamometer designed to minimize changes in knee angle during force development. As such, true isometric force assessment is difficult (impossible) to obtain, and current protocols are designed to minimize joint angle changes by limiting the amount of padding on the dynamometer and immobilizing participants with straps to maintain constant body

positioning [389,391,392]. Hand-held dynamometers are also used in research settings, but are less reliable than commercial isokinetic dynamometers [393].

Dynamic strength testing is typically quantified using free weights or exercise machines, and as such is relatively inexpensive to assess. In addition, it may be the most functionally relevant method of muscular strength assessment in that the multi-joint actions most closely mimic everyday movements (e.g., leg press and standing from a seated position). However, dynamic strength testing requires the participant to perform multiple attempts with incrementally increasing resistance until task failure is achieved. The protocol for dynamic strength assessment includes adding weight after each successful trial (typically in the range of 2.5-10 kg) based on feedback from the participant and expertise of the investigator overseeing the assessment [394]. This is in contrast to dynamometer testing where the participant gives maximal effort for a set number of trials, and changes as small as 0.68 N-m can be detected [395]. Dynamic strength testing is, therefore, less precise than dynamometer testing, and does not allow for offline analysis because force traces are not digitized. Additionally, there is a general concern regarding injury risk during one repetition maximum strength assessment, particularly in older adults [396]. However, injury risk has been shown to be low in older adults when proper precautions are taken [397]. In determining the appropriate method of strength assessment for answering a specific research question, the unique strengths and limitations of each approach should be considered.

Muscular power. Power is defined as work/time or force•velocity, and thus is a scalar quantity that is expressed as a rate (e.g., m/s, Watts) [92]. It can be assessed for a

variety of muscle groups with pneumatic resistance machines with high reliability [398,399]. Peak power is usually attained at a resistance that is 70-75% of a participant's one repetition maximum [234], and thus strength assessment is a prerequisite for power assessment when using this approach. Power can also be calculated from isokinetic torque measurements (because velocity is a known quantity), but some investigations reporting muscle power are actually reporting peak isokinetic torque at different velocities [400]. When done correctly, isokinetic power assessment can provide insight into age-related deficits undetected by strength measurements. For example, Clark et al. [235] calculated muscle power from the mean torque values produced between 55° and 70° of knee flexion at 60°, 90°, 180°, and 240°/second, and found that mobility-limited older adults had significantly lower muscle power than healthy older adults, despite no significant difference in absolute torque production. A wholly different approach to power assessment involves the use of flywheels. This can be easily performed on a cycle ergometer that is interfaced with a computer [374], though the cycling motion may lack functional relevance in older adults.

Rate of force development. There are two common methodological approaches designed to quantify voluntary ballistic RFD, including calculating peak RFD from the steepest portion of the force-time curve and calculating the slope of the force-time curve over specified time points [9]. The latter method typically uses 50 ms time windows, and RFD is either calculated over sequential (e.g., 0-50 ms, 50-100 ms) or overlapping epochs (e.g., 0-50 ms, 0-100 ms) [9]. The reliability of these methods is high to moderate (coefficient of variation = 4-16%), with the greatest variability reported in the first 50 ms

[401]. RFD is strongly associated with muscle strength [241], and absolute RFD is regularly normalized to an individual's MVC in order to compare the explosive force generating capacity of groups of differing strength levels [9]. Other normalization techniques rely on the maximal force attained during individual ballistic trials, wherein RFD is quantified as the slope of the force-time curve during specific proportions of the contraction (e.g., RFD from 5-40% of peak force [402]), or simply as time to reach percentages of peak force (e.g., amount of time to reach 30% of peak force [403]). Each of these calculation methods (with the exception of peak RFD) rely on the accurate assessment of contraction onset, with several methodological iterations having been used with regularity [9]. Arguably the most accurate method of onset detection includes visual identification of actual force onset using a precise definition. For example, Folland et al. [241] defined force onset as the last trough before the force signal deflected above the baseline noise, and reported that both intra- and interobserver reliability was high (standard deviation < 1 ms). In contrast, automated methods are typically less precise, detecting onsets up to 60 ms later than manual methods [404,405]. Automated methods of contraction onset typically use absolute or relative thresholds, wherein onset is defined as the point where force exceeds a specific value [406] or a percentage of maximal force [407].

Voluntary ballistic RFD can be classified as an assessment of muscle power as it is a combination of force and velocity. Muscle power has traditionally been assessed during dynamic contractions [408]. However, joint angle, contraction velocity, and limb acceleration interact with the force-time curve in a non-linear manner during dynamic

movements, and it is difficult to control for these confounding effects when assessing RFD [9]. As such, RFD is most commonly assessed under isometric conditions, with few exceptions [244,406,409]. Even still, there are several variables that can affect isometric voluntary RFD assessment, including joint angle [391], the muscle being tested [248], and whether the contraction is maximal or sub-maximal [410,411]. Further, methodological factors (e.g., surface stability, type of instruction, pretension and counter-movement) have the potential to introduce variability into RFD assessment [244]. Consequently, methodological recommendations to improve the accuracy of RFD assessment have recently been published [9,244].

VO_2max. VO_2max is one of the most commonly measured markers of physical fitness [275]. Assessment of VO_2max consists of a graded exercise test (GXT), wherein the participant begins exercising at a low intensity that systematically increases until they are no longer able to continue the exercise [412]. The protocol for the GXT can be either ramp (resistance increases continuously at a predetermined rate) or step (resistance increases at specified intervals), and can be performed on a variety of exercise devices (e.g., treadmill, cycle ergometer) [413]. During the test the participant breathes into a tube that leads to a gas analyzer capable of detecting the relative proportion of oxygen and carbon dioxide in the participant's expired air, among other relevant variables (e.g., air volume, breathing rate). Ultimately, this leads to the determination of a participant's maximal capacity for oxygen utilization during exercise (i.e., VO_2max) [412]. Oxygen utilization during exercise is directly related to active muscle, and thus, activities that utilize a greater amount of muscle mass produce higher VO_2max values. For example,

recorded VO_2max is 8-15% higher when assessed on a treadmill compared to a cycle ergometer [413]. Using the same line of reasoning, individuals with a large amount of muscle mass generally have higher VO_2max values than those with low muscle mass [277]. To control for this, VO_2max is regularly normalized to body weight (i.e., relative VO_2max) in order to directly compare individuals of different size. However, this method does not take into account the fact that most of the oxygen consumed during a GXT is used by the limb muscles, and fat mass does not significantly contribute to oxygen utilization [414]. As a result, relative VO_2max is likely underestimated in individuals or populations who have a higher proportion of fat mass (e.g., older adults), and normalizing VO_2max to appendicular lean mass may be more appropriate [277]. This also has relevance in regard to exercise-induced adaptations in oxygen utilization, as a decrease in body weight with no change in absolute VO_2max would still result in a greater relative VO_2max. Despite these issues, relative VO_2max is still regularly reported, though best practice dictates that both absolute and relative VO_2max values are reported to ensure accurate interpretation [412].

It can be difficult to assess if a VO_2max value obtained during a GXT represents an individual's maximal oxygen utilization capacity because it relies on the effort level of the participant. However, there are objective criteria used to assess effort level to give investigators a better idea of whether or not participant effort was maximal at the end of the test. These include a plateau in VO_2 despite increased exercise intensity, and achieving predetermined thresholds for heart rate, blood lactate, and respiratory exchange ratio [412]. Less than 50% of individuals who perform a GXT actually have a plateau in

VO_2, however, and a GXT is still considered successful if the other criteria are met [412]. Assessment of VO_2max requires expensive equipment and some technical training, which makes it unfeasible for studies with a large number of participants (without substantial funding and manpower). As a result, simple physical function tests that require little or no equipment have been tested as proxy measures of VO_2max in unique populations. For example, the 6-minute step test, which consists of stepping onto and off of a raised platform as many times as possible in six minutes, was moderately correlated with VO_2max in adults with interstitial lung disease (r = 0.52) [415], and strongly correlated with VO_2max in generally healthy older adults (r = 0.75) [416]. While assessment of VO_2max during a GXT is the gold standard, proxy methods can be useful for determining general fitness levels.

Assessment of neuromuscular form and function.

Muscle size. The most accurate methods of quantifying skeletal muscle size *in vivo* are, arguably, magnetic resonance imaging (MRI) and computed tomography (CT), as these technologies have been shown to be near perfectly correlated with cadaveric values (r = 0.99) when full-length limb scans were used [417,418]. The high level of radiation that accompanies CT makes MRI a more attractive option as it uses a magnetic field to capture images and does not involve radiation, however, both methods are still commonly used [419,420]. A large number of transverse images are produced as a result of these scans that must be individually analyzed, the number of which depends on slice thickness, inter-slice distance, and region of interest [10]. For example, MRI of the thigh with a slice thickness of 5 mm and no inter-slice distance can result in upwards of 65

slices in adults [10]. The amount of time required for scan analysis is substantial (as much as five hours per participant for the quadriceps muscle [10]), making it unfeasible for studies including a large number of participants. However, muscle volume estimates from only eight slices, adjusted based on the mathematical principles of cylinder volume (i.e., the Cavalieri principle), have been shown to be nearly identical to estimates using all slices, reducing the required analysis time considerably [10,421]. Accuracy of MRI single-slice anatomical CSA has also been demonstrated in multiple muscle groups [422,423]. Unfortunately, the high cost of instrumentation, lack of equipment availability, and the expertise required to operate the equipment and analyze the data often preclude the use of these devices in many research settings, particularly late-stage, large-scale clinical trials [424]. As such, dual-energy X-ray absorptiometry (DXA) has gained popularity over the years through its ease of use, reduced cost, and accessibility. However, DXA is unable to separate muscle groups and can only quantify the mass of transverse sections of the body (i.e., total lean soft tissue mass from hip to knee). DXA is, by far, the most common method to assess skeletal muscle mass/size in randomized control trials evaluating muscle mass/size [25], and DXA has recently been recommended as the "reference standard" for the measurement of muscle mass [26]. While MRI and CT measures are estimates of muscle volume or CSA, depending on the variable of interest, DXA measures fat mass, bone mineral content, and lean mass, the last including connective tissue, water and organs [419,425].

A multitude of skeletal muscle size comparative studies have been conducted between DXA and MRI, as well as between DXA and CT, in a variety of populations

[27,28,426–428]. These comparative studies reported strong associations (correlation coefficient values ranging from 0.86 – 0.97), for both whole-body and regional scans [27,28,426–428]. However, these above-mentioned studies were based on cross-sectional designs, and less is known about the agreement between various indices of change in skeletal muscle size over time. Only a few studies have directly compared longitudinal changes in skeletal muscle size using both DXA and MRI/CT [28,426,429–432]. These studies reported strong associations between temporally matched measures (e.g., MRI- and DXA-derived absolute measures at baseline) [28,426,429,430]. However, when comparing percent-change over time, these studies have indicated discrepant findings with a few studies suggesting much lower associations (explained variances [R^2-values] on the order of 4-33%) [28,429,430], while others suggested higher levels of concordance (61% and 77%) [426,432]. The lack of understanding about how well various assessment methods of skeletal muscle size agree in longitudinal studies is a serious problem as it relates to the design and interpretation of clinical trials. For instance, in one study it was reported that 3-months of resistance exercise training in adolescents resulted in a significant increase in appendicular skeletal muscle size when MRI was used as the quantification method, but no significant difference in lean mass with DXA [429]. Another study, this time in older adults, reported increases in thigh muscle size after a 1-year resistance training protocol when measured with CT, but not DXA [431]. These findings are similar to the recently reported results of a clinical trial that investigated the effectiveness of a myostatin-inhibitor for enhancing skeletal muscle size in older adults [433]. In this study, the primary outcome was total body lean mass assessed *via* DXA,

while a secondary outcome was thigh skeletal muscle size assessed *via* MRI. The data were discrepant, with the DXA-derived estimate indicating no significant increase, whereas the MRI-derived estimate demonstrated an increase [433]. While these measures are not always directly comparable, results of this nature highlight the need to better understand how DXA- and MRI-derived measures of change in skeletal muscle mass and size compare, as this knowledge has implications for interpreting findings as well as the design of clinical trials. One potential source of inaccuracy between MRI/CT and DXA relates to the assumptions of DXA technology. Error seen in response to same-day repeated measures is attributed exclusively to machine error and rater error [434], however, one cannot assume that this holds true after an intervention, as exercise training can induce tissue changes that may impact X-ray attenuation [432].

Urinary creatinine excretion is an alternative method of muscle mass estimation not reliant on expensive imaging (e.g., MRI) or assumptions of a compartment model (e.g., DXA) [420,435]. Creatine, the precursor of creatinine, is synthesized in the liver and kidney [436], is found almost exclusively in the muscle [437], and rapidly defuses from muscle into plasma with no reuptake into muscle [437]. Additionally, the concentration of creatine is relatively constant in muscle, and creatine pool size is proportional to skeletal muscle mass [437,438]. Accurate quantification of creatinine excretion in humans has historically been difficult due to methodological constraints, specifically, multiple days of diet restriction, prolonged urine collection procedures, and daily variation in the rate of excretion [420,439]. However, recently a novel method of creatinine quantification (i.e., D_3-Cr dilution) resistant to these previous limitations has

been shown to be strongly associated with cross-sectional and longitudinal measures of MRI-derived muscle mass in rats (r = 0.92 and 0.98, respectively) [440], as well as physical performance and mobility in older humans [435]. Additional low-cost methods of indirect muscle mass assessment are still occasionally used (e.g., bioelectrical impedance, anthropometry), though confounding physiological variables and the relatively high error associated with these methods severely undermines their utility in aging or diseased populations [419,420].

Contractile properties. Voluntary force production is a function of both neural drive and the capacity of a muscle to produce force [92]. Electrical stimulation can induce muscular contraction by activating peripheral motor nerves and/or motor axon terminal branches [441], effectively isolating skeletal muscle contractile properties from the confounds of neural drive (assuming no peripheral nerve disease is present). In human subjects research, electrical currents are delivered to the muscle transcutaneously through electrodes placed on the skin over muscle [442], or percutaneously with small needles penetrating the skin in order to deliver the electrical current closer to the muscle and/or motor nerve [443]. Motor units that are within the electrical field that is produced by the stimulation are activated, resulting in the contraction of their innervated muscle fibers. However, in contrast to voluntary efforts where motor units are recruited in order of their size [116], electrical stimulation recruits motor units in reverse order, with larger, fast contracting motor units being activated first [444]. Distance from the site of stimulation (i.e., axonal branches that are deeper in the muscle) can also affect order of activation [445]. The relevance of this issue can be visualized in Figure 2.9, wherein the muscle

fibers proximal to the electrodes are activated, but those more distal are not [446]. With this in mind, it is extremely difficult, if not impossible, to activate the entire muscle with transcutaneous muscle stimulation. For optimal results, stimulating electrodes should be placed over the motor point (i.e., the location on the muscle where the motor branch of a nerve enters the muscle belly) [447]. Motor point maps indicating the location of motor points in a variety of muscles have been published and are widely used [448]. However, due to the inter-subject variability in motor point locations it is recommended that motor points be detected for individual subjects using a low-frequency stimulus generator in place of using the motor point maps [447,449].

In characterizing contractile properties of skeletal muscle, it is necessary to determine the intensity of stimulation that elicits a maximal response. This typically consists of an electrical pulse (or multiple pulses) being delivered to the muscle at incrementally increasing intensities until force does not increase with increased stimulation intensity [402]. Supramaximal twitch (single pulse) or doublet (two pulses) stimulation is often used to assess the contractile properties of muscle, providing insight into the relative fiber type proportion of a given muscle [450]. Commonly reported variables, including peak torque, contraction time, and half relaxation time [451], have been shown to be highly reliable (coefficient of variation = 3-7%) [401]. Further, the comparison of postactivation potentiated to non-potentiated stimulation responses can assist with this determination. The effects of PAP have been discussed previously (see Part II), but some considerations should be reiterated. Maximal potentiation typically requires a maximal-effort conditioning contraction of 5-10 seconds, followed by

stimulation of the muscle within a few seconds of relaxation [108]. PAP declines relatively quickly, with a half-life of ~28 seconds [109], but effects can be seen up to 10 minutes afterward [108]. Therefore, if a non-potentiated twitch response is desired, it is essential that adequate time passes between voluntary efforts and electrical stimulation.

There are several technical variables that must be considered when using electrical stimulation in human subjects research, including pulse duration (μs), voltage (V or mV), stimulation frequency (Hz), and current (mA), to name a few [452]. Summation of force occurs when the frequency of stimulation is higher than the rate of relaxation, and a stimulation frequency of at least 150 Hz is required to reach maximal electrically stimulated muscle force [453,454]. Interestingly, maximal electrically stimulated RFD requires a stimulation frequency of at least 300 Hz [453,454]. However, it is possible that a maximal force and/or RFD could be elicited at lower stimulation frequencies by modifying the pulse duration, though this has not been adequately studied. For example, Gregory et al. [455] reported that electrically stimulated torque was affected by both pulse frequency and pulse duration. Using a series of pulse frequency and duration combinations, they concluded that the total electrical charge delivered to the muscle was the strongest predictor of torque production. That is, there was no difference in force production between 30-Hz/500-μs and 50-Hz/300-μs trains [455]. Of note, these stimulation parameters produced submaximal force, and it is unclear if the phenomenon is valid at the high stimulation frequencies required to elicit maximal force and/or RFD (i.e., 150+ Hz).

Nervous system properties. EMG is a technique used to quantify the electrical activity from active motor units in order to characterize the neural control of movement [456]. The surface EMG signal (i.e., interference signal) comprises the sum of electrical contributions made by active MUs (as detected by the electrodes on the surface of the skin), and reflects both peripheral and central properties of the neuromuscular system, while indwelling electrodes isolate individual MUs [8]. EMG has several useful applications in characterizing the neural control of movement, including, but not limited to, determining if muscle activation is present, estimating neural drive to the muscle, and characterizing MU properties using different recruitment strategies (e.g., explosive vs. ramped contractions) [456]. While several landmark findings have come about as a result of EMG work, there are limitations that prevent the accurate interpretation and generalizability of surface EMG signals between and within individuals (Table 2.2) [8]. Many of these factors cannot be controlled or determined under experimental conditions, and there is high risk of misleading conclusions and erroneous generalizations by research groups without a strong understanding of these limitations due to the ease of data collection and low cost of EMG systems [8,456,457].

Three specific factors, amplitude cancellation, cross talk, and subcutaneous fat, warrant special attention in regard to research in older adults. Signal amplitude cancellation (also termed “superposition”), a result of cancellation of positive and negative phases of MU action potentials (Figure 2.10), is unpredictable and can be as high as 62% when the muscle is fully activated and up to 85% during fatiguing contractions [458]. Normalization of the interference EMG signal (either to the amplitude

of the EMG signal at maximal muscle activation or to M-wave peak-to-peak amplitude) can largely control for signal cancellation, though not entirely [458]. As a result, changes in surface EMG in response to an intervention may not rigorously reflect altered neural drive to the muscle [8]. Similarly, subcutaneous fat thickness can have substantial effects on the amplitude of the signal, with higher levels of subcutaneous fat dampening the signal to a greater extent [459]. Reducing subcutaneous fat would, therefore, increase signal amplitude independent of any actual change in neural drive. This is not necessarily an issue in cross-sectional studies, at least for within-subject values recorded over a few days. However, any intervention with the potential to alter both subcutaneous fat levels and neural drive (e.g., exercise) becomes problematic, as attributing increased signal amplitude only to increased neural drive may be erroneous if one does not control for subcutaneous fat. For example, several reports indicate that changes in EMG and RFD with resistance training are similar [406,460–466], while others indicate no change in EMG with training [467–470]. Consideration of the methodological approaches used in these reports reveals that the vast majority of studies reporting changes in EMG with training did not normalize the signal to a reference value [406,460–464]. Reports of this nature highlight the complexity of the surface EMG signal and the interpretation thereof.

Historically, single MU activity has been quantified via indwelling needle electrodes [471]. However, in recent decades decomposition algorithms have been developed to separate the neural drive from the shape of the MU action potentials [472,473]. Early iterations of this process detected relatively few MUs per contraction (i.e., ≤ 5) [473], but both accuracy and yield have increased as the technique has been

refined [474]. More recently high-density surface EMG (HDsEMG) systems, sometimes with over 100 electrodes covering individual muscles, have been developed and validated, resulting in a greater number of detected motor units (i.e., ≥ 20) [475,476]. Decomposition of the surface EMG signal is superior to MU analysis via indwelling electrodes in many ways (e.g., noninvasive, larger sample size, ability to generalize MU function [474]), however, it is currently limited to sub-maximal contractions [474].

Electromyography has also been used to estimate the number of MUs in specific muscle groups. The most accurate method of motor unit quantification is arguably anatomical counts, however, studies of this nature are rare and can only be done postmortem, which greatly limits the experimental applications [205]. The *in vivo* MUNE technique, first developed by McComas et al. in 1971 [477], is based on the theory that stimulating a motor nerve with progressively stronger stimuli produces a progressively stronger compound muscle action potential (CMAP) due to an increase in the number of active MUs. They argued that averaging this series of increases resulted in a value that was representative of the average single motor unit potential (SMUP) for the given muscle, ultimately allowing for quantification of the motor unit pool by dividing the maximal CMAP by the average SMUP [477]. Since that time several MUNE methods have been developed, nearly all of which are based on the incremental method developed by McComas and colleagues [478]. MUNE does not appear to be particularly useful as a diagnostic tool due to the wide range of values in the general population [478], however, its ability to track progression of motor neuron disease (e.g., amyotrophic lateral sclerosis, spinal muscular atrophy [479–481]) makes it an invaluable tool. MUNE

techniques have also elucidated age-related MU loss and remodeling longitudinally in animal models [202,482], and cross-sectionally in humans [203,206,483,484]. While the MUNE method has proven to be useful in tracking disease progression, the theoretical underpinnings of the technique have been questioned extensively [485]. Specifically, the McComas incremental method assumes that each MU is approximately the same size (i.e., the innervation ratio), and that the proportion of slow and fast MUs are equal within a muscle [477]. These assumptions have proven to be false [92], and currently MUNE is truly considered an estimate of the MU pool not a count of actual MUs [485].

Chapter 3: Changes In DXA-Derived Lean Mass and MRI-Derived Cross-Sectional Area of the Thigh Are Modestly Associated

The material in this chapter has been published in *Scientific Reports* [29].

Abstract

Dual-energy X-ray absorptiometry (DXA) derived measures of lean mass demonstrate strong associations with magnetic resonance imaging (MRI) derived measures of muscle volume (MV) in cross-sectional studies, however, few studies have compared *changes* in response to an intervention. The purpose of this study was to determine the accuracy of DXA at detecting changes in lean mass, using MRI-derived MV as a reference standard. 10 male and 16 female participants (29.2 ± 9.5 years) underwent DXA and MRI scans before and after a 10-week resistance training intervention. DXA thigh lean mass was compared to MRI mid-thigh MV, and percent change in size was compared between MRI and DXA. There was a strong correlation between measures cross-sectionally (r=0.89) in agreement with previous investigations. However, there was a modest correlation of percentage change over time between methods (r=0.49). Bland-Altman plots revealed that the amount of random error increased as the magnitude of the change from baseline increased. DXA measures of change in lean mass were modestly associated with MRI measures of change in MV. While there are several advantages to using DXA for the measurement of lean mass, the inability to accurately detect changes over time calls into question its use in clinical trials.

Introduction

Skeletal muscle plays a critical role in health as it permits the performance of exercise, as well as the activities of daily living. It also plays a central role in whole-body protein metabolism and glucose-regulation [486,487]. Scientists and clinicians have long had an interest in quantifying skeletal muscle tissue mass or size [488,489], and today the change in skeletal muscle mass or size is the primary outcome in many clinical trials due to its relationship with disease progression and physical function [490–492]. Accordingly, it is crucial that the most appropriate technology for quantifying muscle mass or size be chosen for studies of this nature.

The most accurate methods of quantifying skeletal muscle size *in vivo* are, arguably, magnetic resonance imaging (MRI) and computed tomography (CT), as these technologies have been shown to be near perfectly correlated with cadaveric values (r=0.99) when full-length limb scans were used [417,418]. Accuracy of MRI single-slice anatomical cross-sectional area (CSA) has also been demonstrated in multiple muscle groups [422,423]. These methods are ideal in terms of accuracy, but the high cost of instrumentation, lack of equipment availability, and the expertise required to operate the equipment and analyze the data often preclude the use of these devices in many research settings, particularly late-stage, large-scale clinical trials [424]. As such, dual-energy X-ray absorptiometry (DXA) has gained popularity over the years through its ease of use, reduced cost, and accessibility. While MRI and CT measures are estimates of muscle volume (MV) or CSA, depending on the variable of interest, DXA measures fat mass, bone mineral content, and lean mass, the last including connective tissue, water and

organs [419,425]. For simplicity, these estimates will, at times, be collectively referred to as “muscle size” in this article.

A multitude of skeletal muscle size comparative studies have been conducted between DXA and MRI, as well as between DXA and CT, in a variety of populations [27,28,426–428]. These comparative studies reported strong associations (correlation coefficient values ranging from 0.86 – 0.97) for both whole-body and regional scans [27,28,426–428]. However, these above-mentioned studies were based on cross-sectional designs, and less is known about the agreement between various indices of change in skeletal muscle size over time.

Only a few studies have directly compared longitudinal changes in skeletal muscle size using both DXA and MRI/CT [28,426,429–432]. These studies reported strong associations between temporally matched measures (e.g., MRI- and DXA-derived absolute measures at baseline) [28,426,429,430]. However, when comparing percent-change over time, these studies have indicated discrepant findings with a few studies suggesting much lower associations (explained variances [R^2-values] on the order of 4-33%) [28,429,430], while others suggested higher levels of concordance (61% and 77%) [426,432]. The lack of understanding about how well various assessment methods of skeletal muscle size agree in longitudinal studies is a serious problem as it relates to the design and interpretation of clinical trials. For instance, in one study it was reported that three months of resistance exercise training in adolescents resulted in a significant increase in appendicular skeletal muscle size when MRI was used as the quantification method, but no significant difference in lean mass with DXA [429]. Another study, this

time in older adults, reported increases in thigh muscle size after a 1-year resistance training protocol when measured with CT, but not DXA [431].These findings are similar to the recently reported results of a clinical trial that investigated the effectiveness of a myostatin-inhibitor for enhancing skeletal muscle size in older adults [433]. In this study, the primary outcome was total body lean mass assessed *via* DXA, while a secondary outcome was thigh skeletal muscle size assessed *via* MRI. The data were discrepant, with the DXA-derived estimate indicating no significant increase, whereas the MRI-derived estimate demonstrated an increase. While these measures are not always directly comparable, results of this nature highlight the need to better understand how DXA- and MRI-derived measures of change in skeletal muscle mass and size compare, as this knowledge has implications for interpreting findings as well as the design of clinical trials. This is particularly alarming when one considers that DXA is, by far, the most common method to assess skeletal muscle mass/size in randomized control trials evaluating muscle mass/size [25], and DXA has recently been recommended as the "reference standard" for the measurement of muscle mass [26]. Accordingly, the purpose of this study was to examine the relationship between MRI- and DXA-derived measures of thigh skeletal muscle size/mass in a cross-sectional analysis as well as a longitudinal analysis where resistance exercise served as a stimulus for skeletal muscle adaptation.

Methodology

Study participants & exercise program. Twenty-six adults (29.2 ± 9.5 years, 16 females and 10 males) participated in this study. The data for this manuscript was derived from a previously described randomized control trial comparing two different exercise

programs for effectiveness in inducing skeletal muscle adaptations [493,494]. See Table 3.1 for participant characteristics. Study participants were randomly assigned to one of two exercise intervention groups, and a whole-body DXA scan (thigh region used for analysis) and a mid-thigh MRI were obtained before and after the 10-week exercise program. The use of human participants and all experimental procedures were approved by the Ohio University Institutional Review Board committee and written informed consent was obtained from each participant.

Study participants were randomly assigned to either a low-load blood flow restricted resistance training group (n = 12) or a low-load resistance training control group (n = 14). Supervised exercise training sessions were conducted twice per week for 10 weeks, with exercise intensity set at 25% of each of the participant's maximal voluntary isometric strength (MVC). MVC was obtained at baseline and reassessed during the fifth week of the training schedule. Participants performed three sets of leg extensions, calf raises, and arm curls to failure with 60 seconds rest between sets. The BFR group had pressure applied to the proximal limbs by a KAATSU Master device (KAATSU Training Japan Co., Ltd., Tokyo, Japan) until circulation was impeded, but not occluded. For full details on the exercise program and other trial design parameters please see our in depth description of the protocol/trial [493,494].

Assessment of skeletal muscle size

Magnetic Resonance Imaging. MRI scans were performed with a 0.25-Tesla Musculoskeletal MRI system (Esaote G-Scan Brio, Genoa, Italy) to acquire contiguous transverse T-1 weighted spin echo image slices in the thigh region with a slice thickness

of 10 mm and an inter-slice distance of 10 mm. To ensure consistency among participants, the isocenter was positioned at mid-thigh, midway between the patella and the inguinal crease, and the participants were supine. Images were transferred to a computer for calculation of whole-thigh muscle anatomical CSA. Beginning with the slide with the first discernable visual of the rectus femoris and including the subsequent four proximal slides, total thigh muscle area was traced using a polygon tool, excluding bone, as well as fat tissue surrounding the muscles (MIPAV version 7.3.0) (Figure 3.1). Intramuscular fat was then subtracted by applying a shading correction to each slide, determining average voxel density and standard deviation voxel density from a sample of the lightest area of fat tissue, computing a cutoff value at three standard deviations darker than the sample voxel density, and excluding all pixels with a voxel density at or below the computed value. Pre- and post-intervention slides were displayed simultaneously, and slides were visually compared to ensure that tracing patterns were identical and that the same structures were excluded (i.e., neurovascular bundle, intermuscular fat) on both slides before CSA values were recorded. This process was completed for each analyzed slide, resulting in five measures of whole-thigh CSA with intermuscular and intramuscular fat excluded for both pre and post time points. Muscle volume was calculated using the Cavalieri method [$MV = T (A_1 + A_2 + A_3 + A_4 + A_5)$], where MV=muscle volume, T=the known distance between slices, and A=area [421].

Dual-Energy X-Ray Absorptiometry. DXA scans (Hologic Discovery QDR model Series, Waltham, MA, USA) were performed to assess thigh lean mass immediately after the MRI was performed, and at least three days after the last exercise

session. A whole-body scan was performed, and whole-body and regional lean tissue mass was determined using the system's software package (Hologic APEX, Version 4.0.2). The thigh region was defined and isolated for analysis using a method similar to those reported previously (Fig. 3.1) [495,496]. Participants were scanned at the same time of day pre- and post-intervention and were encouraged to maintain a similar sleeping and eating schedule for both scans. Participants were advised to report to the laboratory in a hydrated state and they were given scrubs to wear during the scan. They were also given the opportunity to use the restroom prior to the scan. Care was taken to follow The International Society for Clinical Densitometry guidelines for positioning during the scan [497].

Repeatability of MRI and DXA Measures. All scans were analyzed by the same investigator blinded to the experimental group of the participants. Repeated measurement coefficient of variation (CV) was calculated for the investigator based on repeated measures of randomly selected MRI and DXA scans from 10 participants on different days separated by at least two weeks. The MRI method had a CV of 1.3%, while the DXA method had a CV of 0.96%. It should be noted that this method only assessed measurement variability of the investigator and not machine variability, as the same scan was analyzed twice at different time points. True CV is expected to be higher if machine variability were to be included.

Statistical analysis. The statistics software SPSS version 24.0 (SPSS Incorporated, Chicago IL) was used for statistical analysis. Pearson correlations were calculated to determine strength of association between MRI and DXA measures at

baseline and after the 10-week exercise program. Percent-change from baseline was then calculated for MRI and DXA measures for each participant (e.g., $[MRI_{post} - MRI_{pre}] / MRI_{pre} * 100$), and a Pearson correlation was calculated between percent-change MRI and percent-change DXA values. For the purpose of our analyses, left and right legs were considered independent of one another, and as such there were 52 data points for comparison.

We utilized the limits of agreement (LOA) method (a measure of absolute reliability), which is a statistical technique that is useful in partitioning out systematic bias vs. random error [498,499]. In doing this, Bland–Altman plots were generated for each variable and analyzed for the presence of heteroscedasticity (heteroscedasticity: residuals are not equally distributed; homoscedasticity: residuals are approximately equal). This was determined by examining the association (R^2) between the absolute differences and the mean values. R^2 values between 0 and 0.1 were considered homoscedastic (no relation between error and the size of the measured variable) [499]. R^2 values greater than 0.1 were heteroscedastic (amount of random error increases as the measured values increases) and the LOA ratio were then calculated [499]. The LOA ratio is calculated using the following equation: LOA ratio = $AVG_{means} \pm (SD_{diffs} * 1.96)$. Where SD_{diffs} is the standard deviation of all of the difference scores (%Δ MRI – %Δ DXA calculated for each participant), AVG_{means} is the average of all of the mean scores (mean of %Δ MRI and %Δ DXA for each participant), and the factor of 1.96 represents the inclusion of 95% of observations of the difference score. The LOA ratio is interpreted as ‘‘any two tests will differ due to measurement error by no more than X% either in the

positive or negative direction'' [499].

Results

There was a strong positive correlation between whole-thigh MRI- and DXA-derived measures at baseline (r=0.89, p<0.001; Figure 3.2) and after the 10-week exercise program (r=0.90, p<0.001). However, there was only modest positive correlation of the percentage change calculated over time between the two respective techniques (r=0.49 p<0.001; Figure 3.3). Bland-Altman plots revealed that there was a relationship between the error (i.e., the difference between the two techniques) and the mean percent change in skeletal muscle size, signifying heteroscedasticity (Figure 3.4). The ratio LOA was 10.40, indicating that differences due to measurement error should be no more than 10.40% in either direction in 95% of cases. Examination of individual cases demonstrated that only 46% of scans agreed within 3%. For those differing in their estimate by > 3%, we noted that in 43% of cases the MRI resulted in higher estimate of percent change, whereas in 57% of cases the DXA resulted in a higher estimate of percent change. Further, for those differing in their estimate by > 3%, five cases displayed hypertrophy via MRI but atrophy via DXA, while seven cases displayed hypertrophy via DXA but atrophy via MRI.

When comparing DXA percent change against MRI percent change, DXA displayed an average error of 4.16%, and overestimated muscle mass in 52% of cases. Average error of overestimations was 4.21% with a maximum difference from MRI of 11.32%, while average error of underestimations was 4.11% with a maximum difference from MRI of 13.77%. This resulted in a negligible bias of 0.21% in the negative

direction.

Discussion

The purpose of this study was to examine the relationship between MRI- and DXA-derived measures of thigh MV and lean mass, respectively, in a cross-sectional analysis as well as a longitudinal analysis where resistance exercise served as a stimulus for skeletal muscle adaptation. Our study demonstrates a strong association between MRI- and DXA-derived values when compared at a single time point, in agreement with previous cross-sectional validation studies comparing MRI and DXA measurements of appendicular muscle size. However, DXA-derived measures of percent change in lean mass were modestly associated with MRI-derived measures of MV, and less than half agreed within 3%. These findings demonstrate the imprecision of DXA as a tool to measure changes in appendicular muscle size in response to exercise. Muscle size and change in muscle size have been synonymous terms in clinical trial research, at least in relation to measurement strategies and recommendations. Very recently DXA was recommended as the "reference standard" for measuring lean mass based on the premise that there is a strong association between cross-sectional DXA and MRI or CT measurements [26]. These authors further recommended DXA as a tool to monitor changes in lean mass. Our findings strongly question the validity of this recommendation at least as it relates to quantifying changes in muscle mass over time (e.g., active muscle wasting).

While cross-sectional validation comparisons are common, there have been a limited number of investigations that directly compared DXA-derived changes in lean

mass against MRI or CT over time, with conflicting results. Nindl et al. [432] reported a strong correlation (r = 0.88) between single-slice MRI and DXA measures of percent change in muscle size in adult women after a 6-month exercise program. However, in this study the lower leg and foot were included in the DXA regional analysis, and whole-leg muscle mass was directly compared to an MRI-derived mid-thigh single-slice CSA. Bridge et al. [426] also reported a strong association (r = 0.78) between MRI- and DXA-derived percent change in muscle size, but with a more equivalent analysis. They compared MRI scans of the middle one-third of the thigh against a DXA-customized region of interest (ROI) of the middle one-third of the thigh in children over a three-year period. It is of note that these changes were not in response to an exercise intervention, but rather due to the normal maturation process. Further, these children exhibited increases in muscle size in the range of 50-100% over the three-year follow-up period [426]. In contrast, we report no significant intervention-related increase in muscle size *per se* using either DXA or MRI data; however, there was a large degree of heterogeneity in response to the intervention with some individuals exhibiting MRI-derived increases as high as ~13% and others exhibiting decreases on the order of ~7% with only modest agreement between measures (r = 0.49). Therefore, it appears that DXA may be able to detect very large changes in lean mass (i.e. >50%), though it should be noted that differences between the two measures in the previous study were still quite large despite the high level of agreement, with thigh mass increasing 55.7% from baseline as measured by DXA but 73.5% as measured by MRI [426]. Additionally, changes of this magnitude are limited to unique scenarios (e.g., adolescent development), and DXA has been unable

to accurately detect smaller changes in lean mass. For instance, a 3-month resistance training program in obese adolescents resulted in significant increases in leg muscle size as measured by MRI, but not DXA [429]. Investigators reported a modest association between whole-body MRI and DXA measures of change in muscle/lean mass ($r = 0.57$), but a weak association between appendicular (leg) measures of change in muscle/lean mass ($r = 0.20$) [429].

In recent years there has been a growing interest in the aging field to quantify muscle mass for the diagnosis of sarcopenia [500,501], as well as evaluation of the effectiveness of exercise, nutrition and pharmaceutical interventions to enhance muscle mass in older adults to prevent or treat sarcopenia [502,503]. Within the older adult population several studies have compared changes in muscle using various imaging modalities. Specifically, Hansen et al. [28] and Delmonico et al. [430] compared changes in muscle size/mass in older adults after surgical repair of the femoral neck and in response to resistance training, respectively. CT- and DXA-derived percent change values were only modestly associated in both studies ($r = 0.51$ and 0.53), and Delmonico and colleagues reported a ratio LOA of 7.2%. These findings, combined with our own results, demonstrate the lack of sensitivity in detecting small to modest changes in muscle size inherent in DXA. The previous discrepancy in reported agreement between DXA and MRI can be largely explained by the discordance of anatomical regions (e.g., whole-leg mass vs. mid-thigh 10 mm single-slice CSA) assessed by the two measures, as well as the magnitude of reported change.

There are several possible reasons for the differences seen between cross-sectional and longitudinal correlations of muscle mass measurements between DXA and MRI. One source of inaccuracy likely stems from the compounding error of multiple DXA measurements. The relatively small error inherent in a DXA cross-sectional measurement of muscle size can be compounded when multiple measures are taken and converted to percent-change in size. Another source of inaccuracy relates to the assumptions of DXA technology. Error seen in response to same-day repeated measures is attributed exclusively to machine error and rater error [434], however, one cannot assume that this holds true after an intervention, as exercise training can induce tissue changes that may impact X-ray attenuation [432]. The component profile for lean soft tissue mass includes proteins, glycogen, soft tissue minerals, and water, and altering the relative composition of these components through an intervention (i.e., exercise or weight loss) changes the attenuation coefficient, potentially violating the assumptions necessary for the accurate use of DXA technology [504]. For example, weight loss can induce fluid retention, and DXA may misinterpret the relatively greater hydrated lean soft tissue mass as a higher-density tissue [505]. It is likely that further changes in the relative composition of the components of lean mass occur in response to different interventions, but additional work is needed to develop a greater understanding of the variables that can affect DXA measures over time.

As mentioned earlier, there was a heterogeneous response to the resistance training in our study, resulting in muscle hypertrophy in some participants, as would be expected, but atrophy in others. We believe this is due to several reasons. First, the

exercise interventions were not overall strenuous in terms of the intensity/load as well as the total volume. This is particularly the case for the low-load resistance training control group. Second, the muscle groups utilized during exercise training were somewhat different than the muscle groups analyzed herein. One of the benefits of using the MRI is the ability to isolate individual muscles or muscle groups for analysis. In our study participants performed knee extension exercises, but not knee flexion exercises. Using the MRI, we would be able to analyze changes in the quadriceps muscle group, excluding all other muscles/tissues. However, the DXA is unable to separate muscle groups and can only quantify the mass of transverse sections of the body (i.e., total lean soft tissue mass from hip to knee). With this in mind, we chose to analyze all muscles of the thigh from the MRI scans to make our analysis more comparable to the DXA scans. Including all thigh muscles in the MRI analysis when only the knee extensors were targeted likely resulted in a misleading interpretation of the effect of the chosen exercise on muscle hypertrophy. However, the intention of this manuscript is to determine the level of agreement between two measurement techniques, and not necessarily to determine if a certain exercise protocol resulted in muscle hypertrophy. Therefore, we believe the heterogeneity of the sample strengthens our findings, as detecting decreases in muscle size is equally important to detecting increases, at least in sarcopenic populations.

This does raise the question of how large of a change in muscle size is considered clinically relevant. Roth et al. [336] reported that MRI-derived thigh muscle volume increased on average 4-6% in older adults and 4-8% in younger adults after six months of resistance training performed three times per week. Additionally, in a meta-analysis

Isodori et al. [506] reported a 2.7% increase in fat free mass in response to testosterone treatment. While statistically significant, it remains unclear how large of an increase should be considered clinically meaningful [507]. A full discussion on the clinical relevance of muscle size and changes in muscle size is outside the scope of this manuscript, however, based on past reports we believe that a change in muscle size of 3% is at least experimentally relevant. Therefore, tools to measure change in muscle size should be highly accurate to detect such small changes.

Our findings, when considered in light of the extant literature, raise questions around prior studies and trials using DXA to detect changes in muscle mass. This is exemplified in previous studies demonstrating changes in muscle size when measured by MRI or CT, but not by DXA [429,431,433]. In light of our findings and similar observations from others [28,429,430], we recommend that future studies give strong consideration to their selection of modalities for assessing muscle mass. While DXA may be a cost- and time-effective method to assess muscle mass cross-sectionally, a more in-depth investigation into the accuracy of DXA at detecting changes in muscle mass should be performed prior to its widespread acceptance as the "reference standard".

Limitations. In the present study the full-thigh DXA lean mass was compared against five-slice MRI-derived MV taken from the middle third of the thigh. It should be noted that middle third of the quadriceps is where most hypertrophy occurs, compared with proximal and distal regions [508], and we limited our analysis to that region. To determine whether differences in the ROI influenced our findings we created a customized ROI to analyze the middle third of the thigh using the DXA software, making

MRI and DXA scans directly comparable. We found that there was even less agreement between the two measures when using the customized ROI. As such, we do not believe that the differences in the ROI drove the findings of a weak association between the two techniques for assessing percent change in muscle size.

Another potential limitation is the field strength of the magnet used in the current study. Our 0.25-tesla magnet will produce lower quality scans than a stronger-tesla magnet, ultimately reducing the accuracy of scan analysis. However, this potential limitation was compensated for by increasing the scan time to reduce the signal-to-noise ratio [509].

Conclusion

The purpose of this study was to examine the relationship between MRI- and DXA-derived measures of thigh skeletal muscle size in a cross-sectional analysis as well as a longitudinal analysis where resistance exercise served as a stimulus for skeletal muscle adaptation. The measurement of lean mass in the thigh region via DXA was highly correlated to MRI-derived MV values, as has been seen in previous cross-sectional studies. However, DXA was unable to accurately detect changes in lean mass in response to resistance exercise. These findings suggest that future studies must give strong consideration to their selection of modalities for assessing lean mass. Further, these findings strongly question the recent suggestion that DXA be considered the “reference standard” for measuring muscle.

Chapter 4: Evaluation of an Experimental Approach for Assessing Neural Deficits in Ballistic Rate of Force Development

The material in this chapter is under review with the *Journal of Applied Physiology*.

Abstract

Rate of force development (RFD) is associated with human performance in athletic, older, and diseased populations. However, the neural and muscular mechanisms that influence RFD are not well understood and difficult to assess. We sought to examine and standardize a method of assessing neural deficits in knee extensor ballistic RFD by expressing voluntary RFD to electrically stimulated RFD, which we dubbed the Central Activation Ballistic (CAB) Force Ratio. We examined the relationships between voluntary RFD, surface electromyography (EMG) measurements, and the CAB Force Ratio during sequential 50 ms windows (range 0-200 ms) in 20 young (22.0 ± 1.7 years) and 16 older (72.3 ± 7.5 years) men and women, as well as in 15 older men and women (72.3 ± 7.7 years) in response to 12 weeks of progressive resistance training. Average EMG amplitude was associated with RFD and CAB in the first 50 ms in young adults (r = 0.46 and 0.69, respectively), but only with RFD in older adults (r = 0.54). Additionally, rate of rise of the EMG signal was associated with RFD and CAB in the first 50 ms in young adults (r = 0.57 and 0.58, respectively), but not in older adults. In contrast, CAB was associated with RFD at all time points, and the CAB Force Ratio method revealed a significant neural deficit from 100-150 ms in older adults when compared to young adults. These findings provide proof-of-concept for the CAB Force Ratio as a tool for identifying neural deficits of the knee extensors during isometric ballistic contractions.

Introduction

Voluntary force production as a biological construct can be broadly separated into neural and musculotendinous components, both of which contain numerous sites where voluntary output can be affected (Figure 4.1) [92,212]. Accordingly, as stated by Merton more than a half-century ago, “In voluntary efforts it is not known for certain whether the force that can be exerted is limited by the capacity of the nervous centres and conducting pathways to deliver motor impulses to the muscle fibres or by the intrinsic contractile properties of the fibres themselves” [510]. A concerted effort has been undertaken by the scientific community to identify these contributions, in large part because understanding the mechanisms limiting human performance in both health and disease is necessary to develop targeted, therapeutic approaches to maintain and restore function, as well as optimize performance. To this end, the capability of the nervous system to fully activate skeletal muscle has been extensively assessed using various superimposed stimulation methods (e.g., interpolated twitch, interpolated doublet, burst superimposition) [511–523]. The basis of these is supramaximal electrical stimulation to the motor nerve during the plateau phase of an maximal voluntary isometric contraction, with any stimulus-evoked increase in force indicating that some motor units have not been recruited or are not firing fast enough to produce maximal tetanic force [510]. As such, it has been used to estimate the relative contribution of neural and musculoskeletal factors in regulating muscle strength/weakness associated with fatigue (e.g., [511,522]), aging (e.g., [515,516,518,522]) neuromuscular diseases (e.g., [524,519,523]), and in association with exercise, injury, and disuse (e.g., [513,514,518,519,521]).

In recent years there has been increasing interest in the performance of ballistic movements (i.e., contractions that exhibit high velocities and accelerations over a very short period of time) [9,244]. Just as for maximal voluntary strength, ballistic movement (also referred to as rapid or explosive movement) is regulated by both neural and musculotendinous mechanisms. Many of the tools used to assess these contributions (e.g., electrical stimulation) are most readily and successfully applied under isometric conditions, and thus isometric ballistic force production is often used as a proxy for ballistic movement. In this paper, we propose a simple, innovative approach for assessing the neural deficits in ballistic rate of force development (RFD), based on the same conceptual framework as the above-described superimposed stimulation techniques, that we argue is relatively simple and innovative. We first describe why the capability to produce ballistic force is an important quantity in the sensorimotor system and then briefly review the strengths and weaknesses of the approaches that have previously been used to assay the neural deficits in ballistic RFD. We then present empirical data supporting the premise of this approach.

The capability to produce ballistic force is an important quantity in the sensorimotor system. The capability to perform ballistic movements is robustly related to survival across the animal kingdom, either through increasing hunting success of ambush predators or increasing chances of escape by prey [230]. For example, in adult garter snakes ‘maximal burst speed’ (the fastest speed recorded when a snake was being chased along a computerized race track) significantly predicts survival, whereas endurance (time crawled on a treadmill at a constant speed) does not [231]. In humans,

success and optimization of certain time-constrained tasks (e.g., jumping, recovering from tripping) requires rapid force generation during the first 200 ms (or thereabout) of muscle action [232,233]. Indeed, high voluntary RFD has been shown to predict greater athletic performance [404,525–527]. Moreover, deficits in RFD are associated with fall risk [232,238] and reduced capability to perform functional tasks [239]. While RFD and power (the product of the force and velocity of muscle contraction) are closely associated with maximal strength (maximal force generation) in healthy adults [240–242], injury and aging can reduce this association [241–243]. For example, deficits in RFD are still apparent six months after knee surgery even when strength has recovered to pre-injury values [243], and subjective knee function is related to RFD ($r = 0.58$) but not maximal force production six months after total knee replacement [528]. Additionally, muscle power declines earlier and more precipitously with advancing age compared to maximal muscle strength, and has emerged as an important predictor of functional limitations in older adults [229]. Together, these findings strongly suggest that the relative contribution of neural and muscular determinants of peak force/torque production and RFD are different. In light of these reports there has been increasing interest in understanding the physiological determinants of ballistic RFD, both for athletic performance and physical function assessment in response to injury, disease, and aging [9,244]. At present, however, the physiological determinants of ballistic RFD are poorly understood.

The nervous system is a critical determinant of maximal voluntary RFD. In the past two years elegant work from Dario Farina's laboratory has provided strong evidence that speed of recruitment and maximal discharge of motor neurons determine

maximal ballistic RFD [529,530]. In the first of these papers, Del Vecchio et al. used electromyographic (EMG) decomposition techniques on data obtained from high-density arrays of electrodes and observed that the peak motor unit discharge rate was strongly associated with RFD variables and, somewhat surprisingly, occurred before the onset of force production [529]. More specifically, a greater number of discharges per motor unit per second in the first 35 ms of motor neuron activity was associated with faster motor unit recruitment and, ultimately, ballistic RFD (explained variance 29-72%). However, none of the neural variables measured after 35 ms predicted explosive force [529]. In the second of these papers, Dideriksen et al. leveraged the power of computational modeling-based simulations and concluded that the capacity for ballistic RFD is limited mainly by neural properties, with the physiological variation of the rate by which motor units are recruited during ballistic contractions being the main determinant for the variability in RFD across individuals [530]. Interestingly, the effect of recruitment rate on simulated RFD was fourfold higher than that of increasing the initial discharge rate and fivefold higher than that of increasing the chance of doublets [530]. Collectively these findings suggest that neural input to the muscle is a critical determinant of maximal ballistic RFD.

Surface EMG is commonly used to assay nervous system contribution to ballistic RFD, but this approach has notable limitations. Relatively few scientists have the specialized instrumentation and technical expertise to successfully obtain and interpret motor unit firing data as described in the preceding section. Thus, the majority of studies that have investigated neural mechanisms of ballistic RFD have largely relied on examining the average amplitude or rate of rise of the global surface EMG signal as a

proxy for neural drive [233,241,249,253,406,411,460,461,463–470]. For instance, Gerstner et al. [249] reported average EMG amplitude explained 19-35% of the variance in RFD variables in a combined group of young and older men, though individual group associations were not reported. Similarly, moderate associations (explained variance 15-50%) have been reported in combined groups of older and young adults between rate of EMG rise (the slope of the filtered, rectified interference EMG signal over discrete time periods) and RFD [253,411]. However, when analyzed individually the association between rate of EMG rise and voluntary RFD was weaker in older adults than young adults (explained variance < 6% in older adults and ~33% in young adults [253,411]). Further, while multiple groups have reported concomitant increases in average EMG amplitude and/or rate of EMG rise and RFD in response to strength or power training [406,460,463–466], others have reported no change in EMG variables with training, despite substantial improvements in RFD [461,467–470]. The surface EMG signal represents the electrical activity generated in muscle fibers in response to the activation provided by innervating motor neurons. However, the traditional surface EMG technique underestimates the net neural activation to the muscle and has notable limitations that make accurate retrieval of the embedded neural code extremely challenging [8,457]. In addition, there are several non-physiological influences on the surface EMG signal that limit the utility of the garnered information (e.g., subcutaneous adipose tissue, location of the electrodes over the muscle, amount of crosstalk from nearby muscles) [8,457]. As a result, differences in surface EMG between groups or changes in response to an

intervention may not accurately reflect differences or changes in neural drive to the muscle [8].

The Central Activation Ballistic Force Ratio. An alternative approach to the use of EMG for parsing out the neural deficits during ballistic RFD is comparing RFD during maximal voluntary isometric ballistic contractions to the RFD during electrically stimulated contractions [391,233,466]. Theoretically, expressing the rate of voluntary force development ($RFD_{VOLUNTARY}$) to the peak rate of force development (i.e., yank [531]) of a potentiated, electrically-stimulated contraction ($Yank_{EVOKED}$) yields a value representing the nervous system's capability to rapidly produce ballistic force, with a smaller value indicative of a greater neural deficit in ballistic RFD. $RFD_{VOLUNTARY}$ is comprised of both neural and musculoskeletal contributors, while $Yank_{EVOKED}$ is largely independent of neural contributions and primary reflects the muscular contributions. This approach is straightforward, conceptually similar to the commonly used superimposition stimulation techniques for assessing deficits in neural activation during an isometric strength task, and relatively simple (i.e., non-invasive, not overly burdensome to a human participant, and can be done with limited instrumentation). This concept has been introduced previously, albeit briefly (e.g., [233,391,401,466]), but has not gained widespread adoption despite the growing interest in understanding the independent contributions of the nervous and musculoskeletal systems to ballistic RFD. Accordingly, in this article we describe and propose a standardized methodological approach for quantifying what we dub herein the Central Activation Ballistic (CAB) Force Ratio.

In addition to describing our approach, we empirically examine the relationships between common surface EMG measures (believed to reflect the neural contribution to ballistic RFD) and our proposed CAB Force Ratio in both a young and older adult population. We first examine these relationships using a cross-sectional (between-subjects) study design and subsequently examine the relationships among the resistance exercise-induced changes in a cohort of older adults (within-subjects). Based on the extant literature and theoretical underpinnings discussed above, we hypothesized that we would observe 1) a robust relationship between surface EMG measures, CAB, and RFD among healthy young adults, but that the relationship between EMG and RFD would be substantially weaker among community dwelling older adults while the relationship between CAB and RFD would remain robust; and 2) a modest relationship between the exercise-induced changes in surface EMG measures, CAB, and RFD among community-dwelling older adults. The results of the study were consistent with our hypotheses. Collectively, these findings suggest that the CAB Force Ratio may be a simple and discriminative approach for assessing differences in neural contributions to ballistic RFD, separate from those obtained from traditional voluntary activation assessment, without the limitations of surface EMG.

Methodology

Participants and General Overview. Twenty young adults (22.0 ± 1.7 years; 13 female, 7 male) and 16 older adults (72.3 ± 7.5 years; 10 female, 6 male) volunteered for this study and performed all tests during a single session. The older adults began the 12-week exercise program within two weeks of completing testing, and completed follow-up

testing within one week of finishing the exercise program. The post-testing EMG data from one older adult had abnormally high noise in the EMG signal and was not included in the final calculations. As a result, all post-testing data is based on N = 15. To be considered for the study, participants had to be either 18-25 years old or ≥ 60 years of age, have a body mass index between 18 and 40 kg/m^2, and be willing to undergo transcutaneous electrical stimulation. Additionally, older adults had to be willing to perform supervised progressive resistance exercise on site two days per week for 12 weeks, as well as undergo a variety of other health and performance measures before and after training. Study participants had to be living independently, free of major musculoskeletal, neurological, cardiac, pulmonary, renal, psychiatric, and cognitive disease or disorders and able to perform traditional activities of daily living (e.g., feeding oneself, showering). Participants were instructed to abstain from drinking caffeinated beverages for at least 4 hours prior and alcohol at least 24 hours prior to testing. The Ohio University Institutional Review Board approved this study, and all participants provided informed consent prior to their involvement in the study. Data for this report were derived from part of a larger study/dataset (The UNCODE Study; NCT02505529).

Study Design.

Dual-Energy X-ray Absorptiometry (DXA). DXA scans (Hologic Discovery QDR model Series, Waltham, MA, USA) were performed to assess body composition as a descriptive measure. A whole-body scan was performed and whole-body fat mass was determined using the system's software package (Hologic APEX, Version 4.0.2). Participants were advised to report to the laboratory in a hydrated state and were given

scrubs to wear during the scan and encouraged to use the restroom prior to the scan. Care was taken to follow The International Society for Clinical Densitometry guidelines for positioning during the scan [497]. Two young adults did not complete baseline scans.

Isometric Maximal Voluntary Contraction (MVC). Participants were seated in a Biodex System 4 Dynamometer (Biodex Medical Systems, Inc., Shirley, NY) with the non-dominant leg immobilized at 90° of flexion and the knee axis of rotation in alignment with the rotational axis of the Biodex torque motor. A lap belt was applied to prevent movement at the hip, and the participant's lower leg was affixed to a lever arm attached to the Biodex torque motor. Additionally, we custom-modified the Biodex to minimize compliance in the system. The manufacturer recommendations for leg extension testing involves the participant exerting force into padding that is secured to a rigid lever arm restraint with Velcro straps. We did not follow these recommendations, but rather we reversed the lever arm and had the participants exert force directly into the lever arm restraint, which had some padding to minimize discomfort while dramatically reducing lever arm compliance. The torque signal was scaled to maximize its resolution (208.7 mV/ Nm; Biodex Researchers Tool Kit Software) and sampled at 625 Hz (MP150 Biopac Systems). Participants received visual feedback of torque on a monitor located 1 m in front of them and were instructed to "push out as hard as you can for about 5-seconds easing into the contraction over the first second". Participants performed three isometric MVCs with one minute of rest between each effort, and the peak torque (N-m) of these three trials was considered the isometric MVC for the analysis (Figure 4.2).

Ballistic Voluntary Contractions. Participants completed three ballistic voluntary contractions, separated by at least 60 seconds, with the knee immobilized at 90° of flexion. Participants were instructed to "kick out as fast and hard as possible", with the emphasis on contraction speed as per prior suggestion [9]. Force onset was visually identified (see *Data Analysis*), and torque (N-m) was measured from onset to 50, 100, 150, and 200 ms. RFD (Δforce/Δtime) was quantified by subtracting force at each time point from force at the previous time point, and then dividing by 0.05 s (i.e., 50 ms), resulting in sequential RFD measures (N-m/s), defined as $RFD_{0\text{-}50}$, $RFD_{50\text{-}100}$, $RFD_{100\text{-}150}$, and $RFD_{150\text{-}200}$. The ballistic contraction with the highest $RFD_{0\text{-}50}$ was used for analysis and values were expressed in absolute terms, relative to MVC (i.e., NRFD), and relative to $Yank_{EVOKED}$ (i.e., CAB). Any trial with a counter movement where force exceeded 1N or duration exceeded 500 ms was excluded from analysis.

Electrically Evoked Doublet Contractions and Voluntary Activation (VA). Single-pulse electrical stimuli were delivered to the knee extensors via transcutaneous stimulation to evoke a maximal twitch response, as we have previously described [532,533]. Here, large self-adhesive electrodes (e.g., 5 cm x 9 cm or 7.5 cm x 13.5 cm depending on the size of the quadriceps being tested; Axelgaard ValuTrode, CA, USA; product #CF5090 and CF7515) were applied over the motor points of the vastus medialis and rectus femoris muscles of the quadriceps. Single pulses (200 μs duration, initial current 50 mA) were applied at incrementally increasing current (50 mA) with voltage held constant (400 V) and at least 10 seconds between pulses until a plateau was reached in the evoked force output (DS7AH; Digitimer, Hertfordshire, UK) (Figure 4.2). The

stimulus intensity that produced maximal twitch force was used for subsequent doublet stimulation. VA was then assessed using the interpolated doublet technique, as has been described previously [533]. Briefly, participants were instructed to perform a 5-second MVC, at the peak of which a 100-Hz supramaximal doublet was superimposed. Participants were instructed to relax upon stimulation, and a second 100-Hz supramaximal doublet was delivered 2-3 seconds after the MVC to ensure that the muscle was potentiated. Participants completed two VA trials with at least 120 seconds rest between trials (Figure 4.2). The doublet with the highest $Yank_{EVOKED}$ (quantified using the peak torque/time integral of the doublet contraction recording) was used for all subsequent $Yank_{EVOKED}$ analyses. VA was calculated by expressing the doublet-induced added force relative to the potentiated doublet after the MVC using the equation VA = [1 – (doublet force evoked during MVC / potentiated doublet force)] x 100, and average VA of the two trials was used for all subsequent VA analyses.

Surface EMG. Skin preparation included shaving, abrading, and cleaning the skin with alcohol. EMG was recorded from the vastus lateralis muscle of the non-dominant leg using bipolar surface electrodes (8-mm diameter Ag/AgCl electrodes with a 35-mm interelectrode distance; Trace 1, Nikomed USA, Inc.) placed longitudinally along the distal end of the muscle. A reference electrode was placed over the dominant patella. The EMG signals were amplified (1,000x), band-pass filtered (10–500 Hz), and sampled at 10,000 Hz (MP150, Biopac Systems Inc., Goleta, CA).

Exercise Training. All older adult participants also completed 12 weeks of progressive resistance training, prior to which each underwent an electrocardiogram and

completed the PAR-Q survey [534] for determination of inclusion/exclusion. The progressive resistance exercise-training program followed a protocol that was recently published by our group [285], wherein participants completed one-on-one supervised resistance training exercises two times per week for 12 weeks. Each day participants completed 10 exercises (from a list of ~25 exercises) using free weights and/or machine weights that targeted the major muscle groups (i.e., chest, back, arms, shoulders, upper legs, and lower legs). The intensity of the exercise was determined based on the participant's ability to complete the prescribed sets and repetitions, where the target intensity was designed to result in task failure, or near-failure, in the range of repetitions provided. Briefly, Phase I (familiarization) typically lasted 2 weeks and participants completed one set of 12-15 repetitions. They were then advanced to phase II, which typically lasted from weeks 3-6, and participants completed two sets of 12-18 repetitions. Phase III typically lasted from weeks 7-10 and consisted of three sets of 8-12 repetitions, and phase IV typically lasted from weeks 11-12 and consisted of three sets of 6-10 repetitions. It should be noted, however, that the progression from phase to phase (and even specific exercises) was made on a case-by-case basis reliant on individual progression and adaptation. Weekly team meetings were held to discuss individual participant progression.

Each exercise session included a brief medical safety check conducted prior to exercise to identify potential contraindications for exercise (e.g., acute illness or worsening of chronic illness, chest discomfort or angina, arrhythmias, dyspnea, dizziness, edema, fatigue, moderate to severe pain in muscle or joint), and vital signs were obtained.

If the readings prior to the exercise exceeded 170 mmHg for systolic blood pressure or 100 mmHg for diastolic blood pressure the participant was not be permitted to exercise on that day and the study physician was notified. Participants were not allowed to continue the exercise program until they were cleared by the study physician. After the medical safety check participants completed a 5-minute warm-up on a treadmill or bicycle ergometer (or walking outside or in the building if preferred), and they completed a similar 5-minute cool down at the end of the exercise session.

Data Analysis. Voluntary and evoked force signal onsets were identified manually using a two-point differential, wherein onset was defined as the final trough before the force signal deflected above the baseline mean, a method that has shown to be highly repeatable [241,404]. Force recordings were consistently viewed with the *x*-axis scale criteria defined as baseline noise filling ~25% of the viewing window, and the y-axis scale set at ~250 ms. The same investigator analyzed all signal onsets. Voluntary RFD was expressed relative to $Yank_{EVOKED}$ at sequential time points, resulting in the CAB Force Ratio, a value we hypothesized to be representative of the central nervous system's ability to rapidly produce force. The equation to calculate CAB is:

$$[CAB = RFD / Yank_{EVOKED}]$$

The interference EMG signal was notch filtered at 60 Hz, corrected for baseline offset, and the root mean square (RMS) was calculated with a 50 ms moving window. Average baseline noise was determined over a 100 ms window beginning ~200 ms before force onset to account for electromechanical delay (EMD). EMG signal onset was defined as the point where the signal exceeded 5 standard deviations above the average

baseline noise. Due to the observed variability in EMD in older adults we also assessed EMG variables using a standardized method of EMG onset detection by assuming a constant EMD of 50 ms and setting EMG onset 50 ms prior to force onset. Statistics were run on EMG variables using both detection methods and the standardized method was more strongly associated with RFD variables than the manual detection method. Thus, all reported EMG variables are based on the standardized method. Rate of rise of the EMG signal (EMG_{RR}) was calculated from signal onset (force onset -50 ms) to force onset as Δamplitude/Δtime, but not at later time points due to a decrease in the EMG signal amplitude after ~70-100 ms, as has been reported previously [406]. Additionally, average amplitude of the RMS EMG signal (EMG_{AVG}) was recorded at 50 ms time intervals from signal onset, defined as $EMG_{AVG0-50}$, $EMG_{AVG50-100}$, $EMG_{AVG100-150}$, and $EMG_{AVG150-200}$. The EMG values from the voluntary ballistic contractions were normalized to the EMG signal during peak force of the MVCs. Briefly, average RMS EMG amplitude during a 500 ms window surrounding peak force (250 ms prior and 250 ms after) was calculated from all three MVCs and averaged to produce a reference EMG signal to which all other EMG values were relatively expressed.

Statistical Analysis. Descriptive statistics are presented as mean ± standard deviation and all analyses were performed using SPSS version 26 (SPSS, Inc., Chicago, IL, USA), with alpha level set at 0.05 for all statistical tests. In line with other proof-of-concept investigations, no statistical control for type-I error from multiple comparisons were considered. Voluntary RFD values are expressed in absolute terms and relative to MVC (NRFD). Age-related differences in MVC, contractile properties, RFD, NRFD,

EMG, and CAB were assessed with unpaired t-tests, while exercise-induced changes in these variables were assessed with paired t-tests; results of these tests can be found in Table 4.1. Pearson's bivariate correlations were performed between RFD, EMG, and CAB for young and older adults separately. Resulting correlation coefficients were statistically compared for differences between age groups using the method developed by Eid et al. for comparing correlations from independent samples [535,536]. Additionally, Pearson's bivariate correlations were performed between changes in EMG, CAB, and RFD variables in older adults. The strength of the relationship was defined as follows: ≤ 0.29 = weak; 0.30-0.49 = modest; 0.50-0.69 = moderate; 0.70-0.89 = strong; ≥ 0.90 = very strong [537]. Further, Pearson's bivariate correlations were performed between VA and CAB variables to determine the degree to which the two measures were related. One older adult and one young adult participant did not meet the threshold for accurate VA assessment (i.e., the superimposed stimulus was not delivered at peak force in either trial), and as such N = 19 for young adults and N = 15 for older adults for any cross-sectional analyses involving VA. Additionally, Pearson's correlations between MVC, contractile properties, RFD, and NRFD variables were performed and can be found in Table 4.2, while correlations between exercise-induced changes in these variables can be found in Table 4.3.

Results

Descriptive statistics of the study participants and effects of the resistance exercise intervention. Descriptive statistics are shown in Table 4.1. Notably, the older adults were weaker (43.9% lower MVC), had slower voluntary RFD (34.0-51.5% lower),

and reduced doublet force and $Yank_{EVOKED}$ (36.2% and 38.7%, respectively). Additionally, the CAB Force Ratio was 9.5% lower in older adults than younger adults from 100-150 ms, though there were no significant differences at any other time point. There were no age-related differences in EMG_{AVG}, EMG_{RR}, or NRFD. In regard to the exercise intervention, there was a significant increase in gait speed (+0.14 m/s), SPPB score (+0.5) and MVC (+15.5%). However, there were no significant exercise-induced changes in any of the other measured variables (Table 4.1).

Relationships between early-phase EMG and CAB are moderate in young adults, weak/modest in older adults. There was a moderate relationship between EMG_{AVG} and CAB, as well as between EMG_{RR} and CAB, from 0-50 ms in young adults ($r = 0.69$, $p = 0.001$; and $r = 0.58$, $p = 0.007$, respectively). However, in older adults only a modest, non-significant association was observed between $EMG_{AVG0\text{-}50}$ and $CAB_{0\text{-}50}$ ($r = 0.31$, $p = 0.244$) (Figure 4.3), while EMG_{RR} and $CAB_{0\text{-}50}$ were not related ($r = 0.00$, $p = 0.994$). There were no significant associations between EMG variables and CAB at any other time point (all p-values > 0.05; Table 4.4). When the 0-50 ms correlation coefficients were statistically compared, we found that the EMG_{RR}/CAB relationship was significantly stronger in young adults than older adults ($p = 0.035$), but the age-related difference in the EMG_{AVG}/CAB relationship did not reach significance ($p = 0.073$).

Relationships between exercise-induced change in early-phase EMG and CAB are modest/moderate in older adults. The exercise-induced changes in EMG_{AVG} and CAB in older adults were moderately associated in the 0-50 ms window ($r = 0.65$, $p = 0.009$) (Figure 4.3), while there was only a modest, non-significant association between

EMG_{RR} and $CAB_{0\text{-}50}$ (r = 0.41, p = 0.131). There were no significant associations between exercise-induced change in EMG variables and CAB at any other time point (all p-values > 0.05; Table 4.5).

Relationships between early-phase EMG and RFD are modest in young adults, moderate in older adults. EMG_{AVG} was modestly associated with RFD from 0-50 ms (r = 0.46, p = 0.042) and 150-200 ms (r = 0.45, p = 0.046) in young adults, and moderately associated with RFD at 0-50 ms in older adults (r = 0.54, p = 0.030) (Figure 4.3). EMG_{RR} was moderately associated with $RFD_{0\text{-}50}$ in young adults (r = 0.57, p = 0.008) but not older adults (r = -0.03, p = 0.922). The $EMG_{RR}/RFD_{0\text{-}50}$ relationship was significantly stronger in young adults than older adults (p = 0.03), but there was no difference in the strength of the $EMG_{AVG0\text{-}50}/CAB_{0\text{-}50}$ relationship between young and older adults (p = 0.379). There were no significant associations between EMG variables and RFD at any other time point (all p-values > 0.05; Table 4.2).

Relationships between exercise-induced change in early-phase EMG and RFD are moderate/strong in older adults. The exercise-induced changes in $RFD_{0\text{-}50}$ were strongly associated with changes in $EMG_{AVG0\text{-}50}$ (Figure 4.3), but not with changes in EMG_{RR} (r = 0.70, p = 0.003; r = 0.40, p = 0.14, respectively). Additionally, exercise-induced change in EMG_{RR} was moderately, negatively associated with changes in $RFD_{100\text{-}150}$ and $CAB_{100\text{-}150}$ (r = -0.67, p = 0.006; r = -0.61, p = 0.016, respectively). There were no significant associations between exercise-induced changes in EMG variables and RFD at any other time point (all p-values > 0.05; Table 4.3).

Relationships between CAB and RFD are moderate/strong at all time points in young and older adults, and relationships between exercise-induced changes in CAB and RFD are strong in older adults. CAB was moderately or strongly associated with RFD at all time points measured in both young (average *r*-value 0.75 ± 0.11, all *p*-values < 0.01) and older adults (average *r*-value 0.64 ± 0.16, all *p*-values < 0.05) (Figure 4.4), and there were no differences in the strength of the relationships between young and older adults (all *p*-values > 0.05). The relationships resulting from exercise-induced changes in CAB and RFD were strong at all time points (average *r*-value 0.76 ± 0.05, all *p*-values < 0.01) (Figure 4.4).

Relationships between CAB and VA are weak/modest in young and older adults, and relationships between exercise-induced changes in CAB and VA are weak/modest in older adults. CAB was weakly or modestly associated with VA at all time points measured in both young (average *r*-value 0.04 ± 0.24, all *p*-values > 0.05) and older adults (average *r*-value -0.08 ± 0.24, all *p*-values > 0.05). The relationships resulting from exercise-induced changes in CAB and VA were also weak or modest at all time points (average *r*-value = -0.17 ± 0.15, all *p*-values > 0.05).

Discussion

In this article we describe a standardized approach utilizing voluntary and electrically evoked RFD measurements to quantify neural deficits in isometric ballistic RFD of the knee extensors. While RFD is not a new measure by any means, our understanding of its prognostic value in regard to human performance in both health and disease is still emerging. Central to the development of this understanding is accurate

characterization of neural and muscular contributors to RFD throughout the force-time curve. We evaluated the difference in, and relationships among, RFD, EMG, and the CAB Force Ratio, both cross-sectionally between young and older adults, as well as in response to a 12-week progressive resistance exercise intervention in older adults. The results provide empirical evidence as proof-of-concept of our approach. We found that EMG-derived estimates of neural drive (EMG_{AVG} and EMG_{RR}) were not significantly different in young and older adults in the first 200 ms of ballistic contractions (Table 4.1). EMG_{AVG} was associated with RFD and CAB in the first 50 ms in young adults, but only with RFD in older adults (Figure 4.3), while EMG_{RR} was associated with $RFD_{0\text{-}50}$ and $CAB_{0\text{-}50}$ in young adults, but with neither in older adults. Additionally, exercise-induced changes in CAB and RFD were associated with EMG_{AVG} in the first 50 ms, but not with EMG_{RR} in older adults. This phenomenon has been reported previously (with EMG_{RR} and RFD) [253,411], and suggests that age-related neuromuscular adaptations negatively affect surface EMG readings. In contrast, we found the CAB Force Ratio to be both associative and discriminative with regard to age-related differences in RFD (Figure 4.4). Additionally, we found that the CAB Force Ratio and VA were only weakly, non-significantly associated, suggesting these two measures quantify separate aspects of voluntary activation capacity (i.e., ballistic and maximal force production). Together, these findings indicate that our novel CAB Force Ratio method and surface EMG are related in their ability to quantify the nervous system's contribution to ballistic RFD, but that the two measures are not interchangeable. Our findings underscore the weaknesses of using surface interference pattern EMG to determine the nervous system's contribution to

ballistic RFD, particularly cross-sectionally, and provide evidence to support the use of the CAB Force Ratio as an alternative method to quantify neural deficits in ballistic RFD that can be compared between groups and potentially in response to an intervention. Additionally, just as muscle power is a more robust predictor of physical function decline in older adults than muscle strength [229], so too may voluntary ballistic activation capacity overtake traditional methods of VA assessment.

The CAB Force Ratio method has three notable strengths. First and foremost, our findings indicate that it demonstrates both associative and discriminative sensitivity, expanding our understanding of the neural contribution to RFD throughout the force-time curve (discussed above). Second, our proposed approach that utilizes a post-activation potentiated doublet for the normalization appears tolerable. We are not the first to express voluntary RFD relative to evoked RFD as a measure of voluntary ballistic activation [233,391,401,466], although this prior work used a 300 Hz octet stimulation protocol. While this approach has scientific merit in that it will yield a true maximal evoked RFD, it is also very painful/uncomfortable, likely limiting utility in many settings. For example, Tillin et al. [466] reported that 30% of young, healthy adults that they studied did not complete the stimulation protocol due to discomfort. In contrast, we have successfully applied 100-Hz doublet stimulation protocols in a variety of muscle groups to hundreds of younger and older adults over the past two decades, and we estimate that ~90% were able to successfully complete and tolerate the testing [392,402,516,532,533,538–540]. While our doublet protocol is unlikely to elicit a true maximal RFD, we believe that our potentiated doublet technique strikes the ideal balance between scientific rigor and

tolerability/subject burden, particularly if it is to be applied to a non-athletic population who may be less apt to tolerate painful electrical stimulation protocols [541]. Lastly, the instrumentation and approach to quantifying the CAB Force Ratio are relatively standard and inexpensive, the data can be collected with minimal time (~ 20-30 mins in our experience), and the technique is non-invasive. In fact, it can easily be incorporated within a standard interpolated doublet VA assessment paradigm, and may, arguably, be a more accurate measure of one's ability to fully activate a muscle because it does not rely on the precise timing of stimulation delivery.

There are some limitations to our CAB Force Ratio method that should be noted. First, it does not provide insight into the specific neural mechanisms causing the deficit, although the same is true for commonly used surface interference EMG measurements [8,457]. That is, it does not delineate whether a deficit is due to supraspinal or spinal-mediated mechanisms *per se*. Second, this method may be better suited for scientific purposes (e.g., characterizing differences between populations) as opposed to serving as a clinical diagnostic tool at the individual level. Third, within the context of what we describe, this method is specific to the knee extensors and it is unclear how translatable it would be to other muscle groups. Fourth, we did not find any exercise-induced changes in RFD, EMG, VA, or CAB variables, and thus we were unable to determine if neural deficits could be improved. As the resistance training program did not include a power component, a consistent change in these variables was not necessarily expected, and future work could expand on this concept. Lastly, we did not examine the repeatability of CAB in this study, though at least one other study using a similar approach to our own

has reported moderate variability (coefficient of variation = 18.8%), primarily driven by the higher variability of the early phase of the voluntary effort (0-50 ms) [401]. However, methodological recommendations for RFD evaluation have recently been published [9], and adherence to these recommendations should greatly improve reliability.

In summary, our aim was to investigate and characterize an alternative method of assessing neural deficits in knee extensor ballistic RFD by isolating the muscular contributions through electrical stimulation. In so doing, we examined the relationships between voluntary RFD, EMG, and our novel CAB Force Ratio during sequential 50 ms windows from 0-200 ms in both young and older adults, and in response to 12 weeks of progressive resistance training in older adults. EMG_{AVG} was associated with both RFD and CAB in the first 50 ms in young adults, but only with RFD in older adults, while EMG_{RR} was associated with $RFD_{0\text{-}50}$ and $CAB_{0\text{-}50}$ in young adults, but not in older adults. Exercise-induced changes in RFD and CAB were associated with changes in EMG_{AVG} in the first 50 ms, but not with EMG_{RR}. Additionally, there were no differences in EMG measures between young and older adults at any time point. In contrast, CAB was associated with RFD at all time points, and older adults demonstrated a 10% neural deficit from 100-150 ms when compared to young adults. These findings provide proof-of-concept for the CAB Force Ratio as a tool for identifying neural deficits and/or voluntary activation capacity of the knee extensors during ballistic contractions as an alternative to surface EMG recordings or traditional methods of VA assessment.

Chapter 5: A Randomized Clinical Trial Comparing Three Different Exercise Strategies for Optimizing Aerobic Capacity and Skeletal Muscle Performance in Older Adults: The DART Study

Portions of the material in this chapter have been published in *Frontiers in Medicine* [542] and are under review for the *Journal of Frailty and Aging*.

Abstract

The aim of this pilot study was to determine the effect of 12 weeks of stationary bicycle high-intensity interval training, stationary bicycle moderate-intensity continuous training, and resistance training on cardiorespiratory, muscular, and physical function measures in insufficiently active older adults (N=14; 66.4±3.9 years; 3 male, 11 female). Participants were tested at baseline and randomly assigned to one of the three exercise groups. High-intensity interval training and moderate-intensity continuous training had small-to-large effect sizes on cardiorespiratory/endurance and physical function measures, but very small effect sizes on muscular measures. Resistance training had small-to-large effect sizes on cardiorespiratory, muscular, and physical function measures. While this was a pilot study that should be interpreted cautiously, findings suggest that resistance exercise may be the most effective standalone exercise strategy for older adults as it can induce beneficial adaptations across multiple domains. These effect sizes can be used to determine optimal sample sizes for future investigations.

This study is registered with clinicaltrials.gov (NCT03978572).

Introduction

The number of Americans 65 years and older is projected to nearly double by 2060 [1]. This doubling will significantly impact the health care system, as 42% of older adults report limitations in their ability to perform functional tasks that are essential for maintaining independence [543]. Exercise is effective at maintaining physical function [18], and national guidelines encourage adults of all ages to perform moderate-intensity aerobic exercise for at least 150 minutes each week and muscle strengthening exercises at least two days per week [290]. Unfortunately, less than 13% of older adults meet the aerobic and muscle strengthening guidelines concurrently, while an additional 31% only meet one of the two [287]. Consequently, the majority of older adults are not getting the muscular and/or cardiorespiratory health benefits that accompany regular participation in aerobic and muscle strengthening exercise. With this in mind, a more pragmatic approach of emphasizing a single exercise strategy with the greatest effect on overall health may be a reasonable solution to optimize outcomes and improve adherence [30].

High-intensity interval training (HIIT) is an exercise strategy that improves cardiorespiratory fitness and lowers cardiovascular disease risk equal to, or greater than, traditional aerobic training [376], and has also been shown to improve knee extensor strength [380], insulin resistance [544], and skeletal muscle metabolism [545] in young adults. However, the potential for HIIT to induce muscular benefits in older adults has not been adequately explored. As such, the aim of the DART Pilot Study (Dual-benefits of Aerobic and Resistance Training) was to examine whether stationary bicycle HIIT was a more efficient standalone exercise strategy to improve cardiovascular and lower

extremity muscular function than established resistance training (RT) or moderate-intensity continuous training (MICT) programs in older adults. Specifically, we hypothesized that **1)** HIIT bicycle exercise would exhibit improvements in cardiorespiratory/endurance measures equal to MICT bicycle exercise and greater than RT; **2)** HIIT bicycle exercise would exhibit improvements in muscular strength, power, and size equal to the RT and greater than the MICT bicycle exercise; and **3)** HIIT bicycle exercise would exhibit improvements in physical function measures greater than either the MICT or RT groups.

Methodology

Participant characteristics. Twenty-two participants aged 60-75 years of age that were generally healthy but insufficiently active were recruited, enrolled, and randomized in this study, with 14 participants (66.4 ± 3.9 years; 3 male, 11 female) completing the study (Figure 5.1). One participant was removed for starting a new blood pressure medication while on the study protocol, and seven participants were interrupted prior to completion due to the COVID-19 pandemic and were unable to resume the study. Participants were recruited from the local community by way of flyers, community events, or emails to individuals who had previously participated in studies with the Ohio Musculoskeletal and Neurological Institute at Ohio University in Athens, Ohio. All interested individuals completed a pre-screening phone interview, and all that were not ruled ineligible were invited for in-person screening. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. Ethical

Approval for this study has been obtained from the Ohio University Institutional Review Board (protocol number 18-F-55).

Eligible participants were insufficiently active. We defined *insufficiently active* as not meeting either the endurance or resistance exercise recommendations for older adults, as set forth by the ACSM, for three consecutive months. The current ACSM recommendations for endurance training are 150-300 minutes per week of moderate-intensity activity (perceived exertion of 5-6 on 0-10 scale), or 75-150 minutes per week of vigorous-intensity activities (perceived exertion of 7-8 on 0-10 scale) [23]. The recommendations for resistance training include at least two days per week of progressive weight training activities that use the major muscle groups (perceived exertion of 5-8 on 0-10 scale) [23]. Additionally, the participants could not be highly active outside of a structured exercise program (e.g., consistent hard physical labor). All had a six-minute walk distance within a normal range for adults aged 60-75. We calculated a normal six-minute walk range by averaging six-minute walk values from multiple studies, plus or minus one standard deviation [546–549]. The calculated six-minute walk range for females was 400-675 meters, and for males was 450-725 meters. Table 5.1 describes inclusion and exclusion criteria in detail. These criteria were designed to recruit a generally healthy, but insufficiently active population, and exclude individuals with poor health and physical function where there could be concerns about a participant's ability to appropriately perform the testing and exercise prescription.

Design. This is a single-blinded (outcomes assessor), single-site, randomized control trial. It is a three (group) by two (time) repeated measures factorial design. The

overall study is illustrated in Figure 5.2. Participants were randomized in a 1:1:1 ratio to receive a 12-week exercise program consisting of either RT, MICT on a stationary bicycle, or HIIT on a stationary bicycle. Due to the small sample size in each arm, permuted-block randomization was used via computer-generated random numbers to ensure equal sample size. The allocation sequence was generated by a biostatistician, who created blocks of three with each treatment permuted within each block. The study participants were enrolled and assigned to their respective interventions by an unblinded project manager. Due to the nature of the intervention neither participants nor staff could be blinded to allocation. The outcomes assessor and data analyst were blinded after study completion by having the participants' demographic and intervention group information coded.

Sample size. In this proof-of-concept, proof-of-mechanism trial, we report sample size estimates based on previous recommended literature [550]. A sample size of approximately 8 participants per group was calculated to detect a moderate effect. Sample size calculation was based on expected effect sizes for the HIIT bicycle, MICT bicycle, and RT groups for the primary outcomes of maximal oxygen consumption (VO_2max) and thigh muscle cross-sectional area (CSA). Consistent with our dual-benefits hypothesis we assumed, based on the literature, an 8% increase in thigh CSA for both the HIIT and RT groups [551] and 2% increase for the MICT group [552] and common SD across all groups of 4%. With the assumption of a 2-sided test and alpha level of significance equal to 0.05, an n=7/group yields power of 0.83. With respect to VO_2max we assumed a 32% increase in the HIIT group, a 15% increase in the MICT

group, and a 10% increase in the RT group and common SD across all groups of 14% [553]. With the assumption of a two-sided test and alpha level of significance equal to 0.05, an n=8/group yields power of 0.80. In line with other proof-of-concept, proof-of-mechanism trials, no statistical control for type-I error from multiple comparisons were considered, and p-values were interpreted with care, as descriptive weights of evidence rather than as confirmatory claims. Lastly, this proof of concept, proof of mechanism trial is needed to test the complex interventions proposed, and the effect sizes calculated from this trial could be used to estimate sample size for a future large-scale clinical trial. Accordingly, we planned to enroll N = 10 participants per group, but were only able to enroll N = 4-5 per group due to COVID-19.

Study timeline. This study had a screening/baseline assessment period of 21 days (maximum) with three sessions spaced at least 48 hours apart, a 12-week exercise training period, and a post-intervention assessment period of 10 days (maximum) with two sessions spaced at least 48 hours apart. Participants visited Ohio University's Clinical and Translational Research Unit facilities prior to the intervention for baseline assessments. During Visit 1 we obtained informed consent and conducted a full medical history screening and a short physical performance battery (SPPB) [554] to determine if candidates met the inclusion/exclusion criteria. Participants who met the criteria were enrolled in the study and completed a series of clinical and physiological outcome measures over the three baseline visits. Upon completion of baseline assessments, participants were randomized into one of the three exercise groups for the 12-week exercise intervention. All exercises were performed on site and supervised by an exercise

professional three days per week. All baseline assessments were repeated upon completion of the exercise intervention. A table of events for the study is illustrated in Table 5.2.

Primary outcomes.

Knee extensor isokinetic strength. Isokinetic strength was measured from the knee extensors. Peak torque was recorded from the non-dominant leg using a Biodex System 4 Dynamometer (Biodex Medical Systems, Inc., Shirley, NY). The participant's leg was immobilized against the lever arm with the distal end of the lever arm secured three inches superior to the medial malleolus. The axis of the lever arm was centered at the lateral knee joint and knee range of motion was obtained by having the participant extend their knee as far as possible against the lever arm. The maximal knee extension angle was recorded, and the knee extension limit was set 10° less than the maximal knee extension angle. The participant then relaxed to allow the leg to return to neutral position and the knee flexion limit was set at 80° of knee flexion. The speed of the lever arm was set at 60°/second for both extension and flexion. The participant then extended the knee with maximal effort until they reached the knee extension limit, and then immediately flexed the knee with maximal effort until they reached the knee flexion limit. The time-series torque signal was collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). The participant completed six isokinetic trials, with 30 seconds rest between trials. The average of the three highest peak torque values for extension was recorded at baseline and post-intervention for analysis.

VO_2max. VO_2max was obtained with a ParvoMedics TrueOne 2400 metabolic measuring system with a Hans Rudolf 3813 pneumotachometer (Shawnee Mission, KS, USA) to measure ventilation. The TrueOne 2400 is a mixing chamber system that uses a paramagnetic oxygen analyzer (range 0-25%) and an infrared, single beam, single wavelength carbon dioxide analyzer (range 0-10%). Prior to each test the system was allowed to heat up for 30 minutes, and then was calibrated according to the manufacturer's recommendations. This consisted of a room air auto-calibration and a gas calibration with a single gas tank (16.000% O_2, 4.008% CO_2). Additionally, the flow meter was calibrated with a 3.000-liter Hans Rudolf 5530 series syringe, with a 5-stroke calibration using different flow rates for each stroke. Ten electrocardiograph (ECG) electrodes were placed on the participant's body according to Mason-Likar procedures [555,556]. Prior to placement, the areas were shaved with a disposable razor, wiped with an alcohol pad, and then lightly abraded with fine-grit sandpaper. The participant was fitted with a Hans Rudolf Oro-Nasal reusable facemask (Shawnee Mission, KS, USA) with a two-way non-rebreathing valve connected to the metabolic cart with large-bore, low-resistance tubing. The testing protocol was explained to the participant, as well as how to communicate with investigators while wearing the facemask (i.e., hand signals). Resting VO_2 values were collected from the participant after three minutes of rest in a seated position on the cycle ergometer. Resting blood pressure was taken immediately after resting VO_2 was obtained. At the onset of the graded exercise test (GXT) the participant began cycling on a magnetically braked cycle Lode Corival CPET ergometer (Lode B.V., Groningen, NL) at 60-80 RPMs (depending on participant comfort level)

with a starting power output of 15 watts (W) for one minute, collecting VO_2 values every 20 seconds. Power output was increased by 15 W every minute until the participant could no longer continue the test or the criteria had been met (Table 5.3). With 20 seconds remaining in each stage, the participant indicated their rating of perceived exertion (RPE) on a 6-20 Borg scale [557]. With 10 seconds left in each stage, heart rate was recorded from ECG readouts. Blood pressure was taken at the beginning of even numbered stages. Participants were verbally encouraged throughout the test, and airflow was provided through the use of a rotating fan. After test termination, the participant performed a five-minute cycling cool down at 30 W. Blood pressure was taken every five minutes for 20 minutes after the completion of the GXT. Maximal heart rate was determined from ECG readings during the final stage of the GXT for all participants.

Quadriceps muscle volume. Quadriceps partial muscle volume was obtained via magnetic resonance imaging (MRI) scans performed with a 0.25-Tesla Musculoskeletal MRI system (Esaote G-Scan Brio, Genoa, Italy) to acquire contiguous transverse T-1 weighted spin echo image slices in the thigh region with a slice thickness of 10 mm and an inter-slice distance of 10 mm. The isocenter was positioned at mid-thigh, midway between the patella and the inguinal crease, and the participants were supine. Images were transferred to a computer for calculation of quadriceps anatomical CSA. Beginning with the slide with the first discernable visual of the rectus femoris and including the subsequent four proximal slides, quadriceps muscle area was traced using a polygon tool, excluding bone, as well as fat tissue surrounding the muscles (MIPAV version 7.3.0). Intramuscular fat was then subtracted by applying a shading correction to each slide,

determining average voxel density and standard deviation voxel density from a sample of the lightest area of fat tissue, computing a cutoff value at three standard deviations darker than the sample voxel density, and excluding all pixels with a voxel density at or below the computed value. Pre- and post-intervention slides were displayed simultaneously, and slides were visually compared to ensure that tracing patterns were identical and that the same structures were excluded (i.e., neurovascular bundle, intermuscular fat) on both slides before CSA values were recorded. This process was completed for each analyzed slide, resulting in five measures of quadriceps CSA with intermuscular and intramuscular fat excluded for both pre and post time points. Partial muscle volume was then calculated using the Cavalieri method [$MV = T (A_1 + A_2 + A_3 + A_4 + A_5)$], where MV=muscle volume, T=the known distance between slices, and A=area [421].

Secondary outcomes.

Knee extensor isometric strength. Maximal isometric force production was measured via three maximal voluntary contractions (MVCs) of the knee extensors while the participant was positioned in the Biodex Dynamometer as described above, with the lever arm immobilized at 90° of knee flexion. The participant was instructed to gradually increase force for the first second, and then exert maximal effort for ~3-4 seconds. The participant performed three MVCs with a 30-60 second rest period between contractions. Verbal encouragement was provided during each trial. The time-series torque signal was collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). The trial with the highest value was recorded at baseline and post-intervention and used for analysis.

Knee extensor isokinetic fatigue. Fatigue resistance of the knee extensors was measured with the participant positioned in the Biodex dynamometer as described above. Participants were asked to perform a series of isokinetic leg extensions at 120°/second (the flexion component was passive at a speed of 240°/second). First, study participants performed three isokinetic extensions recorded as pre-fatigue peak torque values. Next, study participants were given three minutes of rest before beginning the fatigue portion of the test. For the fatigue test, participants performed 120 consecutive maximal isokinetic leg extension contractions (test time ~ 4-minutes). Peak force for each of the 120 contractions was recorded and work performed in the final third of the test (contractions 81-120) were summed and expressed relative to work performed in the first third of the test (contractions 1-40). The participant was verbally encouraged throughout the test. The time-series torque signal was collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA).

Body composition. Total body fat mass was obtained via whole-body dual-energy X-ray absorptiometry (DXA) scans (Hologic Discovery QDR model Series, Waltham, MA, USA) using the system's software package (Hologic APEX, Version 4.0.2). Participants were scanned at the same time of day pre- and post-intervention and were encouraged to maintain a similar sleeping and eating schedule for both scans. Participants were advised to report to the laboratory in a hydrated state and were given scrubs to wear during the scan. They were also given the opportunity to use the restroom prior to the scan. Care was taken to follow The International Society for Clinical Densitometry

guidelines for positioning during the scan [497]. Total body fat mass was recorded at baseline and post-intervention, and percent change from baseline was used for analysis.

Physical function outcomes.

Six-minute walk distance (6MW). A 6MW distance test was performed in a 30-meter hallway marked off with cones at either end, and distance marked every three meters. The participant started at the end of the hallway (starting cone) and was instructed to walk as quickly as they could for six minutes. They walked towards the end of the hallway, around the second cone, and then back towards the starting cone. The participant completed as many laps as possible within six minutes, rounding the cones each lap. Participants were given feedback on elapsed/remaining time every 30 seconds and were encouraged to continue walking as quickly as possible. Distance covered in six minutes was recorded to the nearest meter at baseline and post-intervention for analysis.

4-square step test (4SST). The 4SST is a test that challenges motor planning and initiation as well as motor sequencing and recall [558,559]. A four-foot by four-foot square was marked with athletic tape and split into quadrants. The participants started in square 1 (bottom left), facing square 2 (top left). The participant then stepped forward into square 2, laterally to square 3 (top right), backwards to square 4 (bottom right), laterally to square 1, laterally to square 4, forwards to square 3, laterally to square 2, and backwards to square 1, facing the same direction throughout the entire sequence. Participants placed both feet in the specified quadrant before they could move into the next square, and had at least one foot on the ground at all times. The test was timed with a stopwatch to the nearest 0.01 seconds. When the participant was ready the investigator

said “ready, set, go”. The timer was started at “Go” and stopped when the participant had placed both feet back in square 1 after completing a clockwise and counter-clockwise cycle. The participant performed two trials with 30 seconds rest between trials, and the fastest was recorded at baseline and post-intervention.

Grip strength. Maximal grip strength was obtained with a Jamar hydraulic grip strength dynamometer (Performance Health, LLC, Akron, OH USA). Handle position of the dynamometer was standardized at position II [560,561]. Hand dominance was determined by asking the participant if they were right-handed or left-handed, and the non-dominant hand was tested. The participant was seated with the shoulder in neutral position and the elbow unsupported and flexed to 90° with the forearm and wrist in neutral position [561]. The participant squeezed the dynamometer handle as hard as possible for 3 seconds. Maximal force was recorded in kg. The participant performed three trials, and 15 seconds rest was allowed between trials. Peak force of the three trials for each hand was recorded at baseline and post-intervention.

Exercise intervention. Each participant performed their prescribed exercise three times per week for 12 weeks. Time between visits was generally 48-72 hours, but some ranged from 24-96 hours to meet the demands of the participant’s schedule. Participants were allowed to exercise on consecutive days one time per week, but were required to have at least 48 hours before a third exercise session was completed (for example, Monday, Tuesday, Thursday was acceptable, but Monday, Tuesday, Wednesday was not). Prior to each exercise session an exercise supervisor performed a brief medical safety check. If the participant reported changes in health status that were concerning,

had abnormal vital signs (blood pressure readings that exceeded 170 mmHg for systolic blood pressure or 100 mmHg for diastolic blood pressure), or had changed medications/dosage, they were not be allowed to continue exercising until the study physician had reviewed the changes and cleared the participant. After the medical safety check, the participant performed a 5-minute warm-up on the stationary bike at a low intensity (i.e., output 50% or less of their maximal output during the GXT). After the participant had completed the prescribed exercise for the day they performed a 5-minute cooldown at a low intensity on the stationary bicycle. For the RT and MICT exercise protocols we employed a pragmatic trial design that followed the recommendations set forth by the ACSM, but that were not necessarily matched by time. There are currently no recommendations for HIIT in older adults, and the exercise sessions were shorter in duration due to the high-intensity nature. Successful adherence was defined as a study participant who achieved at least an 80% attendance rate (i.e., attended 29 of 36 exercise sessions).

High-intensity interval training. HIIT was performed on a stationary bicycle (Peloton Interactive, Inc. New York City, NY, USA) interfaced with a computer monitor that played selected pre-recorded "spin classes". Heart rate and power output were displayed in real time. The progression of the 12-week program went as follows. Heart rate reserve (HRR) was calculated by subtracting the participant's resting heart rate (obtained during medical history check at visit 1) from their maximal heart rate (obtained during the GXT). Participants cycled continuously for 20-30 minutes at 50-60% of their HRR for the first week, and 20-30 minutes at 55-65% of HRR for the second week,

similar to the MICT group. During the subsequent weeks, participants performed bouts of higher intensity cycling (target intensity of 80-100% of their HRR) interspersed with low-intensity rest periods (target intensity of 40-60% of their HRR). Here, the duration of the bout (or interval) ranged from as little as 15 seconds and upwards of 1 minute, and rest periods were matched in a work/rest ratio of 2:1, 1:1, or 1:2 (1:1 on average). During weeks 3-4 the intensity was at 80-95% of their HRR and the overall duration lasted 15-20 minutes. Weeks 5-8 the intensity was at 80-100% of their HRR with a duration of 15-30 minutes. During weeks 9-12 intensity was at 85-100% of their HRR and duration lasted 20-30 minutes. When the participant began week three, the target average output was determined by multiplying the participant's best average output from the first two weeks by 1.2. The participant cycled at the target average output for the first 2-3 minutes of each session, at 150-300% of their target average output during the high-intensity intervals, and at 25-50% of their target average output during their low-intensity intervals in order to achieve the target heart rate ranges. The target average output for each subsequent week of exercise sessions was the highest average output from the previous week. If there was no increase from the previous week, target average output was manually increased by 3%. Participants completed 15, 20, or 30-minute sessions throughout the study (Table 5.4) and began each session by cycling at the target average output for 2-4 minutes, followed by several high- and low-intensity intervals. Participants cycled at a high intensity for ~6 total minutes during 15-minute sessions, ~8 total minutes during 20-minute sessions, and ~10 total minutes during 30-minute sessions, maintaining an average work/rest ratio of 1:1. The participant followed the cadence recommendations

(ranging 50-100 RPMs) of the spin class instructor while the in-person exercise supervisor modified the resistance to ensure the output was maintained within the target ranges. It was uncommon for the participant's heart rate to reach 40-60% of HRR during the rest intervals, however, an output that was 25-50% of the target average output would typically elicit a heart rate that was 40-60% of HRR under normal conditions. Therefore, rest intervals were long enough that the participant's heart rate decreased by 5-20 beats per minute prior to the start of the next high-intensity interval. At the end of each exercise session participants were asked their perceived effort level for the entire session on a 1-10 scale and a Borg 6-20 scale.

Moderate-intensity continuous training. Participants in the MICT group used the same stationary bicycle setup as in the HIIT group. The goal of MICT was to maintain an output that elicited the prescribed heart rate throughout the exercise session. The progression of the 12-week program went as follows. For the first week participants cycled for 20-30 minutes at 50-60% of their HRR. Week 2 participants cycled for 20-30 minutes at 55-65% of their HRR. Weeks 3-4 participants cycled for 30 minutes at 60-70% of their HRR. Weeks 5-8 participants cycled for 30-45 minutes at 65-75% HRR, and in weeks 9-12 participants cycled for 45 minutes at 70-75% HRR. These ranges were based on recommendations from a meta-analysis describing a dose-response relationship between exercise intensity and VO_2max adaptations in older adults [562]. An exercise supervisor oversaw each exercise session. The target output was determined during the first exercise session by having the participant cycle for five minutes at a cadence of 75 RPMs with a resistance that produced 50 W. Once a consistent heart rate was established

(using a chest-strap heart rate monitor), we increased or decreased the resistance until the target heart rate was maintained. The target output for each subsequent session was the highest average output from the previous week. If there was no increase from the previous week, target average output was manually increased by 3%. If the participant was maintaining the target output but was not achieving the target heart rate, output was increased incrementally by 2-5 W until the heart rate was maintained in the target range. During each exercise session the participant followed the cadence recommendations of the spin class instructor (ranging 50-100 RPMs) while the in-person exercise supervisor modified the resistance to ensure the output was maintained within the target range. At times the participant was cycling at the target output but had a heart rate that exceeded the target. In these cases, heart rate range took precedence over output range, and output was decreased until the target heart rate was maintained. This sometimes occurred when cycling at higher cadences (i.e., 90-100 RPMs). At the end of each exercise session participants were asked their perceived effort level for the entire session on a 1-10 scale and a Borg 6-20 scale. See Table 5.4 for exercise duration of each session.

Resistance training. Participants performed 10 resistance exercises for the major muscle groups (Table 5.5). The training program was lower extremity-focused, with 70% of exercises isolating the lower extremities. For the first two weeks participants performed 1-2 sets of 15 repetitions for each of the ten exercises, using a weight that elicited an RPE at the end of the final repetitions of the respective sets of 5-6 (0-10 scale) as reported by the participant. Rest between sets/exercises ranged from 30-60 seconds. Weeks 3-4 participants performed 2-3 sets of 12-20 repetitions at an RPE of 5-8 with 60-

90 seconds rest between sets. Weeks 5-8 participants performed 3-4 sets of 10-20 repetitions at an RPE of 6-8 with 60-90 seconds rest between sets. Weeks 9-12 participants performed 3-5 sets of 6-20 repetitions at an RPE of 7-8 with 60-90 seconds rest between sets. Contraction velocity for each exercise was moderate (180-240°/sec), with duration lasting approximately two seconds for concentric actions and two seconds for eccentric actions [563]. This protocol met the ACSM resistance training recommendations for older adults [23], and progressed from entry-level to a more demanding protocol. Duration of each session lasted ~45-75 minutes. Participants were occasionally asked their perceived effort level on 1-10 scale after individual exercise sets to ensure that they were exercising at the prescribed intensity.

Concomitant exercise and diet. Some participants were participating in some low-volume physical activity prior to study enrollment without meeting the current recommendations (e.g., yoga, yard work). To control for this, all participants were encouraged to continue normal activity outside of the study throughout their enrollment in the study. Similarly, study participants were asked to maintain their normal diet throughout the study.

Statistical analysis. The planned analysis for this study was to compare differences in group means using one-way ANOVA. However, because we were unable to complete the study due to COVID 19 our sample size was not adequately powered for this type of analysis. Therefore, descriptive statistics, percent change from baseline (primary and secondary outcomes), absolute change from baseline (Table 5.6), and corrected Hedge's g effect sizes for small samples are reported [564]. Effects sizes were classified as very small

(0.01-0.19), small (0.20-0.49), moderate (0.5-0.79), large (0.8-1.19), and very large (>1.20) [565]. 95% confidence intervals for descriptive statistics can be found in Table 5.7.

Results

Adverse events. Adverse events (AEs) were defined as an unexpected medical problem that happened during the course of the study related or unrelated to the intervention or assessments. All AEs occurring after informed consent was signed and until study completion were recorded. A serious adverse event (SAE) was defined as an unexpected medical problem that was believed by the investigators to be causally related to the study intervention or assessments and resulted in any of the following: a life-threatening condition, severe or permanent disability, prolonged hospitalization, or death. During the course of the study nine AEs were reported, two of which occurred during testing procedures and seven occurred during the exercise intervention. No SAEs were reported during the study. The first AE that occurred during testing was due to an equipment malfunction that resulted in prolonged, high-frequency electrical stimulation of the quadriceps muscle of one participant for approximately 4 seconds during the baseline testing Visit 3. The participant reported pain during the stimulation, but no pain 5 minutes after the incident or anytime thereafter and was able to finish all assessments, with the exception of further electrical stimulation. The second AE that occurred during testing was an abnormal heart rhythm that was detected by the exercise physiologist who was monitoring the ECG during the graded exercise test. The test was stopped, and the heart rhythm returned to normal when the heart rate approached resting levels. The participant reported no symptoms during the test or afterward, and followed up with their

primary care physician following the event. This participant was removed from the study. Both of the AEs that occurred during testing were reported to the Ohio University IRB. Of the remaining seven AEs, only one was likely related to the intervention (one participant in the RT group reported mild muscle soreness for ~24 hours after an exercise session), and all were classified as *Mild*. The remaining six AEs included respiratory illness (4), knee injury occurring at work (1), and a foot injury occurring at home (1).

Adherence. All 14 participants met the minimal adherence standard of 80%, and adherence was not different between groups. The HIIT group attended 93.9% of exercise sessions (range: 86-100%), the MICT group attended 92.4% of exercise sessions (range: 89-100%), and the RT group attended 93.4% of exercise session (range: 89-97%). The one-on-one supervised training sessions ensured that target training intensities were met each session.

Stationary bicycle total output. Total output during the 12-week exercise intervention was recorded for the HIIT and MICT groups. During the first two weeks of the program both groups performed the same moderate-intensity exercise, and as such output from the first two weeks was excluded from analysis. From weeks 3-12 the HIIT group produced 94.79 ± 14.56 kJ/session, while the MICT group produced 210.83 ± 46.01 kJ/session, on average. Output from the HIIT group was 45% of the output from the MICT group.

Primary outcomes. RT had a moderate effect on knee extensor isokinetic strength (+12.0 ± 23.1%, ES = 0.56), while HIIT and MICT had very small effects (-0.2 ± 5.6%, ES = -0.01; and +7.2 ± 8.1%, ES = 0.11, respectively) (Figure 5.3A). HIIT and RT

had small effects on VO_2max (+10.3 ± 9.2%, ES = 0.44; and +9.9 ± 6.1%, ES = 0.41, respectively), while MICT had a very small effect (+6.5 ± 10.6%, ES = 0.16) (Figure 5.3B). MICT and RT had small effects on quadriceps muscle volume (+10.8 ± 1.2, ES = 0.21; and +7.3 ± 7.6%, ES = 0.28, respectively), while HIIT had a very small effect (+4.3 ± 8.7%, ES = 0.19) (Figure 5.3C).

Secondary outcomes. RT had a large effect on knee extensor isometric strength (+26.2 ± 18.2%, ES = 0.99), while HIIT and MICT had very small, negative effects (-5.7 ± 18.6%, ES = -0.17; and -2.1 ± 6.6%, ES = -0.04, respectively) (Figure 5.3D). HIIT and MICT had large effects on fatigue resistance (+9.6 ± 6.6%, ES = 1.13; and +10.8 ± 11.1%, ES = 0.90, respectively), while RT had a small effect (+5.6 ± 7.1%, ES = 0.39) (Figure 5.3E). HIIT, MICT, and RT had very small effects on fat mass (-0.6 ± 5.3%, ES = 0.02; -0.8 ± 2.1%, ES = 0.02; and -4.7 ± 8.2%, ES = 0.14, respectively) (Figure 5.3F).

Physical function outcomes. HIIT had a large effect on 6MW distance (+43.2 ± 13.3 m, ES = 1.08), while MICT and RT had small effects (+13.3 ± 12.7 m, ES = 0.20; and +27.8 ± 21.7 m, ES = 0.39, respectively) (Figure 5.3G). MICT had a moderate effect on 4SST time (-0.74 ± 0.52 s, ES = 0.65), RT had a small effect (-0.74 ± 0.81 s, ES = 0.32), and HIIT had a very small, negative effect (+0.02 ± 0.63 s, ES = -0.02) (Figure 5.3H). HIIT had a moderate effect on non-dominant grip strength (+2.00 ± 3.84 kg, ES = 0.51), while MICT and RT had very small effects (+1.38 ± 3.12 kg, ES = 0.17; and +1.30 ± 2.36 kg, ES = 0.12, respectively) (Figure 5.3I).

Discussion

The purpose of this study was to determine the effect of stationary bicycle HIIT on cardiorespiratory/endurance and muscular measures, as well as physical function adaptations, compared to MICT or RT in a generally healthy but insufficiently active older adult population. Though terminated early due to COVID-19 restrictions, the diverse data that we were able to collect allowed us to calculate effect sizes for future investigations. First, HIIT had a greater effect on VO_2max than MICT (ES = 0.44 and 0.16, respectively), and a similar large effect on fatigue resistance (ES = 1.13 and 0.90, respectively). MICT has long been promoted as an essential element in healthy aging [291], and it is becoming more and more clear that HIIT is also a safe aerobic exercise regimen that is highly effective at improving cardiac, respiratory, and metabolic function in an older adult population [376,566]. In our study, there was potential for participants to have negative expectations of MICT relative to HIIT if they believed that HIIT was the experimental group and MICT was the control group, and therefore less effective. However, during the informed consent process we framed the study as an exploratory investigation with the goal of determining which exercise strategy had the greatest effect on overall health and function. In so doing, none of the exercise strategies were described in a way that could be interpreted as preferential, although we could not control for the participants' preexisting beliefs.

A somewhat unexpected finding of this study, however, was the effect of RT on VO_2max. The benefits of aerobic and resistance training have historically been considered independent of each other, and as such there has been relatively little attention

given to the effects of RT on cardiorespiratory variables [30]. The small effect of RT on fatigue resistance seen in our study suggests that there may be more crossover benefits on top of the well-accepted improvements in strength associated with RT, particularly for older adults. RT has a greater effect on VO_2max in older adults than young adults [30], which may be related to their higher responsiveness to RT-induced mitochondrial adaptations [567]. It should be noted that none of the exercise interventions had substantial effects on fat mass. However, as our subjects were asked to maintain their standard diet for the duration of the study a substantial reduction in fat mass was not expected, based on a previous report indicating that exercise-induced weight loss is minimal without the addition of caloric restriction [306].

Skeletal muscle is the primary target for insulin-mediated glucose uptake [487], and exercise that increases muscle mass also improve insulin sensitivity and glycogen synthase activity [568]. These improvements cannot be explained by increase in muscle mass alone, however, indicating that muscular contraction also improves skeletal muscle glucose uptake [568]. This contraction-mediated glucose uptake is stimulated through separate signaling cascades than those induced by insulin [569]. Further, different exercise modalities can have diverse chronic effects on both insulin- and contraction-mediated glucose uptake. For example, HIIT has a greater effect than MICT on whole body insulin sensitivity and insulin-mediated glucose uptake in obese and non-obese elderly individuals [376,568,570,571], while RT has similar, but blunted effects [568]. However, recent work indicates that these differing effects may be largely due to volume and intensity of exercise, and not necessarily a specific modality [572]. That is, both

higher intensity and higher volume of exercise independently enhance contraction-mediated glucose uptake adaptations [572]. This may be related to the greater activation of type II muscle fibers under these conditions. For example, HIIT can fully activate type II fibers, and can therefore induce mitochondrial biogenesis and improve oxidative capacity in both type I and type II fibers [573]. In contrast, MICT only activates a portion of type II fibers, resulting in a blunted metabolic response relative to HIIT [574]. In addition, type II fibers are similarly activated with RT using heavy (i.e., 80% of 1RM) and light (i.e., 30% of 1RM) loads if each set is performed to failure [575]. Considering the detrimental effect aging has on type II fibers [154], exercise strategies that target these fibers (i.e., HIIT, RT) would likely have the greatest effect on muscular health, including strength, function, and glucose uptake. Indeed, our results support this notion.

Stationary bicycling is an ideal form of aerobic exercise for older adults due to its effectiveness at inducing cardiorespiratory adaptations and the relative low risk of injury [576], and it has also been shown to elicit strength improvements in older adults in response to MICT [310,312] and HIIT [383]. We expected a similar response to our cycling protocols, however, our low-volume bicycle HIIT protocol had a very small effect on muscular strength and size at the group level. There was a diverse response to HIIT at the individual level, wherein some participants showed substantial increases while others demonstrated substantial declines in muscle strength and size (Figure 5.3). It is unclear why our cycling protocols did not consistently result in improved strength, as has been reported previously [310–312,383], although there are several methodological factors that may affect muscular adaptations (e.g., resistance, cadence). Theoretically, a

low cadence, high resistance protocol would be more likely to elicit strength adaptations than a high cadence, low resistance protocol [577]. However, the effect of training cadence on maximal strength is rarely measured, and, to our knowledge, has only been reported in trained cyclists [578,579]. Consequently, the ideal cadence/resistance combination to induce muscular changes in older adults is unclear and should be investigated.

Due to the relatively recent interest in HIIT for older adults there are few studies reporting effects on physical function measures, though those that do appear to indicate beneficial effects [383,385–388]. This proof-of-concept pilot study demonstrates that HIIT had a large effect on 6MW distance and a moderate effect on grip strength. A change in 6MW of 14-30 meters is considered clinically important in a variety of patient populations, including in older adults with a fear of falling [580]. The fact that 12 weeks of HIIT improved 6MW by 42 meters, on average, is encouraging, particularly considering our participants were free of overt physical function limitations prior to enrollment. This may translate into substantial improvements in physical function capacity in mobility-limited older adults, and future work should investigate this possibility.

Conclusion. Individuals who perform both endurance and muscle strengthening exercise can delay cardiorespiratory, muscular, and physical function decline, however, most older adults who do exercise only perform one type of exercise. HIIT is a time-efficient exercise strategy that has the potential to produce both cardiorespiratory and muscular improvements, but few groups have investigated this potential. Our low-volume

HIIT protocol did not consistently induce muscular adaptations but improved cardiorespiratory/endurance and physical function measures comparable to MICT with half of the time commitment. Additionally, an unexpected finding of our study was that RT had small-to-moderate effects on cardiovascular/endurance measures along with the expected improvements in muscular strength, suggesting that it may be considered a "dual-benefit" exercise strategy. Although a preliminary study, this is, to our knowledge, the first randomized controlled trial to directly compare muscular and cardiorespiratory adaptations of bicycle HIIT to adaptations seen in response to traditional MICT or RT in generally healthy older adults. Future work should include strength and physical function measures to better characterize the adaptations to HIIT in order to determine if it is an effective and efficient exercise strategy for healthy and mobility-limited older adults.

Chapter 6: Conclusions and Future Directions

A portion of this chapter has been published in *Frontiers in Physiology* [30].

Conclusions

This dissertation was designed for me to develop proficiency in three distinct areas related to the systematic assessment of exercise-induced and/or age-related neuromuscular adaptations. In Experiment 1 I challenged the recommendation that dual-energy X-ray absorptiometry (DXA) should be used as the reference standard for quantifying exercise-induced changes in muscle size by comparing DXA-derived measures against those derived from magnetic resonance imaging (MRI), the gold-standard. In Experiment 2 I evaluated and standardized a technique for detecting neural deficits in voluntary rate of force development (RFD). In Experiment 3 I developed and implemented a proof-of-concept, proof-of-mechanism clinical trial to assess the effectiveness of high-intensity interval training (HIIT) in an older adult population, relative to moderate-intensity continuous training (MICT) and resistance training (RT). A summary of the findings, and the conclusions drawn from these experiments, are discussed in the following sections.

Experiment 1: Accurate assessment of exercise-induced changes in muscle size. The change in skeletal muscle size is a primary outcome in many clinical trials due to its relationship with disease progression and physical function [490–492]. DXA is a relatively inexpensive and simple method of lean mass quantification, and DXA-derived values are strongly associated with computed tomography (CT) and MRI-derived values when assessed cross-sectionally (r = 0.86-0.97) [27,28,426–428]. As such, DXA has

recently been recommended as the reference standard for the measurement of muscle mass by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis working group on frailty and sarcopenia [26]. Specifically, this group recommended DXA be widely used in order to increase comparability of studies and to improve diagnosis of sarcopenia, as well as to monitor development of muscle mass in healthy, athletic, and sick subjects [26]. However, these recommendations are based solely on cross-sectional assessments on the association of DXA with MRI or CT, and DXA reliability studies. Thus, the capability of DXA to accurately assess changes in muscle size in response to exercise and/or disease is not addressed. The purpose of Experiment 1 was to determine the level of agreement between MRI and DXA-derived measures of muscle size, both cross-sectionally and longitudinally, where resistance exercise served as a stimulus for skeletal muscle adaptation. To achieve this goal, we measured thigh muscle size with DXA and MRI in 26 adults (29.2 ± 9.5 years) before and after 10 weeks of low-load resistance exercise.

The results of this study matched previous cross-sectional investigations reporting strong positive correlations between DXA and MRI-derived measures of muscle size. We found that DXA and MRI measures were strongly associated, both at baseline ($r = 0.89$) and after the 10-week exercise intervention ($r = 0.90$). Considering the strength of these associations at both time points, it would be reasonable to assume that exercise-induced changes in muscle size would also be strongly associated. However, we found that the strength of the relationship was much lower ($r = 0.49$), and that less than half of the scans agreed within 3%. In addition, we found that DXA displayed an average error of 4.2%,

and a maximum error of 13.8%. There is currently no consensus on the minimal clinically important difference for change in muscle mass [581], though we have proposed a change of 3% to be experimentally relevant [29]. With this in mind, we concluded that DXA is not an accurate tool for quantifying modest changes in muscle size, such as what would be expected in response to exercise and/or other therapeutic approaches for treating conditions like sarcopenia. This has broad implications in the context of both health and disease. First, our findings call into question the results of previous studies that have used DXA to report muscular adaptations. The risk of DXA measurement error is exemplified by previous studies demonstrating changes in muscle size when measured by MRI or CT, but not by DXA [429,431,433]. Second, our findings suggest that DXA would be unable to detect clinically meaningful disease-induced changes in muscle size (e.g., muscle wasting) with accuracy. Particularly concerning is the fact that several of our subjects displayed increases in muscle size when assessed with DXA yet had decreased muscle size when assessed with MRI, underscoring the potential danger of monitoring disease status using DXA. That is, using DXA to assess the efficacy of an intervention to reverse muscle wasting could lead to the continuation of an ineffective treatment, or cessation of an effective treatment. Finally, our findings do not contradict previous work indicating that DXA is useful tool for cross-sectional assessment of muscle size. There are several benefits to using DXA over MRI or CT to assess muscle size, including low cost, accessibility, ease of use, and minimal time required for analysis. In this context, DXA can be an effective tool for the diagnosis of muscle wasting conditions, as well as the

characterization of unique clinical populations. However, its use as a tool to monitor the development of muscle mass in health and disease should not be recommended.

Experiment 2: Standardization of a novel method to assess neural deficits in RFD. The capability to rapidly produce force is related to physical performance in both healthy and diseased populations. Ballistic movement is regulated by both muscular and neural mechanisms, but the relative contribution of each is not well understood. Characterizing these contributions is an essential step in developing targeted approaches for improving physical function and optimizing athletic performance. The majority of studies that have investigated the neural mechanisms of ballistic RFD have used surface electromyography (EMG) to quantify neural drive. However, there are several physiological and non-physiological variables that can influence the interference EMG signal during collection and analysis that limit its usefulness [8], which has ultimately led to equivocal reports in the literature. An alternative approach for quantifying the neural contributions to ballistic RFD is by leveraging electrically evoked contractions. If one were to assume that every variable that influences RFD can be categorized as either neural or muscular, then by defining the muscular contributions one can calculate to what extent the neural system limits RFD. Thus, the purpose of Experiment 2 was to evaluate this theory by comparing electrically evoked RFD (believed to represent the muscular contribution) to voluntary RFD (comprised of both neural and muscular contributions). In so doing we also developed a standardized methodology for this technique that is simple, non-invasive, and not bound by the same limitations as EMG. We calculated voluntary RFD at sequential 50 ms intervals from force onset to 200 ms, and expressed those values

relative to electrically evoked peak RFD (which we termed the Central Activation Ballistic (CAB) Force Ratio) in 20 young adults (22.0 ± 1.7 years) and 16 older adults (72.3 ± 7.5 years) to determine if age-related neural deficits in RFD could be detected with this novel method. Additionally, Pearson's bivariate correlations were performed between CAB, RFD, and EMG to determine the degree to which these variables were related.

Our findings indicate that, relative to young adults, older adults had a 10% neural deficit in ballistic RFD from 100-150 ms, as determined by the CAB Force Ratio. There were no significant differences in EMG measures at any time point between young and older adults. EMG is a simple and inexpensive tool that is widely used in research and clinical settings. However, because it takes very little training to obtain EMG data, many investigators who use this technology do not have an adequate appreciation for its limitations. As such, there is a risk of misinterpretation of results and/or using EMG in ways for which it has not been validated [8]. This, paired with the large amount of between-subject variability in EMG output, can partially explain the equivocal results regarding age-related differences in EMG amplitude (or the lack thereof). Our data indicates that EMG is related to RFD in the very early phase of the force-time curve, but the two measure dissociate after that point. As a result, it appears that EMG is not a reliable indicator of neural drive after 50 ms during ballistic efforts, making it difficult to characterize the neural contribution to RFD in later phases. The CAB Force Ratio is not bound by the same limitations as EMG, and was associated with RFD at all time points.

These results demonstrate that the CAB Force Ratio has both discriminative and associative validity regarding age-related impairments in neural drive during ballistic efforts. In contrast, surface EMG measures were unable to discriminate between young and older adults, and were only associated with RFD in the very early phase of the force time curve. Our understanding of RFD as a predictor of physical function is still emerging. It has been reported that strength and power athletes have higher RFD values from 0-50 ms time window, compared to untrained individuals [404,525]. However, it remains unclear which time window throughout the force-time curve is most relevant in aging populations and/or those with overt health/disease conditions. Our findings suggest that the 100-150 ms time window is a critical determinant of age-related musculoskeletal function decline, and one that deserves further investigation. Upcoming work related to this finding will be discussed in the *Future Directions* subsection.

Experiment 3: Optimization of exercise strategies to improve physical function and overall health in older adults. National exercise guidelines recommend performing both aerobic and muscle strengthening exercise for optimal health benefits [290]. However, exercise participation is low, particularly in the older adult population. HIIT is an exercise strategy that has potential to induce beneficial health adaptations with a fraction of the training volume required for optimal benefits from MICT. In addition, HIIT can improve lower extremity muscle strength when performed on a stationary bicycle, making it an appealing option as a standalone exercise strategy for older adults. However, the potential of HIIT has not been adequately studied with the appropriate measurement techniques. Experiment 3 represents the first investigation into the

cardiorespiratory and muscular adaptations induced by HIIT in older adults, relative to the adaptations seen in response to MICT or RT. 14 older adults (66.4 ± 3.9 years) were randomized to complete a 12-week exercise program consisting of HIIT on a stationary bicycle (N = 5), MICT on a stationary bicycle (N = 4), or RT using free weights and machines (N = 5).

Our findings indicate that HIIT and MICT are equally effective at inducing traditional aerobic adaptations, but have a limited effect on muscular adaptations. In contrast, 12 weeks of RT induced both muscular and cardiorespiratory adaptations, suggesting that RT may be an effective standalone exercise strategy for maximizing health benefits in older adults. Our findings contradict previous reports of increased strength in response to stationary bicycle exercise [310,312,383]. However, we used a low-volume HIIT protocol with relatively short interval durations, which may have impacted the muscular response. Despite this lack of effect, HIIT appears to have substantial effects on clinically relevant physical function measures, and may appeal to older adults as a time-efficient exercise strategy. The implications of our results on future investigations will be discussed in the next section.

Resistance training results in beneficial musculoskeletal adaptations that are not consistently seen in response to MICT, which is the primary reason for their inclusion in the current guidelines for the general population [290]. An emphasis on RT is even more critical for older adults because of the higher risk of sarcopenia and loss of independence in this population [582]. There remains a widespread belief that the benefits of MICT and RT are independent of one another, however, there is mounting evidence that RT is just

as beneficial as MICT at mitigating chronic diseases highly prevalent in the aging population. There are several barriers to physical activity in older adults that can be targeted to increase participation (e.g., time, cost, disinterest, ongoing pain or illness, fear of injury, and feeling too old [583–585]). Additional barriers specific to RT include perceived complexity of RT programs, lack of knowledge, and lack of age-appropriate programs [24,585,586]. Addressing these barriers will be a necessary step in achieving widespread adherence to muscle strengthening guidelines. In contrast, the guidelines for MICT are simplistic and can be met without specialized equipment or training (e.g., walking), though walking activity is affected by seasonal changes [587]. Presumably this is linked to why a greater proportion of older adults in the United States report meeting the MICT guidelines than the muscle strengthening guidelines (39% and 18%, respectively [292]). Interestingly, 71% of older adults who report meeting muscle strengthening guidelines also meet the MICT guidelines, whereas only 33% of older adults who report meeting MICT guidelines also meet muscle strengthening guidelines [292], suggesting that emphasizing RT for older adults may indirectly result in greater MICT participation. Considering the fact that 87% of older adults report either not exercising regularly or only meeting the guidelines for one type of exercise [292], implementing a new approach to increasing exercise participation is necessary. HIIT also has potential to improve musculoskeletal health in older adults, though our understanding of this capability is still emerging.

Additionally, self-isolation due to the ongoing COVID-19 pandemic likely has detrimental effects on physical activity [588], making this a critical time to leverage at-

home exercise programs [589]. Specifically, the creation of simple, age-appropriate, and educational RT programs that are easily accessible would address existing barriers to widespread adherence, protect older adults from exposure by not requiring gym attendance, and reduce sedentary behavior associated with self-isolation. Promoting RT as the exercise type with the greatest overall effect on health is a reasonable strategy, particularly for older adults. Of primary importance is overcoming the widespread belief that the benefits of MICT and RT are independent of one another (Figure 6.1).

Future Directions

Numerous ideas for future studies can stem from the work reported in this dissertation. In the following subsections I provide perspectives on future direction for studies regarding RFD and the CAB Force Ratio, as well as potential modifications to previous HIIT and MICT stationary bicycling protocols that have the potential to induce muscular adaptations to a greater extent than what was seen in the DART study.

RFD and the CAB Force Ratio. The work presented in Chapter 4 represents one of the pioneering efforts to assess the neural contribution to RFD without the need for surface EMG. Our data was obtained in a small sample of young and older adults, which may limit the generalizability of the results as it should not be assumed that all older adults age at the same rate. Thus, future work should separate older adults into sub-groups, not based on their chronological age, but instead based on their biological age (i.e., the phenotypic expression of age-related biological dysregulation). A relatively simple method of categorization would be to use the knee extension strength cutpoints established by Manini et al. [236] to identify older adults who are at risk of developing

future mobility limitations. Once categorized, the relationships between the CAB Force Ratio and physical function/mobility could be examined. The results of such a study would strengthen our initial findings regarding neural deficits from 100-150 ms of the force-time curve and lend construct validity to this novel method. In addition, the effect of exercise on the CAB Force Ratio could be extensively explored. In our initial study, older adults completed 12 weeks of resistance exercise, two days per week. However, the exercises employed did not have a power component, and substantial improvements in RFD were not necessarily expected. Indeed, we did not see significant changes in RFD, EMG, or CAB measures at the end of the intervention. However, there was a moderate-to-strong relationship between the exercise-induced changes in these measures. As such, the CAB Force Ratio may also be relevant as a predictor of performance in an athletic population, and future work should investigate this possibility.

Cadence, resistance, and interval duration during stationary bicycling. Stationary bicycling is an ideal form of aerobic exercise because risk of injury is relatively low, even when exercising at high intensities (e.g., compare to high-intensity walking/running wherein fall-induced injuries are a concern). In addition, muscle hypertrophy and strength improvements have been reported in response to MICT and HIIT in older adults [310,312,383]. However, stationary bicycling does not always result in substantial muscular adaptations [316], as was apparent from our results. In Chapter 5 we raised the possibility that this could be due to the combination of cadence and resistance required to achieve a certain output. For example, a cadence of 100 RPMs and a resistance of 2 kg would produce the same work output as a cadence of 50 RPMs and a

resistance of 4 kg. However, the extent to which these two protocols would rely on the muscular and cardiorespiratory systems would theoretically be different, resulting in divergent adaptations. The effect of cadence/resistance combinations on maximal performance has been investigated (briefly) in elite cyclists [578,579], but not in recreational athletes or older adults. Because of the widespread belief that the benefits of aerobic and resistance exercise are independent of one another, few groups assess muscular adaptations in response to aerobic exercise interventions. Thus, characterization of exercise protocol modifications that increase the muscular response to stationary bicycling represents an area previously unexplored in aging research.

There are a variety of HIIT protocols that have been reported in the literature, typically falling into one of three categories: sprint interval training (SIT), low-volume HIIT, and high-volume HIIT [374]. SIT is characterized by short, high-intensity efforts (10-30 seconds) with longer breaks in between efforts. In contrast, both low-volume and high-volume HIIT consist of longer-duration intervals (ranging from 15 seconds to 4 minutes), wherein the work/rest ratio is 1:2, 1:1, or 2:1. The difference between these two protocols is the total duration of high-intensity effort per session. That is, a session with less than 16 minutes of total high-intensity effort is classified as low-volume, and sessions with more than 16 minutes is classified as high-volume. There has been a recent spike in interest regarding time-efficient exercise strategies for older adults, with HIIT being one such strategy [590]. However, because this avenue of research is so new there is no consensus on the most effective interval durations, work/rest ratios, intensity thresholds, or total high-intensity volume. Should all of the intervals be of the same

duration for the entire session (e.g., 60 seconds) or should they be randomized within a range (e.g., 15-60 seconds)? Is it better to perform short, extremely high efforts with an equally short rest period between intervals, or should the rest be long enough to allow adequate recovery? These questions represent the tip of the iceberg for potential HIIT investigations. Considering what little is known regarding the effect of cadence and resistance combinations, as previously discussed, there is an almost unlimited potential for investigations into the effectiveness of stationary bicycling as an essential exercise strategy for older adults.

References

1. FIFoA-R. Federal Interagency Forum on Aging-Related Statistics. Older Americans 2016: key indicators of well being. Washington DC: US Government Printing Office; 2016.

2. NORC at the University of Chicago. Perceptions of aging during each decade of life after 30. 2017;10.

3. NIA. Fiscal Year 2019 Budget [Internet]. National Institute on Aging; 2020.

4. Sekar K. National Institutes of Health (NIH) Funding: FY1995-FY2021 [Internet]. National Institutes of Health; 2020 p. 19. Report No.: R43341.

5. Glasziou P, Altman DG, Bossuyt P, Boutron I, Clarke M, Julious S, et al. Reducing waste from incomplete or unusable reports of biomedical research. The Lancet. 2014;383(9913):267–76. doi:10.1016/S0140-6736(13)62228-X

6. Begley CG, Ioannidis JPA. Reproducibility in science: Improving the standard for basic and preclinical research. Circ Res. 2015;116(1):116–26. doi:10.1161/CIRCRESAHA.114.303819

7. Pusztai L, Hatzis C, Andre F. Reproducibility of research and preclinical validation: Problems and solutions. Nat Rev Clin Oncol. 2013;10(12):720–4. doi:10.1038/nrclinonc.2013.171

8. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. J Appl Physiol. 2004;96(4):1486–95. doi:10.1152/japplphysiol.01070.2003

9. Maffiuletti NA, Aagaard P, Blazevich AJ, Folland J, Tillin N, Duchateau J. Rate of force development: physiological and methodological considerations. Eur J Appl Physiol. 2016;116(6):1091–116. doi:10.1007/s00421-016-3346-6

10. Barnouin Y, Butler-Browne G, Moraux A, Reversat D, Leroux G, Béhin A, et al. Comparison of different methods to estimate the volume of the quadriceps femoris muscles using MRI. J Med Imaging Health Inform. 2015;5(6):1201–7. doi:10.1166/jmihi.2015.1506

11. Lee D, Artero EG, Xuemei Sui, Blair SN. Review: Mortality trends in the general population: the importance of cardiorespiratory fitness. J Psychopharmacol (Oxf). 2010;24(4_suppl):27–35. doi:10.1177/1359786810382057

12. Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. J Gerontol A Biol Sci Med Sci. 2005;60(3):324–33. doi:10.1093/gerona/60.3.324

13. Anton SD, Woods AJ, Ashizawa T, Barb D, Buford TW, Carter CS, et al. Successful aging: Advancing the science of physical independence in older adults. Ageing Res Rev. 2015;24:304–27. doi:10.1016/j.arr.2015.09.005

14. Sun F, Norman IJ, While AE. Physical activity in older people: A systematic review. BMC Public Health. 2013;13(449). doi:10.1186/1471-2458-13-449

15. Keadle SK, McKinnon R, Graubard BI, Troiano RP. Prevalence and trends in physical activity among older adults in the United States: A comparison across three national surveys. Prev Med. 2016;89:37–43. doi:10.1016/j.ypmed.2016.05.009

16. DiPietro L. Physical activity in aging: changes in patterns and their relationship to health and function. J Gerontol A Biol Sci Med Sci. 2001;56(Supplement 2):13–22. doi:10.1093/gerona/56.suppl_2.13

17. Davidson L, Hudson R, Kilpatrick K, Kuk J, McMillan K, Janiszewski P, et al. Effects of exercise modality on insulin resistance and functional limitation in Older adults: A randomized controlled trial. Arch Intern Med. 2009;169(2):122–31. doi:10.1016/S1073-5437(10)79513-9

18. Mangione KK, Miller AH, Naughton IV. Cochrane Review: Improving physical function and performance with progressive resistance strength training in older adults. Phys Ther. 2010;90(12):1711–5. doi:10.2522/ptj.20100270

19. Solberg PA, Kvamme NH, Raastad T, Ommundsen Y, Tomten SE, Halvari H, et al. Effects of different types of exercise on muscle mass, strength, function and well-being in elderly. Eur J Sport Sci. 2013;13(1):112–25. doi:10.1080/17461391.2011.617391

20. Cadore EL, Casas-Herrero A, Zambom-Ferraresi F, Idoate F, Millor N, Gómez M, et al. Multicomponent exercises including muscle power training enhance muscle mass, power output, and functional outcomes in institutionalized frail nonagenarians. AGE. 2014;36(2):773–85. doi:10.1007/s11357-013-9586-z

21. Serra-Rexach JA, Bustamante-Ara N, Hierro Villarán M, González Gil P, Sanz Ibáñez MJ, Blanco Sanz N, et al. Short-term, light- to moderate-intensity exercise training improves leg muscle strength in the oldest old: a randomized controlled trial. J Am Geriatr Soc. 2011;59(4):594–602. doi:10.1111/j.1532-5415.2011.03356.x

22. Billat V, Dhonneur G, Mille-Hamard L, Le Moyec L, Momken I, Launay T, et al. Case Studies in Physiology: Maximal oxygen consumption and performance in a centenarian cyclist. J Appl Physiol. 2017;122(3):430–4. doi:10.1152/japplphysiol.00569.2016

23. Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, Salem GJ, et al. Exercise and physical activity for older adults. Med Sci Sports Exerc. 2009;41(7):1510–30. doi:10.1249/MSS.0b013e3181a0c95c

24. Phillips SM, Winett RA. Uncomplicated resistance training and health-related outcomes: evidence for a public health mandate. Curr Sports Med Rep. 2010;9(4):208–13. doi:10.1249/JSR.0b013e3181e7da73

25. Moreira OC, de Oliveira CEP, Candia-Luján R, Romero-Pérez EM, de Paz Fernandez JA. Métodos de evaluación de la masa muscular: una revisión sistemática de ensayos controlados aleatorios. Methods of evaluation of muscle mass: a systematic review of randomized clinical trials. Nutr Hosp. 2015;32(3):977–85.

26. Buckinx F, Landi F, Cesari M, Fielding RA, Visser M, Engelke K, et al. Pitfalls in the measurement of muscle mass: A need for a reference standard. J Cachexia Sarcopenia Muscle. 2018;9(2):269–78. doi:10.1002/jcsm.12268

27. Wang W, Wang Z, Faith M, Kotler D, Shih R, Heymsfield S. Regional skeletal muscle measurement: evaluation of new dual-energy X-ray absorptiometry model. J Appl Physiol. 1999;87(3):1163–71.

28. Hansen R, Williamson D, Finnegan T, Lloyd B, Grady J, Diamond T, et al. Estimation of thigh muscle cross-sectional area by dual-energy X-ray absorptiometry in frail elderly patients. Am J Clin Nutr. 2007;86:952–8.

29. Tavoian D, Ampomah K, Amano S, Law T, Clark B. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. Sci Rep. 2019;9:10028. doi:10.1038/s41598-019-46428-w

30. Tavoian D, Russ DW, Consitt LA, Clark BC. Perspective: Pragmatic exercise recommendations for older adults: The case for emphasizing resistance training. Front Physiol. 2020;11:799. doi:10.3389/fphys.2020.00799

31. Ward JM, Youssef SA, Treuting PM. Why animals die: An introduction to the pathology of aging. Vet Pathol. 2016 Mar;53(2):229–32. doi:10.1177/0300985815612151

32. Nichols H, Tavella V. What are the leading causes of death in the US? Medical News Today [Internet]. 2019 [cited 2020 Feb 12].

33. Williams G. Pleiotropy, natural selection, and the evolution of senescence. Evolution. 1957;11(4):398–411.

34. Rose MR, Flatt T, Graves JL, Greer LF, Martinez DE, Matos M, et al. What is aging? Front Genet. 2012;3(134):3. doi:10.3389/fgene.2012.00134

35. Jin K. Modern biological theories of aging. Aging Dis. 2010;1(2):72–4.

36. Crimmins EM. Lifespan and healthspan: past, present, and promise. The Gerontologist. 2015 Dec;55(6):901–11. doi:10.1093/geront/gnv130

37. Manini T. Development of physical disability in older adults. Curr Aging Sci. 2011 Dec 1;4(3):184–91. doi:10.2174/1874609811104030184

38. Fulop T, Larbi A, Khalil A, Cohen AA, Witkowski JM. Are we ill because we age? Front Physiol. 2019;10:1508. doi:10.3389/fphys.2019.01508

39. da Costa J, Vitorino R, Silva G, Vogel C, Duarte A, Rocha-Santos T. A synopsis on aging—theories, mechanisms and future prospects. Ageing Res Rev. 2016 Aug;29:90–112. doi:10.1016/j.arr.2016.06.005

40. Kirkwood T, Austad S. Why do we age? Nature. 2000;408:233–8.

41. Shephard R. Aging, physical activity, and health. Human Kinetics; 1997. 488 p.

42. Fabian D, Flatt T. The evolution of aging. Nat Knowl Educ. 2011;3(10):9.

43. Medawar P. Old age and natural death. Mod Q. 1946;1:30–56.

44. Tanner J. Sequence, tempo, and individual variation in the growth and development of boys and girls aged twelve to sixteen. Daedalus. 1971;100(4):907–30.

45. Bathgate KE, Bagley JR, Jo E, Talmadge RJ, Tobias IS, Brown LE, et al. Muscle health and performance in monozygotic twins with 30 years of discordant exercise habits. Eur J Appl Physiol. 2018 Oct;118(10):2097–110. doi:10.1007/s00421-018-3943-7

46. Li Y, Pan A, Wang DD, Liu X, Dhana K, Franco OH, et al. Impact of healthy lifestyle factors on life expectancies in the US population. Circulation. 2018 Jul 24;138(4):345–55. doi:10.1161/CIRCULATIONAHA.117.032047

47. Austad SN. Methusaleh's Zoo: How nature provides us with clues for extending human health span. J Comp Pathol. 2010 Jan;142(S1):S10-21. doi:10.1016/j.jcpa.2009.10.024

48. Pearl R. The rate of living. New York: AA Knopf; 1928.

49. Buffenstein R. Negligible senescence in the longest living rodent, the naked mole-rat: Insights from a successfully aging species. J Comp Physiol B. 2008 May;178(4):439–45. doi:10.1007/s00360-007-0237-5

50. Harman D. Aging: A theory based on free radical and radiation chemistry. J Gerontol. 1956 Jul 1;11(3):298–300. doi:10.1093/geronj/11.3.298

51. Bagchi K, Puri S. Free radicals and antinoxidants in health and disease. Rev Sante Mediterr Orient. 1998;4(2):350–60.

52. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118–26. doi:10.4103/0973-7847.70902

53. Cheeseman KH, Slater TF. An introduction to free radical biochemistry. Br Med Bull. 1993;49(3):481–93. doi:10.1093/oxfordjournals.bmb.a072625

54. Panth N, Paudel KR, Parajuli K. Reactive oxygen species: A key hallmark of cardiovascular disease. Adv Med. 2016;2016:1–12. doi:10.1155/2016/9152732

55. Liou G-Y, Storz P. Reactive oxygen species in cancer. Free Radic Res. 2010 Jan;44(5):479–96. doi:10.3109/10715761003667554

56. Kaneto H, Katakami N, Matsuhisa M, Matsuoka T. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm. 2010;2010:1–11. doi:10.1155/2010/453892

57. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018 Apr;13:757–72. doi:10.2147/CIA.S158513

58. Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Park Dis. 2013;3(4):461–91. doi:10.3233/JPD-130230

59. Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev. 1998 Apr 1;78(2):547–81. doi:10.1152/physrev.1998.78.2.547

60. Austad SN. The comparative biology of mitochondrial function and the rate of aging. Integr Comp Biol. 2018 Sep 1;58(3):559–66. doi:10.1093/icb/icy068

61. Sergiev P, Dontsova O, Bereskin G. Theories of aging: An ever-evolving field. Acta Naturae. 2015 Mar 1;7(1):9–18. doi:10.32607/20758251-2015-7-1-9-18

62. Depczynski M, Bellwood DR. Shortest recorded vertebrate lifespan found in a coral reef fish. Curr Biol. 2005 Apr;15(8):R288-9. doi:10.1016/j.cub.2005.04.016

63. Nielsen J, Hedeholm RB, Heinemeier J, Bushnell PG, Christiansen JS, Olsen J, et al. Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (Somniosus microcephalus). Science. 2016 Aug 12;353(6300):702–4. doi:10.1126/science.aaf1703

64. Dong X, Milholland B, Vijg J. Evidence for a limit to human lifespan. Nature. 2016 Oct;538(7624):257–9. doi:10.1038/nature19793

65. Life expectancy at birth, at age 65, and at age 75, by sex, race, and Hispanic origin: United States, selected years 1900–2016. Trend Tables. 2017;3.

66. Redfield R. CDC Director's media statement on US life expectancy. 2018 [cited 2020 Feb 13].

67. Organization for Economic Cooperation and Development (OECD). Health spending (indicator). 2020; doi:10.1787/8643de7e-en

68. Organization for Economic Cooperation and Development (OECD). Life expectancy at birth (indicator). 2020; doi:10.1787/27e0fc9d-en

69. Kontis V, Bennet J, Ezzati M. Future life expectancy in 35 industrialized countries: Projections with a Bayesian model ensemble. Lancet. 2017;389(10076):1323–35.

70. Ho J, Hendi A. Recent trends in life expectancy across high income countries: Retrospective observational study. Br Med J. 2018;362(k3622):18.

71. Woolf SH, Schoomaker H. Life expectancy and mortality rates in the United States, 1959-2017. JAMA. 2019 Nov 26;322(20):1996–2016. doi:10.1001/jama.2019.16932

72. U.S. health in international perspective: shorter lives, poorer health. Mil Med. 2016 Sep;181(9):945–6. doi:10.7205/MILMED-D-16-00227

73. Organization for Economic Cooperation and Development (OECD). Life expectancy at 65 (indicator). 2020; doi:10.1787/0e9a3f00-en

74. SSA. Benefits planner: Life expectancy. 2019 [cited 2020 Feb 13].

75. United Nations, Department of Economic and Social Affairs, Population Division. World population prospects, 2019, online edition. 2019. Report No.: Rev. 1.

76. Vijg J, Suh Y. Genetics of longevity and aging. Annu Rev Med. 2005 Feb;56(1):193–212. doi:10.1146/annurev.med.56.082103.104617

77. Austad SN. Why women live longer than men: Sex differences in longevity. Gend Med. 2006 Jun;3(2):79–92. doi:10.1016/S1550-8579(06)80198-1

78. Hill TD, Jorgenson A. Bring out your dead!: A study of income inequality and life expectancy in the United States, 2000–2010. Health Place. 2018 Jan;49:1–6. doi:10.1016/j.healthplace.2017.11.001

79. Sasson I. Trends in life expectancy and lifespan variation by educational attainment: United States, 1990–2010. Demography. 2016 Apr;53(2):269–93. doi:10.1007/s13524-015-0453-7

80. Sasson I. Diverging trends in cause-specific mortality and life years lost by educational attainment: evidence from United States vital statistics data, 1990-2010. Patra J, editor. PLOS ONE. 2016 Oct 4;11(10):e0163412. doi:10.1371/journal.pone.0163412

81. O'Connor KJ, Graham C. Longer, more optimistic, lives: Historic optimism and life expectancy in the United States. J Econ Behav Organ. 2019 Dec;168:374–92. doi:10.1016/j.jebo.2019.10.018

82. Vespa J, Armstrong D, Medina L. Demographic turning points for the United States: population projections for 2020 to 2060. Curr Popul Rep. 2018;15.

83. Wingard DL. The sex differential in morbidity, mortality, and lifestyle. Ann Rev Public Health. 1984;5:433–58.

84. Crimmins EM, Zhang Y, Saito Y. Trends over 4 decades in disability-free life expectancy in the United States. Am J Public Health. 2016;106(7):1287–93. doi:10.2105/AJPH.2016.303120

85. Solé-Auró A, Beltrán-Sánchez H, Crimmins EM. Are differences in disability-free life expectancy by gender, race, and education widening at older ages? Popul Res Policy Rev. 2015 Feb;34(1):18. doi:10.1007/s11113-014-9337-6

86. Mather M, Jacobsen LA, Pollard KM. Aging in the United States. Popul Bull. 2015;70(2).

87. Why population aging matters: A global perspective. National Institute on Aging; 2007 p. 28. Report No.: 07–6134.

88. Meador S. Age-related utilization of advanced life support services. Prehospital Disaster Med. 1991;6(1):9–14. doi:10.1017/S1049023X00028004

89. Alemayehu B, Warner KE. The lifetime distribution of health care costs. Health Serv Res. 2004;39(3):627–42. doi:10.1111/j.1475-6773.2004.00248.x

90. National Health Expenditure Projections 2018-2027 [Internet]. National Health Expenditure Accounts; 2017 p. 3.

91. Cahill K, Giandrea M, Quinn J. Reentering the labor force after retirement. Bur Labor Stat. 2011;34–42.

92. Enoka R. Neuromechanics of human movement. Fifth edition. Champaign, IL: Human Kinetics; 2015.

93. Powers S, Howley E. Exercise physiology: Theory and application to fitness and performance. 9th ed. New York, NY: McGraw Hill Education; 2015.

94. MacIntosh B, Gardiner P, McComas A. Skeletal muscle: Form and function. Second edition. Champaign, IL: Human Kinetics; 2006.

95. Adrian E, Bronk D. The discharge of impulses in motor nerve fibres. J Physiol. 1929;67(2):119–51.

96. Zurlo F, Larson K, Bogardus C, Ravussin E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest. 1990;86(5):1423–7. doi:10.1172/JCI114857

97. Sandri M. Signaling in muscle atrophy and hypertrophy. Physiology. 2008;23(3):160–70. doi:10.1152/physiol.00041.2007

98. Andersen JL. Muscle fibre type adaptation in the elderly human muscle. Scand J Med Sci Sports. 2003;13(1):40–7. doi:10.1034/j.1600-0838.2003.00299.x

99. Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R, Roubenoff R. Skeletal muscle fiber quality in older men and women. Am J Physiol-Cell Physiol. 2000;279(3):C611–8. doi:10.1152/ajpcell.2000.279.3.C611

100. Miller MS, Toth MJ. Myofilament protein alterations promote physical disability in aging and disease. Exerc Sport Sci Rev. 2013;41(2):93–9. doi:10.1097/JES.0b013e31828bbcd8

101. Kamen G, Sison S, Du C, Patten C. Motor unit discharge behavior in older adults during maximal-effort contractions. J Appl Physiol. 1995;79(6):1908–13.

102. Vikne H, Gundersen K, Liestøl K, Maelen J, Vøllestad N. Intermuscular relationship of human muscle fiber type proportions: Slow leg muscles predict slow neck muscles. Muscle Nerve. 2012;45(4):527–35.

103. Simoneau JA, Bouchard C. Human variation in skeletal muscle fiber-type proportion and enzyme activities. Am J Physiol-Endocrinol Metab. 1989;257:E567–72. doi:10.1152/ajpendo.1989.257.4.E567

104. O'Leary TJ, Collett J, Howells K, Morris MG. Endurance capacity and neuromuscular fatigue following high- vs moderate-intensity endurance training: A randomized trial. Scand J Med Sci Sports. 2017;27(12):1648–61. doi:10.1111/sms.12854

105. Brown GL, von Euler US. The after effects of a tetanus on mammalian muscle. J Physiol. 1938;93(1):39–60. doi:10.1113/jphysiol.1938.sp003623

106. Baudry S, Klass M, Duchateau J. Postactivation potentiation influences differently the nonlinear summation of contractions in young and elderly adults. J Appl Physiol. 2005;98(4):1243–50. doi:10.1152/japplphysiol.00735.2004

107. Sweeney HL, Bowman BF, Stull JT. Myosin light chain phosphorylation in vertebrate striated muscle: Regulation and function. Am J Physiol-Cell Physiol. 1993;264(5):C1085–95. doi:10.1152/ajpcell.1993.264.5.C1085

108. Vandervoort AA, Quinlan J, McComas AJ. Twitch potentiation after voluntary contraction. Exp Neurol. 1983;81(1):141–52. doi:10.1016/0014-4886(83)90163-2

109. Blazevich AJ, Babault N. Post-activation potentiation versus post-activation performance enhancement in humans: Historical perspective, underlying mechanisms, and current issues. Front Physiol [Internet]. 2019 [cited 2020 Oct 14];10(1359). doi:10.3389/fphys.2019.01359

110. Duchateau J, Enoka RM. Human motor unit recordings: Origins and insight into the integrated motor system. Brain Res. 2011;1409:42–61. doi:10.1016/j.brainres.2011.06.011

111. Feinstein B, Lindegard B, Nyman E, Wohlfart G. Morphological studies of motor units in normal human muscles. Acta Anat. 1955;23:127–42.

112. Torre M. Number and dimensions of the motor units of the extrinsic eye muscles and, in general, of skeletal muscles connected with the sensory organs. Schweiz Arch Neurol Psychiatr. 1953;72(1–2):362–76.

113. Edstrom L, Kugelberg E. Histochemical composition, distribution of fibres and fatiguability of single motor units: Anterior tibial muscle of the rat. J Neurol Neurosurg Psychiatry. 1968;31(5):424–33. doi:10.1136/jnnp.31.5.424

114. Purves D, Augustine G, Fitzpatrick D, Katz L, LaMantia A-S, McNamara J, et al. The motor unit. In: Neuroscience. Second edition. Sunderland, MA: Sinauer Associates; 2001.

115. Burke RE, Levine DN, Zajac FE, Tsairis P, Engel WK. Mammalian motor units: Physiological-histochemical correlation in three types in cat gastrocnemius. Science. 1971;174(4010):709–12. doi:10.1126/science.174.4010.709

116. Henneman E, Somjen G, Carpenter D. Functional significance of cell size in spinal motoneurons. J Neurophysiol. 1965;28(3):560–80.

117. Burke RE, Levine DN, Salcman M, Tsairis P. Motor units in cat soleus muscle: physiological, histochemical and morphological characteristics. J Physiol. 1974;238(3):503–14. doi:10.1113/jphysiol.1974.sp010540

118. Enoka RM, Fuglevand AJ. Motor unit physiology: Some unresolved issues. Muscle Nerve. 2001;24:4–17.

119. Hoffman P. Über die Beziehungen der Sehnenreflexe zur willkürlichen Bewegung und zum Tonus. Z Biol. 1918;68:351–70.

120. Burke D. Clinical uses of H reflexes of upper and lower limb muscles. Clin Neurophysiol Pract. 2016;1:9–17. doi:10.1016/j.cnp.2016.02.003

121. Enoka R. Morphological features and activation patterns of motor units. J Clin Neurophysiol. 1995;12(6):538–59.

122. Zajac FE, Faden JS. Relationship among recruitment order, axonal conduction velocity, and muscle-unit properties of type-identified motor units in cat plantaris muscle. J Neurophysiol. 1985;53(5):1303–22. doi:10.1152/jn.1985.53.5.1303

123. Milner-Brown HS, Stein RB, Yemm R. The orderly recruitment of human motor units during voluntary isometric contractions. J Physiol. 1973;230(2):359–70. doi:10.1113/jphysiol.1973.sp010192

124. De Luca CJ, LeFever RS, McCue MP, Xenakis AP. Behaviour of human motor units in different muscles during linearly varying contractions. J Physiol. 1982;329(1):113–28. doi:10.1113/jphysiol.1982.sp014293

125. Person R. Rhythmic activity of a group of human motorneurones during voluntary contraction of a muscle. Electroencephalogr Clin Neurophysiol. 1974;36:585–95.

126. Nardone A, Romanò C, Schieppati M. Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. J Physiol. 1989;409(1):451–71. doi:10.1113/jphysiol.1989.sp017507

127. Grimby L. Firing properties of single human motor units during locomotion. J Physiol. 1984;346(1):195–202. doi:10.1113/jphysiol.1984.sp015016

128. Gillespie CA, Simpson DR, Edgerton VR. Motor unit recruitment as reflected by muscle fibre glycogen loss in a prosimian (bushbaby) after running and jumping. J Neurol Neurosurg Psychiatry. 1974;37(7):817–24. doi:10.1136/jnnp.37.7.817

129. Gonyea WJ, Sale DG, Gonyea FB, Mikesky A. Exercise induced increases in muscle fiber number. Eur J Appl Physiol. 1986;55(2):137–41. doi:10.1007/BF00714995

130. Larsson L, Tesch PA. Motor unit fibre density in extremely hypertrophied skeletal muscles in man: Electrophysiological signs of muscle fibre hyperplasia. Eur J Appl Physiol. 1986;55(2):130–6. doi:10.1007/BF00714994

131. Antonio J, Gonyea W. Skeletal muscle fiber hyperplasia. Med Sci Sports Exerc. 1993;25(12):1333–45.

132. McCarthy JJ, Esser KA. Anabolic and catabolic pathways regulating skeletal muscle mass: Curr Opin Clin Nutr Metab Care. 2010;13(3):230–5. doi:10.1097/MCO.0b013e32833781b5

133. Krok-Schoen JL, Archdeacon Price A, Luo M, Kelly OJ, Taylor CA. Low dietary protein intakes and associated dietary patterns and functional limitations in an aging population: A NHANES analysis. J Nutr Health Aging. 2019;23(4):338–47. doi:10.1007/s12603-019-1174-1

134. Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol. 2001;3(11):1014–9. doi:10.1038/ncb1101-1014

135. McPherron AC, Lawler AM, Lee S-J. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature. 1997;387:83–90.

136. Qaisar R, Renaud G, Morine K, Barton ER, Sweeney HL, Larsson L. Is functional hypertrophy and specific force coupled with the addition of myonuclei at the single muscle fiber level? FASEB J. 2012;26(3):1077–85. doi:10.1096/fj.11-192195

137. Hennebry A, Oldham J, Shavlakadze T, Grounds MD, Sheard P, Fiorotto ML, et al. IGF1 stimulates greater muscle hypertrophy in the absence of myostatin in male mice. J Endocrinol. 2017;234(2):187–200. doi:10.1530/JOE-17-0032

138. Jackman RW, Kandarian SC. The molecular basis of skeletal muscle atrophy. Am J Physiol-Cell Physiol. 2004;287(4):C834–43. doi:10.1152/ajpcell.00579.2003

139. Schakman O, Gilson H, Thissen JP. Mechanisms of glucocorticoid-induced myopathy. J Endocrinol. 2008;197:10.

140. Bodine SC. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science. 2001;294(5547):1704–8. doi:10.1126/science.1065874

141. Sato A, Richardson D, Cregor M, Davis H, Au E, McAndrews K, et al. Glucocorticoids induce bone and muscle atrophy by tissue-specific mechanisms upstream of E3 ubiquitin ligases. Endocrinology. 2017;158:664–77.

142. Moustakas A, Souchelnytskyi S, Heldin C-H. Smad regulation in TGF-β signal transduction. J Cell Sci. 2001;114(24):4359–69.

143. Mauro A. Satellite cells of skeletal muscle fibers. J Biophys Biochem Cytol. 1961;9(2):493–5. doi:10.1083/jcb.9.2.493

144. Tedesco FS, Dellavalle A, Diaz-Manera J, Messina G, Cossu G. Repairing skeletal muscle: Regenerative potential of skeletal muscle stem cells. J Clin Invest. 2010;120(1):11–9. doi:10.1172/JCI40373

145. Hawke TJ, Garry DJ. Myogenic satellite cells: Physiology to molecular biology. J Appl Physiol. 2001;91(2):534–51. doi:10.1152/jappl.2001.91.2.534

146. Tierney MT, Sacco A. Satellite cell heterogeneity in skeletal muscle homeostasis. Trends Cell Biol. 2016;26(6):434–44. doi:10.1016/j.tcb.2016.02.004

147. Zammit PS, Golding JP, Nagata Y, Hudon V, Partridge TA, Beauchamp JR. Muscle satellite cells adopt divergent fates. J Cell Biol. 2004;166(3):347–57. doi:10.1083/jcb.200312007

148. McCroskery S, Thomas M, Maxwell L, Sharma M, Kambadur R. Myostatin negatively regulates satellite cell activation and self-renewal. J Cell Biol. 2003;162(6):1135–47. doi:10.1083/jcb.200207056

149. Schmalbruch H, Hellhammer U. The number of nuclei in adult rat muscles with special reference to satellite cells. Anat Rec. 1977;189(2):169–75. doi:10.1002/ar.1091890204

150. Wokke JHJ, van Den Oord CJM, Leppink GJ, Jennekens FGI. Perisynaptic satellite cells in human external intercostal muscle: A quantitative and qualitative study. Anat Rec. 1989;223(2):174–80. doi:10.1002/ar.1092230209

151. Evans WJ, Lexell J. Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci. 1995;50A(Special):11–6. doi:10.1093/gerona/50A.Special_Issue.11

152. Andersen JL, Terzis G, Kryger A. Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. Muscle Nerve. 1999;22:449–54.

153. Davis MG, Fox KR. Physical activity patterns assessed by accelerometry in older people. Eur J Appl Physiol. 2007;100(5):581–9. doi:10.1007/s00421-006-0320-8

154. Lexell J, Taylor CC, Sjöström M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis

muscle from 15- to 83-year-old men. J Neurol Sci. 1988;84(2–3):275–94. doi:10.1016/0022-510x(88)90132-3

155. Nilwik R, Snijders T, Leenders M, Groen BBL, van Kranenburg J, Verdijk LB, et al. The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size. Exp Gerontol. 2013;48:492–8. doi:10.1016/j.exger.2013.02.012

156. Verdijk LB, Koopman R, Schaart G, Meijer K. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. Endocrinol Metab. 2007;292:E151–7.

157. Lexell J, Henriksson-Larsén K, Winblad B, Sjöström M. Distribution of different fiber types in human skeletal muscles: Effects of aging studied in whole muscle cross sections. Muscle Nerve. 1983;6(8):588–95. doi:10.1002/mus.880060809

158. Yarasheski K, Welle S, Nair S. Muscle protein synthesis in younger and older men: Letter to the editor. JAMA. 2002;287(3):317–8.

159. Volpi E, Rasmussen B, Sheffield-Moore M, Wolfe R. Muscle protein synthesis in younger and older men - Reply: Letter to the editor. JAMA. 2002;287(3):317–8.

160. Balagopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. Am J Physiol-Endocrinol Metab. 1997;273(4):E790–800. doi:10.1152/ajpendo.1997.273.4.E790

161. Welle S, Thornton C, Jozefowicz R, Statt M. Myofibrillar protein synthesis in young and old men. Am J Physiol-Endocrinol Metab. 1993;264(5):E693–8. doi:10.1152/ajpendo.1993.264.5.E693

162. Rooyackers OE, Adey DB, Ades PA, Nair KS. Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. Proc Natl Acad Sci. 1996;93(26):15364–9. doi:10.1073/pnas.93.26.15364

163. Volpi E. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. JAMA. 2001;286(10):1206–12. doi:10.1001/jama.286.10.1206

164. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J. 2005;19(3):1–22. doi:10.1096/fj.04-2640fje

165. Fry C, Rasmussen B. Skeletal muscle protein balance and metabolism in the elderly. Curr Aging Sci. 2011;4(3):260–8. doi:10.2174/1874609811104030260

166. Guillet C, Prod'homme M, Balage M, Gachon P, Giraudet C, Morin L, et al. Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. FASEB J. 2004;18(13):1586–7. doi:10.1096/fj.03-1341fje

167. Boirie Y, Dangin M, Gachon P, Vasson M-P, Maubois J-L, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci. 1997;94(26):14930–5. doi:10.1073/pnas.94.26.14930

168. Murton AJ. Muscle protein turnover in the elderly and its potential contribution to the development of sarcopenia. Proc Nutr Soc. 2015;74:387–96. doi:10.1017/S0029665115000130

169. Morley JE, Argiles JM, Evans WJ, Bhasin S, Cella D, Deutz NEP, et al. Nutritional recommendations for the management of sarcopenia. J Am Med Dir Assoc. 2010;11(6):391–6. doi:10.1016/j.jamda.2010.04.014

170. Campbell WW, Trappe TA, Wolfe RR, Evans WJ. The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. J Gerontol A Biol Sci Med Sci. 2001;56(6):M373–80. doi:10.1093/gerona/56.6.M373

171. English KL, Paddon-Jones D. Protecting muscle mass and function in older adults during bed rest: Curr Opin Clin Nutr Metab Care. 2010;13(1):34–9. doi:10.1097/MCO.0b013e328333aa66

172. Chambers TL, Burnett TR, Raue U, Lee GA, Finch WH, Graham BM, et al. Skeletal muscle size, function, and adiposity with lifelong aerobic exercise. J Appl Physiol. 2020;128(2):368–78. doi:10.1152/japplphysiol.00426.2019

173. Sandri M, Barberi L, Bijlsma AY, Blaauw B, Dyar KA, Milan G, et al. Signalling pathways regulating muscle mass in ageing skeletal muscle. The role of the IGF1-Akt-mTOR-FoxO pathway. Biogerontology. 2013;14(3):303–23. doi:10.1007/s10522-013-9432-9

174. Barclay RD, Burd NA, Tyler C, Tillin NA, Mackenzie RW. The role of the IGF-1 signaling cascade in muscle protein synthesis and anabolic resistance in aging skeletal muscle. Front Nutr. 2019;6:146. doi:10.3389/fnut.2019.00146

175. Markofski MM, Dickinson JM, Drummond MJ, Fry CS, Fujita S, Gundermann DM, et al. Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women. Exp Gerontol. 2015;65:1–7. doi:10.1016/j.exger.2015.02.015

176. Castets P, Lin S, Rion N, Di Fulvio S, Romanino K, Guridi M, et al. Sustained activation of mTORC1 in skeletal muscle inhibits constitutive and starvation-induced autophagy and causes a severe, late-onset myopathy. Cell Metab. 2013;17(5):731–44. doi:10.1016/j.cmet.2013.03.015

177. Renault V, Thorne L-E, Eriksson P-O, Butler-Browne G, Mouly V. Regenerative potential of human skeletal muscle during aging. Aging Cell. 2002;1(2):132–9. doi:10.1046/j.1474-9728.2002.00017.x

178. Snijders T, Verdijk LB, Smeets JSJ, McKay BR, Senden JMG, Hartgens F, et al. The skeletal muscle satellite cell response to a single bout of resistance-type exercise is delayed with aging in men. AGE. 2014;36(4):9699. doi:10.1007/s11357-014-9699-z

179. McKay BR, Ogborn DI, Bellamy LM, Tarnopolsky MA, Parise G. Myostatin is associated with age-related human muscle stem cell dysfunction. FASEB J. 2012;26(6):2509–21. doi:10.1096/fj.11-198663

180. Suetta C, Frandsen U, Mackey AL, Jensen L, Hvid LG, Bayer ML, et al. Ageing is associated with diminished muscle re-growth and myogenic precursor cell expansion early after immobility-induced atrophy in human skeletal muscle: Ageing affects human skeletal muscle recovery. J Physiol. 2013;591(15):3789–804. doi:10.1113/jphysiol.2013.257121

181. Leng S, Cappola A, Andersen R, Blackman M, Koenig K, Blair M, et al. Serum levels of insulin-like growth factor-I (IGF-I) and dehydroepiandrosterone sulfate (DHEA-S), and their relationships with serum interleukin-6, in the geriatric syndrome of frailty. Aging Clin Exp Res. 2004;16:153–7.

182. Larsson L, Salviati G. Effects of age on calcium transport activity of sarcoplasmic reticulum in fast- and slow-twitch rat muscle fibers. J Physiol. 1989;419:253–64.

183. Payne AM, Delbono O. Neurogenesis of excitation-contraction uncoupling in aging skeletal muscle. Exerc Sport Sci Rev. 2004;32(1):36–40. doi:10.1097/00003677-200401000-00008

184. D'Antona G. The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. J Physiol. 2003;552(2):499–511. doi:10.1113/jphysiol.2003.046276

185. Piec I, Listrat A, Alliot J, Chambon C, Taylor RG, Bechet D. Differential proteome analysis of aging in rat skeletal muscle. FASEB J. 2005;19(9):1143–5. doi:10.1096/fj.04-3084fje

186. Vann CG, Roberson PaulA, Osburn SC, Mumford PW, Romero MA, Fox CD, et al. Skeletal muscle myofibrillar protein abundance is higher in resistance-trained men, and aging in the absence of training may have an opposite effect. Sports. 2020;8(1):7. doi:10.3390/sports8010007

187. Capitanio D, Vasso M, Fania C, Moriggi M, Viganò A, Procacci P, et al. Comparative proteomic profile of rat sciatic nerve and gastrocnemius muscle tissues in ageing by 2-D DIGE. Proteomics. 2009;9(7):2004–20. doi:10.1002/pmic.200701162

188. Donoghue P, Staunton L, Mullen E, Manning G, Ohlendieck K. DIGE analysis of rat skeletal muscle proteins using nonionic detergent phase extraction of young adult versus aged gastrocnemius tissue. J Proteomics. 2010;73(8):1441–53. doi:10.1016/j.jprot.2010.01.014

189. Lowe DA, Surek JT, Thomas DD, Thompson LV. Electron paramagnetic resonance reveals age-related myosin structural changes in rat skeletal muscle fibers. Am J Physiol-Cell Physiol. 2001;280(3):C540–7. doi:10.1152/ajpcell.2001.280.3.C540

190. Piazzesi G, Reconditi M, Linari M, Lucii L, Bianco P, Brunello E, et al. Skeletal muscle performance determined by modulation of number of myosin motors rather

than motor force or stroke size. Cell. 2007;131(4):784–95. doi:10.1016/j.cell.2007.09.045

191. Lowe DA, Thomas DD, Thompson LV. Force generation, but not myosin ATPase activity, declines with age in rat muscle fibers. Am J Physiol-Cell Physiol. 2002;283(1):C187–92. doi:10.1152/ajpcell.00008.2002

192. Lowe DA, Husom AD, Ferrington DA, Thompson LV. Myofibrillar myosin ATPase activity in hindlimb muscles from young and aged rats. Mech Ageing Dev. 2004;125(9):619–27. doi:10.1016/j.mad.2004.07.002

193. Vandervoort AA, McComas AJ. Contractile changes in opposing muscles of the human ankle joint with aging. J Appl Physiol. 1986;61(1):361–7. doi:10.1152/jappl.1986.61.1.361

194. Petrella RJ, Cunningham DA, Vandervoort AA, Paterson DH. Comparison of twitch potentiation in the gastrocnemius of young and elderly men. Eur J Appl Physiol. 1989;58(4):395–9. doi:10.1007/BF00643515

195. Svensson RB, Heinemeier KM, Couppé C, Kjaer M, Magnusson SP. Effect of aging and exercise on the tendon. J Appl Physiol. 2016;121(6):1353–62. doi:10.1152/japplphysiol.00328.2016

196. Eriksen CS, Henkel C, Svensson RB, Agergaard A-S, Couppé C, Kjaer M, et al. Lower tendon stiffness in very old compared with old individuals is unaffected by

short-term resistance training of skeletal muscle. J Appl Physiol. 2018;125(1):205–14. doi:10.1152/japplphysiol.00028.2018

197. Gonzalez-Freire M, de Cabo R, Studenski SA, Ferrucci L. The neuromuscular junction: Aging at the crossroad between nerves and muscle. Front Aging Neurosci. 2014;6:208. doi:10.3389/fnagi.2014.00208

198. Deschenes MR, Roby MA, Eason MK, Harris MB. Remodeling of the neuromuscular junction precedes sarcopenia related alterations in myofibers. Exp Gerontol. 2010;45(5):389–93. doi:10.1016/j.exger.2010.03.007

199. Tomlinson B, Irving D. The numbers of limb motor neurons in the human lumbosacral cord throughout life. J Neurol Sci. 1977;34:213–9.

200. Einsiedel LJ, Luff AR. Alterations in the contractile properties of motor units within the ageing medial gastrocnemius. J Neurol Sci. 1992;112:170–7.

201. Arnold WD, Taylor RS, Li J, Nagy JA, Sanchez B, Rutkove SB. Electrical impedance myography detects age-related muscle change in mice. Phillips WD, editor. PLOS ONE. 2017;12(10):e0185614. doi:10.1371/journal.pone.0185614

202. Tavoian D, Arnold WD, Mort SC, de Lacalle S. Sex differences in body composition but not neuromuscular function following long-term, doxycycline-induced reduction in circulating levels of myostatin in mice. PLOS ONE. 2019;14(11):e0225283. doi:10.1371/journal.pone.0225283

203. Piasecki M, Ireland A, Coulson J, Stashuk DW, Hamilton-Wright A, Swiecicka A, et al. Motor unit number estimates and neuromuscular transmission in the tibialis anterior of master athletes: Evidence that athletic older people are not spared from age-related motor unit remodeling. Physiol Rep. 2016;4(19):e12987. doi:10.14814/phy2.12987

204. Hepple RT, Rice CL. Innervation and neuromuscular control in ageing skeletal muscle. J Physiol. 2016;594(8):1965–78. doi:10.1113/JP270561

205. Wilkinson DJ, Piasecki M, Atherton PJ. The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. Ageing Res Rev. 2018;47:123–32. doi:10.1016/j.arr.2018.07.005

206. McNeil CJ, Doherty TJ, Stashuk DW, Rice CL. Motor unit number estimates in the tibialis anterior muscle of young, old, and very old men. Muscle Nerve. 2005;31(4):461–7. doi:10.1002/mus.20276

207. Rafuse VF, Gordon T, Orozco R. Proportional enlargement of motor units after partial denervation of cat triceps surae muscles. J Neurophysiol. 1992;68(4):1261–76. doi:10.1152/jn.1992.68.4.1261

208. Gordon T, Hegedus J, Tam S. Adaptive and maladaptive motor axonal sprouting in aging and motoneuron disease. Neurol Res. 2004;26(2):174–85.

209. Lexell J, Downham D, Sjöström M. Distribution of different fibre types in human skeletal muscles: Fibre type arrangement in m. vastus lateralis from three groups of healthy men between 15 and 83 years. J Neurol Sci. 1986;72:211–22.

210. McNeil CJ, Rice CL. Neuromuscular adaptations to healthy aging. Appl Physiol Nutr Metab. 2018;43(11):1158–65. doi:10.1139/apnm-2018-0327

211. Tracy BL, Maluf KS, Stephenson JL, Hunter SK, Enoka RM. Variability of motor unit discharge and force fluctuations across a range of muscle forces in older adults. Muscle Nerve. 2005;32(4):533–40. doi:10.1002/mus.20392

212. Russ DW, Gregg-Cornell K, Conaway MJ, Clark BC. Evolving concepts on the age-related changes in "muscle quality." J Cachexia Sarcopenia Muscle. 2012;3(2):95–109. doi:10.1007/s13539-011-0054-2

213. Clark B, Taylor J. Age-related changes in motor cortical properties and voluntary activation of skeletal muscle. Curr Aging Sci. 2011;4(3):192–9. doi:10.2174/1874609811104030192

214. McGinley M, Hoffman RL, Russ DW, Thomas JS, Clark BC. Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. Exp Gerontol. 2010;45(9):671–8. doi:10.1016/j.exger.2010.04.005

215. Evans W. What is sarcopenia? J Gerontol Biol Sci Med Sc. 1995;50A(Special Issue):5–8.

216. Clark B, Manini TM. Sarcopenia ≠ dynapenia. J Gerontol. 2008;63A(8):829–34.

217. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: Revised European consensus on definition and diagnosis. Age Ageing. 2019;48(1):16–31. doi:10.1093/ageing/afy169

218. Webber SC, Porter MM, Menec VH. Mobility in older adults: A comprehensive framework. The Gerontologist. 2010;50(4):443–50. doi:10.1093/geront/gnq013

219. Vellas B, Sourdet S. Prevention of frailty in aging. J Frailty Aging. 2017;6(4):174–7. doi:10.14283/jfa.2017.42

220. Guralnik J, Alecxih L, Branch L, Wiener J. Independence for older Americans: An investment in our nation's future. Washington DC: Alliance for Aging Reseasrch; 1999 p. 23.

221. van Lummel RC, Walgaard S, Pijnappels M, Elders PJM, Garcia-Aymerich J, van Dieën JH, et al. Physical performance and physical activity in older adults: Associated but separate domains of physical function in old age. Reddy H, editor. PLOS ONE. 2015;10(12):e0144048. doi:10.1371/journal.pone.0144048

222. Katz S. Assessing self-maintenance: activities of daily living, mobility, and instrumental activities of daily living. J Am Geriatr Soc. 1983;31(12):721–7. doi:10.1111/j.1532-5415.1983.tb03391.x

223. FIFoA-R. Older Americans 2016: key indicators of well-being. 2016;204.

224. Clark DJ, Manini TM, Ferris DP, Hass CJ, Brumback BA, Cruz-Almeida Y, et al. Multimodal imaging of brain activity to investigate walking and mobility decline in older adults (mind in motion study): Hypothesis, theory, and methods. Front Aging Neurosci. 2020;11:358. doi:10.3389/fnagi.2019.00358

225. Mackey DC, Cauley JA, Barrett-Connor E, Schousboe JT, Cawthon PM, Cummings SR, et al. Life-space mobility and mortality in older men: A prospective cohort study. J Am Geriatr Soc. 2014;62(7):1288–96. doi:10.1111/jgs.12892

226. Kawamoto R, Doi T. Self-reported functional ability predicts three-year mobility and mortality in community-dwelling older persons: Three-year elderly mobility and mortality. Geriatr Gerontol Int. 2002;2(2):68–74. doi:10.1046/j.1444-1586.2002.00024.x

227. Eekhoff EMW, van Schoor NM, Biedermann JS, Oosterwerff MM, de Jongh R, Bravenboer N, et al. Relative importance of four functional measures as predictors of 15-year mortality in the older Dutch population. BMC Geriatr. 2019;19(1):92. doi:10.1186/s12877-019-1092-4

228. Wei MY, Kabeto MU, Galecki AT, Langa KM. Physical functioning decline and mortality in older adults with multimorbidity: Joint modeling of longitudinal and survival data. J Gerontol Ser A. 2019;74(2):226–32. doi:10.1093/gerona/gly038

229. Reid KF, Fielding RA. Skeletal muscle power: A critical determinant of physical functioning in older adults. Exerc Sport Sci Rev. 2012;40(1):4–12. doi:10.1097/JES.0b013e31823b5f13

230. deVries MS, Murphy EAK, Patek SN. Strike mechanics of an ambush predator: the spearing mantis shrimp. J Exp Biol. 2012;215(24):4374–84. doi:10.1242/jeb.075317

231. Jayne BC, Bennett AF. Selection on locomotor performance capacity in a natural population of garter snakes. Evolution. 1990;44(5):1204–29. doi:10.1111/j.1558-5646.1990.tb05226.x

232. Kamo T, Asahi R, Azami M, Ogihara H, Ikeda T, Suzuki K, et al. Rate of torque development and the risk of falls among community dwelling older adults in Japan. Gait Posture. 2019;72:28–33. doi:10.1016/j.gaitpost.2019.05.019

233. de Ruiter CJ, Vermeulen G, Toussaint HM, De Haan A. Isometric knee-extensor torque development and jump height in volleyball players. Med Sci Sports Exerc. 2007;39(8):1336–46. doi:10.1097/mss.0b013e318063c719

234. Foldvari M, Clark M, Laviolette LC, Bernstein MA, Kaliton D, Castaneda C, et al. Association of muscle power with functional status in community-dwelling elderly women. J Gerontol Med Sci. 2000;55A(4):M192–9. doi:10.1093/gerona/55.4.m192

235. Clark DJ, Patten C, Reid KF, Carabello RJ, Phillips EM, Fielding RA. Impaired voluntary neuromuscular activation limits muscle power in mobility-limited older

adults. J Gerontol A Biol Sci Med Sci. 2010;65A(5):495–502. doi:10.1093/gerona/glq012

236. Manini TM, Visser M, Won-Park S, Patel KV, Strotmeyer ES, Chen H, et al. Knee extension strength cutpoints for maintaining mobility. J Am Geriatr Soc. 2007;55(3):451–7. doi:10.1111/j.1532-5415.2007.01087.x

237. Cress ME, Meyer M. Maximal voluntary and functional performance levels needed for independence in adults aged 65 to 97 years. Phys Ther. 2003;83(1):37–48.

238. Pijnappels M, Bobbert MF, Dieën JH van. Push-off reactions in recovery after tripping discriminate young subjects, older non-fallers and older fallers. Gait Posture. 2005;21(4):388–94. doi:10.1016/j.gaitpost.2004.04.009

239. Osawa Y, Studenski SA, Ferrucci L. Knee extension rate of torque development and peak torque: Associations with lower extremity function. J Cachexia Sarcopenia Muscle. 2018;9(3):530–9. doi:10.1002/jcsm.12285

240. Andersen LL, Aagaard P. Influence of maximal muscle strength and intrinsic muscle contractile properties on contractile rate of force development. Eur J Appl Physiol. 2006;96(1):46–52. doi:10.1007/s00421-005-0070-z

241. Folland JP, Buckthorpe MW, Hannah R. Human capacity for explosive force production: Neural and contractile determinants. Scand J Med Sci Sports. 2014;24(6):894–906. doi:10.1111/sms.12131

242. Fields D, Bemben M, Mayhew J. The influence of muscle group location and race on the relationship between muscle strength and power. J Sports Med Phys Fit. 1997;37(1):24–31.

243. Angelozzi M, Madama M, Corsica C, Calvisi V, Properzi G, McCaw ST, et al. Rate of force development as an adjunctive outcome measure for return-to-sport decisions after anterior cruciate ligament reconstruction. J Orthop Sports Phys Ther. 2012;42(9):772–80. doi:10.2519/jospt.2012.3780

244. Rodríguez-Rosell D, Pareja-Blanco F, Aagaard P, González-Badillo JJ. Physiological and methodological aspects of rate of force development assessment in human skeletal muscle. Clin Physiol Funct Imaging. 2018;38(5):743–62. doi:10.1111/cpf.12495

245. Izquierdo M, Aguado X, Gonzalez R, Lopez JL, Häkkinen K. Maximal and explosive force production capacity and balance performance in men of different ages. Eur J Appl Physiol. 1999;79(3):260–7. doi:10.1007/s004210050504

246. Mau-Moeller A, Behrens M, Lindner T, Bader R, Bruhn S. Age-related changes in neuromuscular function of the quadriceps muscle in physically active adults. J Electromyogr Kinesiol. 2013;23(3):640–8. doi:10.1016/j.jelekin.2013.01.009

247. Jenkins NDM, Buckner SL, Cochrane KC, Bergstrom HC, Palmer TB, Johnson GO, et al. Age-related differences in rates of torque development and rise in EMG are

eliminated by normalization. Exp Gerontol. 2014;57:18–28. doi:10.1016/j.exger.2014.04.015

248. Thompson BJ, Ryan ED, Sobolewski EJ, Conchola EC, Cramer JT. Age related differences in maximal and rapid torque characteristics of the leg extensors and flexors in young, middle-aged and old men. Exp Gerontol. 2013;48(2):277–82. doi:10.1016/j.exger.2012.10.009

249. Gerstner GR, Thompson BJ, Rosenberg JG, Sobolewski EJ, Scharville MJ, Ryan ED. Neural and muscular contributions to the age-related reductions in rapid strength. Med Sci Sports Exerc. 2017;49(7):1331–9. doi:10.1249/MSS.0000000000001231

250. Thompson BJ, Ryan ED, Herda TJ, Costa PB, Herda AA, Cramer JT. Age-related changes in the rate of muscle activation and rapid force characteristics. AGE. 2014;36(2):839–49. doi:10.1007/s11357-013-9605-0

251. Unhjem R, Lundestad R, Fimland MS, Mosti MP, Wang E. Strength training-induced responses in older adults: attenuation of descending neural drive with age. AGE. 2015;37(3):47. doi:10.1007/s11357-015-9784-y

252. Thelen DG, Schultz AB, Alexander NB, Ashton-Miller JA. Effects of age on rapid ankle torque development. J Gerontol A Biol Sci Med Sci. 1996;51A(5):M226–32. doi:10.1093/gerona/51A.5.M226

253. Klass M, Baudry S, Duchateau J. Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. J Appl Physiol. 2008;104(3):739–46. doi:10.1152/japplphysiol.00550.2007

254. Metter EJ, Talbot LA, Schrager M, Conwit R. Skeletal muscle strength as a predictor of all-cause mortality in healthy men. J Gerontol A Biol Sci Med Sci. 2002;57(10):B359–65. doi:10.1093/gerona/57.10.B359

255. García-Hermoso A, Cavero-Redondo I, Ramírez-Vélez R, Ruiz JR, Ortega FB, Lee D-C, et al. Muscular strength as a predictor of all-cause mortality in an apparently healthy population: A systematic review and meta-analysis of data from approximately 2 million men and women. Arch Phys Med Rehabil. 2018;99(10):2100-2113.e5. doi:10.1016/j.apmr.2018.01.008

256. McGrath R, Vincent BM, Hackney KJ, Robinson-Lane SG, Downer B, Clark BC. The longitudinal associations of handgrip strength and cognitive function in aging Americans. J Am Med Dir Assoc. 2020;21(5):634-639.e1. doi:10.1016/j.jamda.2019.08.032

257. Looker AC, Wang C. Prevalence of reduced muscle strength in older U.S. adults: United States, 2011–2012. Hyattsville, MD: National Center for Health Statistics; 2015. Report No.: NCHS Data Brief, no 179.

258. Alonso AC, Ribeiro SM, Luna NMS, Peterson MD, Bocalini DS, Serra MM, et al. Association between handgrip strength, balance, and knee flexion/extension strength in older adults. Sergi G, editor. PLOS ONE. 2018;13(6):e0198185. doi:10.1371/journal.pone.0198185

259. Bohannon RW. Are hand-grip and knee extension strength reflective of a common construct? Percept Mot Skills. 2012;114(2):514–8. doi:10.2466/03.26.PMS.114.2.514-518

260. Hughes VA, Frontera WR, Wood M, Evans WJ, Dallal GE, Roubenoff R, et al. Longitudinal muscle strength changes in older adults influence of muscle mass, physical activity, and health. J Gerontol A Biol Sci Med Sci. 2001;56(5):B209-17.

261. Yeung SSY, Reijnierse EM, Trappenburg MC, Blauw GJ, Meskers CGM, Maier AB. Knee extension strength measurements should be considered as part of the comprehensive geriatric assessment. BMC Geriatr. 2018;18(1):130. doi:10.1186/s12877-018-0815-2

262. Bohannon RW. Muscle strength: Clinical and prognostic value of hand-grip dynamometry. Curr Opin Clin Nutr Metab Care. 2015;18(5):465–70. doi:10.1097/MCO.0000000000000202

263. Kim YH, Kim K-I, Paik N-J, Kim K-W, Jang HC, Lim J-Y. Muscle strength: A better index of low physical performance than muscle mass in older adults. Geriatr Gerontol Int. 2016;16(5):577–85. doi:10.1111/ggi.12514

264. Norman K, Stobäus N, Gonzalez MC, Schulzke J-D, Pirlich M. Hand grip strength: Outcome predictor and marker of nutritional status. Clin Nutr. 2011;30(2):135–42. doi:10.1016/j.clnu.2010.09.010

265. Ploutz-Snyder LL, Manini T, Ploutz-Snyder RJ, Wolf DA. Functionally relevant thresholds of quadriceps femoris strength. J Gerontol A Biol Sci Med Sci. 2002;57(4):144–52. doi:10.1093/gerona/57.4.B144

266. Gouveia ÉR, Ihle A, Gouveia BR, Kliegel M, Marques A, Freitas DL. Muscle mass and muscle strength relationships to balance: The role of age and physical activity. J Aging Phys Act. 2020;28(2):262–8. doi:10.1123/japa.2018-0113

267. Trombetti A, Reid KF, Hars M, Herrmann FR, Pasha E, Phillips EM, et al. Age-associated declines in muscle mass, strength, power, and physical performance: impact on fear of falling and quality of life. Osteoporos Int. 2016;27(2):463–71. doi:10.1007/s00198-015-3236-5

268. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The loss of skeletal muscle strength, mass, and quality in older adults: The health, aging and body composition study. J Gerontol A Biol Sci Med Sci. 2006;61(10):1059–64. doi:10.1093/gerona/61.10.1059

269. Li R, Xia J, Zhang X, Gathirua-Mwangi WG, Guo J, Li Y, et al. Associations of muscle mass and strength with all-cause mortality among US older adults. Med Sci Sports Exerc. 2018;50(3):458–67. doi:10.1249/MSS.0000000000001448

270. Caan BJ, Cespedes Feliciano EM, Prado CM, Alexeeff S, Kroenke CH, Bradshaw P, et al. Association of muscle and adiposity measured by computed tomography with survival in patients with nonmetastatic breast cancer. JAMA Oncol. 2018;4(6):798. doi:10.1001/jamaoncol.2018.0137

271. Son JW, Lee SS, Kim SR, Yoo SJ, Cha BY, Son HY, et al. Low muscle mass and risk of type 2 diabetes in middle-aged and older adults: Findings from the KoGES. Diabetologia. 2017;60(5):865–72. doi:10.1007/s00125-016-4196-9

272. Tyrovolas S, Panagiotakos D, Georgousopoulou E, Chrysohoou C, Tousoulis D, Haro J, et al. Skeletal muscle mass in relation to 10 year cardiovascular disease incidence among middle aged and older adults: The ATTICA study. J Epidemiol Community Health. 2020;74(1):26–31.

273. Cunningham D, Paterson D, Koval J, St. Croix C. A model of oxygen transport capacity changes for independently living older men and women. Can J Appl Physiol. 1997;22(5):439–53.

274. Rogers MA, Hagberg JM, Martin WH, Ehsani AA, Holloszy JO. Decline in VO2max with aging in master athletes and sedentary men. J Appl Physiol. 1990;68(5):2195–9. doi:10.1152/jappl.1990.68.5.2195

275. Radak Z, Torma F, Berkes I, Goto S, Mimura T, Posa A, et al. Exercise effects on physiological function during aging. Free Radic Biol Med. 2019;132:33–41. doi:10.1016/j.freeradbiomed.2018.10.444

276. Ogawa T, Spina RJ, Martin WH, Kohrt WM, Schechtman KB, Holloszy JO, et al. Effects of aging, sex, and physical training on cardiovascular responses to exercise. Circulation. 1992;86(2):494–503. doi:10.1161/01.CIR.86.2.494

277. Proctor DN, Joyner MJ. Skeletal muscle mass and the reduction of VO2max in trained older subjects. J Appl Physiol. 1997;82(5):1411–5. doi:10.1152/jappl.1997.82.5.1411

278. Russ DW, Kent-Braun JA. Is skeletal muscle oxidative capacity decreased in old age? Sports Med. 2004;34(4):221–9. doi:10.2165/00007256-200434040-00002

279. Solomon A, Borodulin K, Ngandu T, Kivipelto M, Laatikainen T, Kulmala J. Self-rated physical fitness and estimated maximal oxygen uptake in relation to all-cause and cause-specific mortality. Scand J Med Sci Sports. 2018;28(2):532–40. doi:10.1111/sms.12924

280. Paterson DH, Govindasamy D, Vidmar M, Cunningham DA, Koval JJ. Longitudinal study of determinants of dependence in an elderly population. J Am Geriatr Soc. 2004;52(10):1632–8. doi:10.1111/j.1532-5415.2004.52454.x

281. Malatesta D, Simar D, Dauvilliers Y, Candau R, Borrani F, Préfaut C, et al. Energy cost of walking and gait instability in healthy 65- and 80-yr-olds. J Appl Physiol. 2003;95(6):2248–56. doi:10.1152/japplphysiol.01106.2002

282. Weiss EP, Spina RJ, Holloszy JO, Ehsani AA. Gender differences in the decline in aerobic capacity and its physiological determinants during the later decades of life. J Appl Physiol. 2006;101(3):938–44. doi:10.1152/japplphysiol.01398.2005

283. Knaeps S, Bourgois JG, Charlier R, Mertens E, Lefevre J, Wijndaele K. Ten-year change in sedentary behaviour, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic risk: independent associations and mediation analysis. Br J Sports Med. 2018;52(16):1063–8. doi:10.1136/bjsports-2016-096083

284. Talbot LA, Metter EJ, Fleg JL. Leisure-time physical activities and their relationship to cardiorespiratory fitness in healthy men and women 18-95 years old. Med Sci Sports Exerc. 2000;32(2):417. doi:10.1097/00005768-200002000-00024

285. Law TD, Clark LA, Clark BC. Resistance exercise to prevent and manage sarcopenia and dynapenia. Annu Rev Gerontol Geriatr. 2016;36(1):205–28. doi:10.1891/0198-8794.36.205

286. Ruegsegger GN, Booth FW. Health benefits of exercise. Cold Spring Harb Perspect Med. 2018;8(7):a029694. doi:10.1101/cshperspect.a029694

287. NCHS. National Center for Health Statistics. Health, United States, 2016: With Chartbook on Long-Term Trends in Health. Hyattsville, MD; 2017.

288. Ackermann RT, Cheadle A, Sandhu N, Madsen L, Wagner EH, LoGerfo JP. Community exercise program use and changes in healthcare costs for older adults. Am J Prev Med. 2003;25(3):232–7. doi:10.1016/S0749-3797(03)00196-X

289. US Department of Health and Human Services. 2008 Physical Activity Guidelines for Americans [Internet]. Washington DC: US Department of Health and Human Services; 2008.

290. US Department of Health and Human Services. Physical Activity Guidelines for Americans, 2nd edition. Washington DC: US Department of Health and Human Services; 2018.

291. PAGAC. 2018 Physical Activity Guidelines Advisory Committee Scientific Report. Washington, DC: U.S. Department of Health and Human Services; 2018.

292. NCHS. Health, United States, 2018. Hyattsville, MD: National Center for Health Statistics; 2018.

293. Troiano RP, Berrigan D, Dodd KW, Mâsse LC, Tilert T, Mcdowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc. 2008;40(1):181–8. doi:10.1249/mss.0b013e31815a51b3

294. Visser M, Brychta RJ, Chen KY, Koster A. Self-reported adherence to the physical activity recommendation and determinants of misperception in older adults. J Aging Phys Act. 2014;22(2):226–34. doi:10.1123/japa.2012-0219

295. Mitchell CJ, Churchward-Venne TA, West DWD, Burd NA, Breen L, Baker SK, et al. Resistance exercise load does not determine training-mediated hypertrophic gains in young men. J Appl Physiol. 2012;113(1):71–7. doi:10.1152/japplphysiol.00307.2012

296. Van Roie E, Delecluse C, Coudyzer W, Boonen S, Bautmans I. Strength training at high versus low external resistance in older adults: Effects on muscle volume, muscle strength, and force–velocity characteristics. Exp Gerontol. 2013;48(11):1351–61. doi:10.1016/j.exger.2013.08.010

297. Hamer M, Lavoie KL, Bacon SL. Taking up physical activity in later life and healthy ageing: the English longitudinal study of ageing. Br J Sports Med. 2014;48(3):239–43. doi:10.1136/bjsports-2013-092993

298. Kelly P, Kahlmeier S, Götschi T, Orsini N, Richards J, Roberts N, et al. Systematic review and meta-analysis of reduction in all-cause mortality from walking and cycling and shape of dose response relationship. Int J Behav Nutr Phys Act. 2014;11(1):132. doi:10.1186/s12966-014-0132-x

299. Hamer M, Chida Y. Walking and primary prevention: A meta-analysis of prospective cohort studies. Br J Sports Med. 2008;42(4):238–43. doi:10.1136/bjsm.2007.039974

300. Sadarangani KP, Hamer M, Mindell JS, Coombs NA, Stamatakis E. Physical activity and risk of all-cause and cardiovascular disease mortality in diabetic adults

from Great Britain: Pooled analysis of 10 population-based cohorts. Diabetes Care. 2014;37(4):1016–23. doi:10.2337/dc13-1816

301. Wahid A, Manek N, Nichols M, Kelly P, Foster C, Webster P, et al. Quantifying the association between physical activity and cardiovascular disease and diabetes: A systematic review and meta-analysis. J Am Heart Assoc. 2016;5:e002495. doi:10.1161/JAHA.115.002495

302. Cornelissen VA, Smart NA. Exercise training for blood pressure: A systematic review and meta-analysis. J Am Heart Assoc. 2013;2:e004473. doi:10.1161/JAHA.112.004473

303. Lin X, Zhang X, Guo J, Roberts CK, McKenzie S, Wu W, et al. Effects of exercise training on cardiorespiratory fitness and biomarkers of cardiometabolic health: A systematic review and meta-analysis of randomized controlled trials. J Am Heart Assoc. 2015;4:e002014. doi:10.1161/JAHA.115.002014

304. Kyu HH, Bachman VF, Alexander LT, Mumford JE, Afshin A, Estep K, et al. Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the Global Burden of Disease Study 2013. BMJ. 2016;354:i3857. doi:10.1136/bmj.i3857

305. Moholdt T, Wisløff U, Lydersen S, Nauman J. Current physical activity guidelines for health are insufficient to mitigate long-term weight gain: more data in the fitness

versus fatness debate (The HUNT study, Norway). Br J Sports Med. 2014;48(20):1489–96. doi:10.1136/bjsports-2014-093416

306. Franz MJ, VanWormer JJ, Crain AL, Boucher JL, Histon T, Caplan W, et al. Weight-loss outcomes: A systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. J Am Diet Assoc. 2007;107(10):1755–67. doi:10.1016/j.jada.2007.07.017

307. Gillespie LD, Robertson MC, Gillespie WJ, Sherrington C, Gates S, Clemson LM, et al. Interventions for preventing falls in older people living in the community. Cochrane Bone, Joint and Muscle Trauma Group, editor. Cochrane Database Syst Rev. 2012;(9):CD007146. doi:10.1002/14651858.CD007146.pub3

308. Benedetti MG, Furlini G, Zati A, Letizia Mauro G. The effectiveness of physical exercise on bone density in osteoporotic patients. BioMed Res Int. 2018;2018:4840531. doi:10.1155/2018/4840531

309. Tak E, Kuiper R, Chorus A, Hopman-Rock M. Prevention of onset and progression of basic ADL disability by physical activity in community dwelling older adults: A meta-analysis. Ageing Res Rev. 2013;12(1):329–38. doi:10.1016/j.arr.2012.10.001

310. Izquierdo M, Ibanez J, Häkkinen K, Kraemer WJ, Larrion JL, Gorostiaga EM. Once weekly combined resistance and cardiovascular training in healthy older men. Med Sci Sports Exerc. 2004;36(3):435–43. doi:10.1249/01.MSS.0000117897.55226.9A

311. Lovell DI, Cuneo R, Gass GC. Can aerobic training improve muscle strength and power in older men? J Aging Phys Act. 2010;18(1):14–26. doi:10.1123/japa.18.1.14

312. Harber MP, Konopka AR, Douglass MD, Minchev K, Kaminsky LA, Trappe TA, et al. Aerobic exercise training improves whole muscle and single myofiber size and function in older women. Am J Physiol-Regul Integr Comp Physiol. 2009;297(5):R1452-9. doi:10.1152/ajpregu.00354.2009

313. Hollings M, Mavros Y, Freeston J, Fiatarone Singh M. The effect of progressive resistance training on aerobic fitness and strength in adults with coronary heart disease: A systematic review and meta-analysis of randomised controlled trials. Eur J Prev Cardiol. 2017;24(12):1242–59. doi:10.1177/2047487317713329

314. Timmons JF, Minnock D, Hone M, Cogan KE, Murphy JC, Egan B. Comparison of time-matched aerobic, resistance, or concurrent exercise training in older adults. Scand J Med Sci Sports. 2018;28(11):2272–83. doi:10.1111/sms.13254

315. Wernbom M, Augustsson J, Thomee R. The influence of frequency, intensity, volume and mode of strength training on whole muscle cross-sectional area in humans. Sports Med. 2007;37(3):225–64. doi:10.2165/00007256-200737030-00004

316. Grgic J, Mcllvenna LC, Fyfe JJ, Sabol F, Bishop DJ, Schoenfeld BJ, et al. Does aerobic training promote the same skeletal muscle hypertrophy as resistance training? A systematic review and meta-analysis. Sports Med. 2019;49(2):233–54. doi:10.1007/s40279-018-1008-z

317. Konopka AR, Suer MK, Wolff CA, Harber MP. Markers of human skeletal muscle mitochondrial biogenesis and quality control: Effects of age and aerobic exercise training. J Gerontol Ser A. 2014;69(4):371–8. doi:10.1093/gerona/glt107

318. Konopka AR, Douglass MD, Kaminsky LA, Jemiolo B, Trappe TA, Trappe S, et al. Molecular adaptations to aerobic exercise training in skeletal muscle of older women. J Gerontol A Biol Sci Med Sci. 2010;65A(11):1201–7. doi:10.1093/gerona/glq109

319. Harber MP, Konopka AR, Undem MK, Hinkley JM, Minchev K, Kaminsky LA, et al. Aerobic exercise training induces skeletal muscle hypertrophy and age-dependent adaptations in myofiber function in young and older men. J Appl Physiol. 2012;113(9):1495–504. doi:10.1152/japplphysiol.00786.2012

320. Konopka AR, Harber MP. Skeletal muscle hypertrophy after aerobic exercise training. Exerc Sport Sci Rev. 2014;42(2):53–61. doi:10.1249/JES.0000000000000007

321. Lee H, Kim I-G, Sung C, Jeon T-B, Cho K, Ha Y-C, et al. Exercise training increases skeletal muscle strength independent of hypertrophy in older adults aged 75 years and older. Geriatr Gerontol Int. 2019;19(3):265–70. doi:10.1111/ggi.13597

322. Ozaki H, Sakamaki M, Yasuda T, Fujita S, Ogasawara R, Sugaya M, et al. Increases in thigh muscle volume and strength by walk training with leg blood flow reduction

in older participants. J Gerontol A Biol Sci Med Sci. 2011;66A(3):257–63. doi:10.1093/gerona/glq182

323. Kubo K, Ishida Y, Suzuki S, Komuro T, Shirasawa H, Ishiguro N, et al. Effects of 6 months of walking training on lower limb muscle and tendon in elderly: Effects of walking on the muscle and tendon. Scand J Med Sci Sports. 2007;18(1):31–9. doi:10.1111/j.1600-0838.2007.00654.x

324. Rooks DS, Kiel DP, Parsons C, Hayes WC. Self-paced resistance training and walking exercise in community-dwelling older adults: Effects on neuromotor performance. J Gerontol A Biol Sci Med Sci. 1997;52A(3):M161–8. doi:10.1093/gerona/52A.3.M161

325. Crane JD, MacNeil LG, Tarnopolsky MA. Long-term aerobic exercise is associated with greater muscle strength throughout the life span. J Gerontol A Biol Sci Med Sci. 2013;68(6):631–8. doi:10.1093/gerona/gls237

326. Brightwell CR, Markofski MM, Moro T, Fry CS, Porter C, Volpi E, et al. Moderate-intensity aerobic exercise improves skeletal muscle quality in older adults. Transl Sports Med. 2019;2(3):109–19. doi:10.1002/tsm2.70

327. Kubo K, Ishida Y, Suzuki S, Komuro T, Shirasawa H, Ishiguro N, et al. Effects of 6 months of walking training on lower limb muscle and tendon in elderly: Effects of walking on the muscle and tendon. Scand J Med Sci Sports. 2007;18(1):31–9. doi:10.1111/j.1600-0838.2007.00654.x

328. Fry CS, Noehren B, Mula J, Ubele MF, Westgate PM, Kern PA, et al. Fibre type-specific satellite cell response to aerobic training in sedentary adults. J Physiol. 2014;592(12):2625–35. doi:10.1113/jphysiol.2014.271288

329. Broskey NT, Greggio C, Boss A, Boutant M, Dwyer A, Schlueter L, et al. Skeletal muscle mitochondria in the elderly: Effects of physical fitness and exercise training. J Clin Endocrinol Metab. 2014;99(5):1852–61. doi:10.1210/jc.2013-3983

330. Peterson MD, Sen A, Gordon PM. Influence of resistance exercise on lean body mass in aging adults: A meta-analysis. Med Sci Sports Exerc. 2011;43(2):249–58. doi:10.1249/MSS.0b013e3181eb6265

331. Peterson MD, Rhea MR, Sen A, Gordon PM. Resistance exercise for muscular strength in older adults: A meta-analysis. Ageing Res Rev. 2010;9(3):226–37. doi:10.1016/j.arr.2010.03.004

332. Churchward-Venne TA, Tieland M, Verdijk LB, Leenders M, Dirks ML, de Groot LCPGM, et al. There are no nonresponders to resistance-type exercise training in older men and women. J Am Med Dir Assoc. 2015;16(5):400–11. doi:10.1016/j.jamda.2015.01.071

333. Steib S, Schoene D, Pfeifer K. Dose–response relationship of resistance training in older adults: A meta-analysis. Med Sci Sports Exerc. 2010;42(5):902–14.

334. Bean JF, Kiely DK, LaRose S, O'Neill E, Goldstein R, Frontera WR. Increased velocity exercise specific to task training versus the National Institute on Aging's

strength training program: Changes in limb power and mobility. J Gerontol A Biol Sci Med Sci. 2009;64A(9):983–91. doi:10.1093/gerona/glp056

335. Walker S, Häkkinen K. Similar increases in strength after short-term resistance training due to different neuromuscular adaptations in young and older men. J Strength Cond Res. 2014;28(11):3041–8. doi:10.1519/JSC.0000000000000381

336. Roth SM, Ivey FM, Martel GF, Lemmer JT, Hurlbut DE, Siegel EL, et al. Muscle size responses to strength training in young and older men and women. J Am Geriatr Soc. 2001;49(11):1428–33. doi:10.1046/j.1532-5415.2001.4911233.x

337. Häkkinen K, Newton RU, Gordon SE, McCormick M, Volek JS, Nindl BC, et al. Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. J Gerontol A Biol Sci Med Sci. 1998;53A(6):B415–23. doi:10.1093/gerona/53A.6.B415

338. Newton RU, Häkkinen K, Häkkinen A, Mccormick M, Volek J, Kraemer WJ. Mixed-methods resistance training increases power and strength of young and older men. Med Sci Sports Exerc. 2002;34(8):1367–75. doi:10.1097/00005768-200208000-00020

339. Lemmer JT, Hurlbut DE, Martel GF, Tracy BL, Ey FM, Metter EJ, et al. Age and gender responses to strength training and detraining. Med Sci Sports Exerc. 2000;32(8):1505–12. doi:10.1097/00005768-200008000-00021

340. Macaluso A, De Vito G, Felici F, Nimmo MA. Electromyogram changes during sustained contraction after resistance training in women in their 3rd and 8th decades. Eur J Appl Physiol. 2000;82:418–24. doi:10.1007/s004210000212

341. Raue U, Slivka D, Minchev K, Trappe S. Improvements in whole muscle and myocellular function are limited with high-intensity resistance training in octogenarian women. J Appl Physiol. 1985;106(5):1611–7.

342. Martel GF, Roth SM, Ivey FM, Lemmer JT, Tracy BL, Hurlbut DE, et al. Age and sex affect human muscle fibre adaptations to heavy-resistance strength training. Exp Physiol. 2006;91(2):457–64. doi:10.1113/expphysiol.2005.032771

343. Evans W. Reversing sarcopenia: How weight training can build strength and vitality. Geriatrics. 1996;51(5):46–54.

344. Hunter GR, McCarthy JP, Bamman MM. Effects of resistance training on older adults. Sports Med. 2004;34(5):329–48. doi:10.2165/00007256-200434050-00005

345. Going SB, Laudermilk M. Osteoporosis and strength training. Am J Lifestyle Med. 2009;3(4):310–9. doi:10.1177/1559827609334979

346. MacDonald HV, Johnson BT, Huedo-Medina TB, Livingston J, Forsyth KC, Kraemer WJ, et al. Dynamic resistance training as stand-alone antihypertensive lifestyle therapy: A meta-analysis. J Am Heart Assoc. 2016;5:e003231. doi:10.1161/JAHA.116.003231

347. Moro T, Brightwell CR, Volpi E, Rasmussen BB, Fry CS. Resistance exercise training promotes fiber type-specific myonuclear adaptations in older adults. J Appl Physiol. 2020;128(4):795–804. doi:10.1152/japplphysiol.00723.2019

348. Snijders T, Nederveen JP, Joanisse S, Leenders M, Verdijk LB, van Loon LJC, et al. Muscle fibre capillarization is a critical factor in muscle fibre hypertrophy during resistance exercise training in older men: Muscle fibre capillarization and exercise training in older men. J Cachexia Sarcopenia Muscle. 2017;8(2):267–76. doi:10.1002/jcsm.12137

349. Andersen LL, Andersen JL, Zebis MK, Aagaard P. Early and late rate of force development: differential adaptive responses to resistance training? Scand J Med Sci Sports. 2009;20(1):e162–9. doi:10.1111/j.1600-0838.2009.00933.x

350. Canepari M, Rossi R, Pellegrino MA, Orrell RW, Cobbold M, Harridge S, et al. Effects of resistance training on myosin function studied by the in vitro motility assay in young and older men. J Appl Physiol. 2005;98(6):2390–5. doi:10.1152/japplphysiol.01103.2004

351. Chesley A, MacDougall JD, Tarnopolsky MA, Atkinson SA, Smith K. Changes in human muscle protein synthesis after resistance exercise. J Appl Physiol. 1992;73(4):1383–8. doi:10.1152/jappl.1992.73.4.1383

352. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol-Endocrinol Metab. 1997;273(1):E99–107. doi:10.1152/ajpendo.1997.273.1.E99

353. Drummond MJ, Dreyer HC, Pennings B, Fry CS, Dhanani S, Dillon EL, et al. Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. J Appl Physiol. 2008;104(5):1452–61. doi:10.1152/japplphysiol.00021.2008

354. Yarasheski KE, Zachwieja JJ, Bier DM. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. Am J Physiol-Endocrinol Metab. 1993;265(2):E210–4. doi:10.1152/ajpendo.1993.265.2.E210

355. Bennie JA, De Cocker K, Teychenne MJ, Brown WJ, Biddle SJH. The epidemiology of aerobic physical activity and muscle-strengthening activity guideline adherence among 383,928 U.S. adults. Int J Behav Nutr Phys Act. 2019;16(1):34. doi:10.1186/s12966-019-0797-2

356. Stamatakis E, Lee I-M, Bennie J, Freeston J, Hamer M, O'Donovan G, et al. Does strength-promoting exercise confer unique health benefits? A pooled analysis of data on 11 population cohorts with all-cause, cancer, and cardiovascular mortality endpoints. Am J Epidemiol. 2018;187(5):1102–12. doi:10.1093/aje/kwx345

357. Liu Y, Lee D-C, Li Y, Zhu W, Zhang R, Sui X, et al. Associations of resistance exercise with cardiovascular disease morbidity and mortality. Med Sci Sports Exerc. 2019;51(3):499–508. doi:10.1249/MSS.0000000000001822

358. Keum N, Bao Y, Smith-Warner SA, Orav J, Wu K, Fuchs CS, et al. Association of physical activity by type and intensity with digestive system cancer risk. JAMA Oncol. 2016;2(9):1146. doi:10.1001/jamaoncol.2016.0740

359. Grøntved A, Rimm EB, Willett WC, Andersen LB, Hu FB. A prospective study of weight training and risk of type 2 diabetes mellitus in men. Arch Intern Med. 2012;172(17):1306. doi:10.1001/archinternmed.2012.3138

360. Gelecek N, İlçin N, Subaşi SS, Acar S, Demir N, Örmen M. The effects of resistance training on cardiovascular disease risk factors in postmenopausal women: A randomized-controlled trial. Health Care Women Int. 2012;33(12):1072–85. doi:10.1080/07399332.2011.645960

361. Tanasescu M, Leitzmann MF, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Exercise type and intensity in relation to coronary heart disease in men. JAMA. 2002;288(16):1994–2000.

362. Yang Z, Scott CA, Mao C, Tang J, Farmer AJ. Resistance exercise versus aerobic exercise for type 2 diabetes: A systematic review and meta-analysis. Sports Med. 2014;44(4):487–99. doi:10.1007/s40279-013-0128-8

363. Shiroma EJ, Cook NR, Manson JE, Moorthy M, Buring JE, Rimm EB, et al. Strength training and the risk of type 2 diabetes and cardiovascular disease. Med Sci Sports Exerc. 2017;49(1):40–6. doi:10.1249/MSS.0000000000001063

364. Smutok MA, Reece C, Kokkinos PF, Farmer C, Dawson P, Shulman R, et al. Aerobic versus strength training for risk factor intervention in middle-aged men at high risk for coronary heart disease. Metabolism. 1993;42(2):177–84. doi:10.1016/0026-0495(93)90032-J

365. Ihalainen JK, Inglis A, Mäkinen T, Newton RU, Kainulainen H, Kyröläinen H, et al. Strength training improves metabolic health markers in older individual regardless of training frequency. Front Physiol. 2019;10:32. doi:10.3389/fphys.2019.00032

366. Villareal DT, Aguirre L, Gurney AB, Waters DL, Sinacore DR, Colombo E, et al. Aerobic or resistance exercise, or both, in dieting obese older adults. N Engl J Med. 2017;376(20):1943–55. doi:10.1056/NEJMoa1616338

367. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JFP, Dela F. Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. Diabetes. 2004;53:294–305.

368. Ozaki H, Loenneke JP, Thiebaud RS, Abe T. Resistance training induced increase in VO2max in young and older subjects. Eur Rev Aging Phys Act. 2013;10(2):107–16. doi:10.1007/s11556-013-0120-1

369. Jackson AS, Sui X, Hébert JR, Church TS, Blair SN. Role of lifestyle and aging on the longitudinal change in cardiorespiratory fitness. Arch Intern Med. 2009;169(19):1781–7. doi:10.1001/archinternmed.2009.312

370. Parry HA, Roberts MD, Kavazis AN. Human skeletal muscle mitochondrial adaptations following resistance exercise training. Int J Sports Med. 2020;41(06):349–59. doi:10.1055/a-1121-7851

371. Ibañez J, Izquierdo M, Argüelles I, Forga L, Larrion JL, García-Unciti M, et al. Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes. Diabetes Care. 2005;28(3):662–7. doi:10.2337/diacare.28.3.662

372. Kelley G, Kelley K. Impact of progressive resistance training on lipids and lipoproteins in adults: A meta-analysis of randomized controlled trials. Prev Med. 2009;48(1):9–19. doi:https://doi.org/10.1016/j.ypmed.2008.10.010

373. Gordon BA, Benson AC, Bird SR, Fraser SF. Resistance training improves metabolic health in type 2 diabetes: A systematic review. Diabetes Res Clin Pract. 2009;83(2):157–75. doi:10.1016/j.diabres.2008.11.024

374. Weston M, Taylor KL, Batterham AM, Hopkins WG. Effects of low-volume high-intensity interval training (HIT) on fitness in adults: A meta-analysis of controlled and non-controlled trials. Sports Med. 2014;44(7):1005–17. doi:10.1007/s40279-014-0180-z

375. Bouaziz W, Malgoyre A, Schmitt E, Lang P, Vogel T, Kanagaratnam L. Effect of high-intensity interval training and continuous endurance training on peak oxygen uptake among seniors aged 65 or older: A meta-analysis of randomized controlled trials. Int J Clin Pract. 2020;74:e13490. doi:10.1111/ijcp.13490

376. Weston KS, Wisløff U, Coombes JS. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. Br J Sports Med. 2014;48(16):1227–34. doi:10.1136/bjsports-2013-092576

377. De Nardi AT, Tolves T, Lenzi TL, Signori LU, Silva AMV da. High-intensity interval training versus continuous training on physiological and metabolic variables in prediabetes and type 2 diabetes: A meta-analysis. Diabetes Res Clin Pract. 2018;137:149–59. doi:10.1016/j.diabres.2017.12.017

378. Stensvold D, Viken H, Steinshamn SL, Dalen H, Støylen A, Loennechen JP, et al. Effect of exercise training for five years on all cause mortality in older adults—the Generation 100 study: randomised controlled trial. BMJ. 2020;371:m3485. doi:10.1136/bmj.m3485

379. Wilson JM, Marin PJ, Rhea MR, Wilson SMC, Loenneke JP, Anderson JC. Concurrent training: A meta-analysis examining interference of aerobic and resistance exercises. J Strength Cond Res. 2012;26(8):2293–307. doi:10.1519/JSC.0b013e31823a3e2d

380. Martinez-Valdes E, Falla D, Negro F, Mayer F, Farina D. Differential motor unit changes after endurance or high-intensity interval training. Med Sci Sports Exerc. 2017;49(6):1126–36. doi:10.1249/MSS.0000000000001209

381. Siahkouhian M, Khodadadi D, Shahmoradi K. Effects of high-intensity interval training on aerobic and anaerobic indices: Comparison of physically active and inactive men. Sci Sports. 2013;28(5):e119-25. doi:10.1016/j.scispo.2012.11.006

382. Burgomaster KA, Howarth KR, Phillips SM, Rakobowchuk M, MacDonald MJ, McGee SL, et al. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans: Metabolic adaptations to sprint or endurance training in humans. J Physiol. 2008;586(1):151–60. doi:10.1113/jphysiol.2007.142109

383. Marzuca-Nassr GN, Artigas-Arias M, Olea M, SanMartín-Calísto Y, Huard N, Durán-Vejar F, et al. High-intensity interval training on body composition, functional capacity and biochemical markers in healthy young versus older people. Exp Gerontol. 2020;141:111096. doi:10.1016/j.exger.2020.111096

384. Nederveen JP, Joanisse S, Séguin CML, Bell KE, Baker SK, Phillips SM, et al. The effect of exercise mode on the acute response of satellite cells in old men. Acta Physiol. 2015;215(4):177–90. doi:10.1111/apha.12601

385. Boukabous I, Marcotte-Chénard A, Amamou T, Boulay P, Brochu M, Tessier D, et al. Low-volume high-intensity interval training versus moderate-intensity

continuous training on body composition, cardiometabolic profile, and physical capacity in older women. J Aging Phys Act. 2019;27(6):879–89. doi:10.1123/japa.2018-0309

386. Keogh JW, Grigg J, Vertullo CJ. Is high-intensity interval cycling feasible and more beneficial than continuous cycling for knee osteoarthritic patients? Results of a randomised control feasibility trial. PeerJ. 2018;6:e4738. doi:10.7717/peerj.4738

387. Buckinx F, Gaudreau P, Marcangeli V, Boutros GEH, Dulac MC, Morais JA, et al. Muscle adaptation in response to a high-intensity interval training in obese older adults: effect of daily protein intake distribution. Aging Clin Exp Res. 2019;31(6):863–74. doi:10.1007/s40520-019-01149-y

388. Buckinx F, Carvalho L, Marcangeli V, Dulac M, Hajj Boutros G, Gouspillou G, et al. High intensity interval training combined with L-citrulline supplementation: Effects on physical performance in healthy older adults. Exp Gerontol. 2020;140:111036. doi:10.1016/j.exger.2020.111036

389. Nitschke JE. Reliability of isokinetic torque measurements: A review of the literature. Aust J Physiother. 1992;38(2):125–34. doi:10.1016/S0004-9514(14)60557-2

390. Maffiuletti NA, Bizzini M, Desbrosses K, Babault N, Munzinger U. Reliability of knee extension and flexion measurements using the Con-Trex isokinetic

dynamometer. Clin Physiol Funct Imaging. 2007;27(6):346–53. doi:10.1111/j.1475-097X.2007.00758.x

391. de Ruiter CJ, Kooistra RD, Paalman MI, de Haan A. Initial phase of maximal voluntary and electrically stimulated knee extension torque development at different knee angles. J Appl Physiol. 2004;97(5):1693–701. doi:10.1152/japplphysiol.00230.2004

392. Moskowitz S, Russ DW, Clark LA, Wages NP, Grooms DR, Woods AJ, et al. Is impaired dopaminergic function associated with mobility capacity in older adults? GeroScience [Internet]. 2020 [cited 2020 Dec 7];Online ahead of print. doi:10.1007/s11357-020-00303-z

393. Kim WK, Kim D-K, Seo KM, Kang SH. Reliability and validity of isometric knee extensor strength test with hand-held dynamometer depending on its fixation: A pilot study. Ann Rehabil Med. 2014;38(1):84. doi:10.5535/arm.2014.38.1.84

394. Wilk M, Golas A, Zmijewski P, Krzysztofik M, Filip A, Coso JD, et al. The effects of the movement tempo on the one-repetition maximum bench press results. J Hum Kinet. 2020;72(1):151–9. doi:10.2478/hukin-2020-0001

395. System 4 Biodex multi-joint system: Operation Manual. Biodex Medical Systems, Inc.; 2017.

396. Pollock ML, Carroll JF, Graves JE, Leggett SH, Braith RW, Limacher M, et al. Injuries and adherence to walk/jog and resistance training programs in the elderly. Med Sci Sports Exerc. 1991;23(10):1194–200.

397. Shaw C, McCully K, Posner J. Injuries during one repetition maximum assessment in the elderly. J Cardpulm Rehabil. 1995;15(4):283–7. doi:10.1097/00008483-199507000-00005

398. Thomas M, Fiatarone M, Fielding R. Leg power in young women: Relationship to body composition, strength, and function. Med Sci Sports Exerc. 1996;28(10):1321–6.

399. Fielding RA, LeBrasseur NK, Cuoco A, Bean J, Mizer K, Singh MAF. High-velocity resistance training increases skeletal muscle peak power in older women. J Am Geriatr Soc. 2002;50(4):655–62. doi:10.1046/j.1532-5415.2002.50159.x

400. Sim Y-J, Byun Y-H, Yoo J. Comparison of isokinetic muscle strength and muscle power by types of warm-up. J Phys Ther Sci. 2015;27(5):1491–4. doi:10.1589/jpts.27.1491

401. Buckthorpe MW, Hannah R, Pain TG, Folland JP. Reliability of neuromuscular measurements during explosive isometric contractions, with special reference to electromyography normalization techniques. Muscle Nerve. 2012;46(4):566–76. doi:10.1002/mus.23322

402. Clark BC, Cook SB, Ploutz-Snyder LL. Reliability of techniques to assess human neuromuscular function in vivo. J Electromyogr Kinesiol. 2007;17(1):90–101. doi:10.1016/j.jelekin.2005.11.008

403. Hannah R, Folland JP. Muscle-tendon unit stiffness does not independently affect voluntary explosive force production or muscle intrinsic contractile properties. Appl Physiol Nutr Metab. 2015;40(1):87–95. doi:10.1139/apnm-2014-0343

404. Tillin NA, Jimenez-Reyes P, Pain MTG, Folland JP. Neuromuscular performance of explosive power athletes versus untrained individuals. Med Sci Sports Exerc. 2010;42(4):781–90. doi:10.1249/MSS.0b013e3181be9c7e

405. Allison GT. Trunk muscle onset detection technique for EMG signals with ECG artefact. J Electromyogr Kinesiol. 2003;13(3):209–16. doi:10.1016/S1050-6411(03)00019-1

406. Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Increased rate of force development and neural drive of human skeletal muscle following resistance training. J Appl Physiol. 2002 Oct 1;93(4):1318–26. doi:10.1152/japplphysiol.00283.2002

407. Johnson ST, Kipp K, Norcross MF, Hoffman MA. Spinal and supraspinal motor control predictors of rate of torque development. Scand J Med Sci Sports. 2015;25(5):623–9. doi:10.1111/sms.12283

408. Clark DJ, Patten C, Reid KF, Carabello RJ, Phillips EM, Fielding RA. Muscle performance and physical function are associated with voluntary rate of neuromuscular activation in older adults. J Gerontol A Biol Sci Med Sci. 2011;66A(1):115–21. doi:10.1093/gerona/glq153

409. Tillin NA, Pain MTG, Folland JP. Contraction type influences the human ability to use the available torque capacity of skeletal muscle during explosive efforts. Proc R Soc B Biol Sci. 2012;279(1736):2106–15. doi:10.1098/rspb.2011.2109

410. Cogliati M, Cudicio A, Toscani F, Gaffurini P, Bissolotti LM, Orizio C, et al. Normalized maximal rate of torque development during voluntary and stimulated static contraction in human tibialis anterior: Influence of age. Exp Gerontol. 2020;138:110999. doi:10.1016/j.exger.2020.110999

411. Kwon M, Senefeld JW, Hunter SK. Attenuated activation of knee extensor muscles during fast contractions in older men and women. Eur J Appl Physiol. 2020;120(10):2289–99. doi:10.1007/s00421-020-04451-0

412. Reibe D, Ehrman JK, Liguori G, Magal M. ACSM's guidelines for exercise testing and prescription. Tenth. Wolters Kluwer; 2018.

413. Huggett DL, Connelly DM, Overend TJ. Maximal aerobic capacity testing of older adults: A critical review. J Gerontol A Biol Sci Med Sci. 2005;60(1):57–66. doi:10.1093/gerona/60.1.57

414. Knight DR, Poole DC, Schaffartzik W, Guy HJ, Prediletto R, Hogan MC, et al. Relationship between body and leg VO2 during maximal cycle ergometry. J Appl Physiol. 1992;73(3):1114–21. doi:10.1152/jappl.1992.73.3.1114

415. Dal Corso S, Duarte SR, Neder JA, Malaguti C, de Fuccio MB, de Castro Pereira CA, et al. A step test to assess exercise-related oxygen desaturation in interstitial lung disease. Eur Respir J. 2006;29(2):330–6. doi:10.1183/09031936.00094006

416. Jones S, Tillin T, Williams S, Coady E, Chaturvedi N, Hughes A. Assessment of exercise capacity and oxygen consumption using a 6 min stepper test in older adults. Front Pharmacol. 2017;8:408. doi:10.3389/fphys.2017.00408

417. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R. Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. J Appl Physiol. 1998;85(1):115–22.

418. Engstrom CM, Loeb GE, Reid JG, Forrest WJ, Avruch L. Morphometry of the human thigh muscles. A comparison between anatomical sections and computer tomographic and magnetic resonance images. J Anat. 1991;176:139–56.

419. Cawthon PM. Assessment of lean mass and physical performance in sarcopenia. J Clin Densitom. 2015 Oct;18(4):467–71. doi:10.1016/j.jocd.2015.05.063

420. Pahor M, Manini T, Cesari M. Sarcopenia: Clinical evaluation, biological markers and other evaluation tools. J Nutr Health Aging. 2009;13(8):724–8. doi:10.1007/s12603-009-0204-9

421. Lund H, Christensen L, Savnik A, Danneskiold-Samsøe B, Bliddal H. Volume estimation of extensor muscles of the lower leg based on MR imaging. Eur Radiol. 2002;12:1982–7.

422. Morse CI, Degens H, Jones DA. The validity of estimating quadriceps volume from single MRI cross-sections in young men. Eur J Appl Physiol. 2007;100(3):267–74. doi:10.1007/s00421-007-0429-4

423. Gacesa JP, Dragnic NR, Prvulovic NM, Barak OF, Grujic N. The validity of estimating triceps brachii volume from single MRI cross-sectional area before and after resistance training. J Sports Sci. 2011;29(6):635–41. doi:10.1080/02640414.2010.549498

424. Clark BC, Tavoian D, Goodpaster BH, Cawthon PM, Hansen RD, Manini TM. Comment on: “Pitfalls in the measurement of muscle mass: A need for a reference standard” by Buckinx et al.: Correspondence. J Cachexia Sarcopenia Muscle. 2018 Dec;9(7):1269–71. doi:10.1002/jcsm.12372

425. Kim J, Wang Z, Heymsfield SB, Baumgartner RN, Gallagher D. Total-body skeletal muscle mass: Estimation by a new dual-energy X-ray absorptiometry method. Am J Clin Nutr. 2002;76(2):378–83.

426. Bridge P, Pocock NA, Nguyen T, Munns C, Cowell CT, Forwood N, et al. Validation of longitudinal DXA changes in body composition from pre- to mid-

adolescence using MRI as reference. J Clin Densitom. 2011;14(3):340–7. doi:10.1016/j.jocd.2011.04.005

427. Freda PU, Shen W, Reyes-Vidal CM, Geer EB, Arias-Mendoza F, Gallagher D, et al. Skeletal muscle mass in acromegaly assessed by magnetic resonance imaging and dual-photon X-ray absorptiometry. J Clin Endocrinol Metab. 2009 Aug;94(8):2880–6. doi:10.1210/jc.2009-0026

428. Zhao X, Wang Z, Zhang J, Hua J, He W, Zhu S. Estimation of total body skeletal muscle mass in Chinese adults: Prediction model by dual-energy X-ray absorptiometry. Yue J, editor. PLoS ONE. 2013 Jan 7;8(1):e53561. doi:10.1371/journal.pone.0053561

429. Lee S, Kuk JL. Changes in fat and skeletal muscle with exercise training in obese adolescents: Comparison of whole-body MRI and dual energy X-ray absorptiometry. Obesity. 2013;21(10):2063–71. doi:10.1002/oby.20448

430. Delmonico MJ, Kostek MC, Johns J, Hurley BF, Conway JM. Can dual-energy X-ray absorptiometry provide a valid assessment of changes in thigh muscle mass with strength training in older adults? Eur J Clin Nutr. 2008;62(12):1372–8.

431. Nelson M, Fiatarone M, Layne J, Trice I, Economos C, Fielding R, et al. Analysis of body-composition techniques and models for detecting change in soft tissue with strength training. Am J Clin Nutr. 1996;63(5):678–86.

432. Nindl BC, Harman EA, Marx JO, Gotshalk LA, Frykman PN, Lammi E, et al. Regional body composition changes in women after 6 months of periodized physical training. J Appl Physiol. 2000;88(6):2251–9.

433. Fielding R, Bhasin S, Basaria S, Storer T, Clark B, Law T, et al. OC3- A randomized, double-blind, placebo-controlled, parallel group, multicenter study of the safety and bioeffect of regn1033 with and without exercise in healthy subjects (NCT01910220). J Frailty Aging. 2015;4(1):25.

434. Knapp KM, Welsman JR, Hopkins SJ, Shallcross A, Fogelman I, Blake GM. Obesity increases precision errors in total body dual-energy X-ray absorptiometry measurements. J Clin Densitom. 2015;18(2):209–16. doi:10.1016/j.jocd.2014.06.001

435. Cawthon PM, Orwoll ES, Peters KE, Ensrud KE, Cauley JA, Kado DM, et al. Strong relation between muscle mass determined by D3-creatine dilution, physical performance, and incidence of falls and mobility limitations in a prospective cohort of older men. J Gerontol Ser A. 2019 May 16;74(6):844–52. doi:10.1093/gerona/gly129

436. Bloch K, Schoenheimer R. Studies in protein metabolism. J Biol Chem. 1939;131:111–9.

437. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol Rev. 2000;80(3):1108–203.

438. Heymsfield SB, Arteaga C, McManus C, Smith J, Moffitt S. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. Am J Clin Nutr. 1983;37(3):478–94. doi:10.1093/ajcn/37.3.478

439. Proctor DN, O'Brien PC, Atkinson EJ, Nair KS. Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. Am J Physiol-Endocrinol Metab. 1999;277(3):E489–95. doi:10.1152/ajpendo.1999.277.3.E489

440. Stimpson SA, Leonard MS, Clifton LG, Poole JC, Turner SM, Shearer TW, et al. Longitudinal changes in total body creatine pool size and skeletal muscle mass using the D_3 -creatine dilution method. J Cachexia Sarcopenia Muscle. 2013;4(3):217–23. doi:10.1007/s13539-013-0110-1

441. Farina D, Blanchietti A, Pozzo M, Merletti R. M-wave properties during progressive motor unit activation by transcutaneous stimulation. J Appl Physiol. 2004;97(2):545–55. doi:10.1152/japplphysiol.00064.2004

442. Gobbo M, Gaffurini P, Bissolotti L, Esposito F, Orizio C. Transcutaneous neuromuscular electrical stimulation: Influence of electrode positioning and stimulus amplitude settings on muscle response. Eur J Appl Physiol. 2011;111(10):2451–9. doi:10.1007/s00421-011-2047-4

443. Burton C, Sajja A, Latthe PM. Effectiveness of percutaneous posterior tibial nerve stimulation for overactive bladder: A systematic review and meta-analysis. Neurourol Urodyn. 2012;31(8):1206–16. doi:10.1002/nau.22251

444. Requena Sánchez B, Padial Puche P, González-Badillo JJ. Percutaneous electrical stimulation in strength training: An update. J Strength Cond Res. 2005;19(2):438. doi:10.1519/13173.1

445. Feiereisen P, Duchateau J, Hainaut K. Motor unit recruitment order during voluntary and electrically induced contractions in the tibialis anterior: Exp Brain Res. 1997;114(1):117–23. doi:10.1007/PL00005610

446. Paillard T. Training based on electrical stimulation superimposed onto voluntary contraction would be relevant only as part of submaximal contractions in healthy subjects. Front Physiol. 2018;9:1428. doi:10.3389/fphys.2018.01428

447. Botter A, Oprandi G, Lanfranco F, Allasia S, Maffiuletti NA, Minetto MA. Atlas of the muscle motor points for the lower limb: Implications for electrical stimulation procedures and electrode positioning. Eur J Appl Physiol. 2011;111(10):2461–71. doi:10.1007/s00421-011-2093-y

448. Konrad P. The ABC of EMG - A practical introduction to kinesiological electromyography. Noraxon; 2006.

449. Forrester BJ, Petrofsky JS. Effect of electrode size, shape, and placement during electrical stimulation. 2004;4(2):346–54.

450. Harridge SDR, Bottinelli R, Canepari M, Pellegrino MA, Reggiani C, Esbjörnsson M, et al. Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans. Pflüg Arch - Eur J Physiol. 1996;432(5):913–20. doi:10.1007/s004240050215

451. Moss C. Comparison of the histochemical and contractile properties of human triceps surae. Med Biol Eng Comput. 1992;30:600–4.

452. Alon G. Principles of electrical stimulation. In: Hayes K, Currier D, Nelson R, editors. Clinical electrotherapy. Upper Saddle River, NJ: Prentice Hall; 1999.

453. de Ruiter CJ, Jones DA, Sargeant AJ, de Haan A. Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. Exp Physiol. 1999;84(6):1137–50. doi:10.1111/j.1469-445X.1999.01895.x

454. Deutekom M, Beltman JGM, de Ruiter CJ, de Koning JJ, de Haan A. No acute effects of short-term creatine supplementation on muscle properties and sprint performance. Eur J Appl Physiol. 2000 Jun 13;82(3):223–9. doi:10.1007/s004210050675

455. Gregory CM, Dixon W, Bickel CS. Impact of varying pulse frequency and duration on muscle torque production and fatigue. Muscle Nerve. 2007;35(4):504–9. doi:10.1002/mus.20710

456. De Luca C. The use of surface electromyography in biomechanics. Jounal Appl Biomech. 1997;13(2):135–63.

457. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG: an update. J Appl Physiol. 2014;117(11):1215–30. doi:10.1152/japplphysiol.00162.2014

458. Keenan KG, Farina D, Maluf KS, Merletti R, Enoka RM. Influence of amplitude cancellation on the simulated surface electromyogram. J Appl Physiol. 2005;98(1):120–31. doi:10.1152/japplphysiol.00894.2004

459. Kuiken TA, Lowery MM, Stoykov NS. The effect of subcutaneous fat on myoelectric signal amplitude and cross-talk. Prosthet Orthot Int. 2003;27(1):48–54. doi:10.3109/03093640309167976

460. Del Balso C, Cafarelli E. Adaptations in the activation of human skeletal muscle induced by short-term isometric resistance training. J Appl Physiol. 2007 Jul;103(1):402–11. doi:10.1152/japplphysiol.00477.2006

461. Blazevich AJ, Horne S, Cannavan D, Coleman DR, Aagaard P. Effect of contraction mode of slow-speed resistance training on the maximum rate of force development in the human quadriceps. Muscle Nerve. 2008 Sep;38(3):1133–1046. doi:10.1002/mus.21021

462. Barry BK, Warman GE, Carson RG. Age-related differences in rapid muscle activation after rate of force development training of the elbow flexors. Exp Brain Res. 2005;162(1):122–32. doi:10.1007/s00221-004-2127-3

463. Gurjão ALD, Gobbi L, Carneiro N, Gonçalves R, de Moura R, Cyrino E, et al. Effect of strength training on rate of force development in older women. Res Q Exerc Sport. 2012;83(2):268–75.

464. Suetta C, Aagaard P, Rosted A, Jakobsen AK, Duus B, Kjaer M, et al. Training-induced changes in muscle CSA, muscle strength, EMG, and rate of force development in elderly subjects after long-term unilateral disuse. J Appl Physiol. 2004;97(5):1954–61. doi:10.1152/japplphysiol.01307.2003

465. Gruber M, Gruber SBH, Taube W, Schubert M, Beck SC, Gollhofer A. Differential effects of ballistic versus sensorimotor training on rate of force development and neural activation in humans. J Strength Cond Res. 2007;21(1):274–82. doi:10.1519/00124278-200702000-00049

466. Tillin NA, Pain MTG, Folland JP. Short-term training for explosive strength causes neural and mechanical adaptations: Neuromuscular adaptations with explosive strength training. Exp Physiol. 2012 May;97(5):630–41. doi:10.1113/expphysiol.2011.063040

467. LaRoche DP, Roy SJ, Knight CA, Dickie JL. Elderly women have blunted response to resistance training despite reduced antagonist coactivation. Med Sci Sports Exerc. 2008;40(9):1660–8. doi:10.1249/MSS.0b013e3181761561

468. Kobayashi Y, Ueyasu Y, Yamashita Y, Akagi R. Effects of 4 weeks of explosive-type strength training for the plantar flexors on the rate of torque development and postural stability in elderly individuals. Int J Sports Med. 2016;37(6):470–5. doi:10.1055/s-0035-1569367

469. Narici MV, Hoppeler H, Kayser B, Landoni L, Claassen H, Gavardi C, et al. Human quadriceps cross-sectional area, torque and neural activation during 6 months strength training. Acta Physiol Scand. 1996;157(2):175–86. doi:10.1046/j.1365-201X.1996.483230000.x

470. Holtermann A, Roeleveld K, Engstrøm M, Sand T. Enhanced H-reflex with resistance training is related to increased rate of force development. Eur J Appl Physiol. 2007;101(3):301–12. doi:10.1007/s00421-007-0503-y

471. Kazamel M, Warren PP. History of electromyography and nerve conduction studies: A tribute to the founding fathers. J Clin Neurosci. 2017;43:54–60. doi:10.1016/j.jocn.2017.05.018

472. Gazzoni M, Farina D, Merletti R. A new method for the extraction and classification of single motor unit action potentials from surface EMG signals. J Neurosci Methods. 2004;136(2):165–77. doi:10.1016/j.jneumeth.2004.01.002

473. De Luca CJ, Adam A, Wotiz R, Gilmore LD, Nawab SH. Decomposition of surface EMG signals. J Neurophysiol. 2006;96(3):1646–57. doi:10.1152/jn.00009.2006

474. Farina D, Holobar A, Merletti R, Enoka RM. Decoding the neural drive to muscles from the surface electromyogram. Clin Neurophysiol. 2010;121(10):1616–23. doi:10.1016/j.clinph.2009.10.040

475. Blok JH, van Dijk JP, Drost G, Zwarts MJ, Stegeman DF. A high-density multichannel surface electromyography system for the characterization of single motor units. Rev Sci Instrum. 2002;73(4):1887–97. doi:10.1063/1.1455134

476. Holobar A, Minetto MA, Farina D. Accurate identification of motor unit discharge patterns from high-density surface EMG and validation with a novel signal-based performance metric. J Neural Eng. 2014;11(1):016008. doi:10.1088/1741-2560/11/1/016008

477. McComas AJ, Fawcett PRW, Campbell MJ, Sica REP. Electrophysiological estimation of the number of motor units within a human muscle. J Neurol Neurosurg Psychiatry. 1971;34(2):121–31. doi:10.1136/jnnp.34.2.121

478. Gooch CL, Doherty TJ, Chan KM, Bromberg MB, Lewis RA, Stashuk DW, et al. Motor unit number estimation: A technology and literature review. Muscle Nerve. 2014;50(6):884–93. doi:10.1002/mus.24442

479. Mitsumoto H, Ulug AM, Pullman SL, Gooch CL, Chan S, Tang M-X, et al. Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. Neurology. 2007;68(17):1402–10. doi:10.1212/01.wnl.0000260065.57832.87

480. Bromberg MB, Swoboda KJ. Motor unit number estimation in infants and children with spinal muscular atrophy. Muscle Nerve. 2002;25(3):445–7. doi:10.1002/mus.10050

481. Carleton SA, Brown WF. Changes in motor unit populations in motor neurone disease. J Neurol Neurosurg Psychiatry. 1979;42(1):42–51. doi:10.1136/jnnp.42.1.42

482. Sheth KA, Iyer CC, Wier CG, Crum AE, Bratasz A, Kolb SJ, et al. Muscle strength and size are associated with motor unit connectivity in aged mice. Neurobiol Aging. 2018;67:128–6. doi:10.1016/j.neurobiolaging.2018.03.016

483. Piasecki M, Ireland A, Jones DA, McPhee JS. Age-dependent motor unit remodelling in human limb muscles. Biogerontology. 2016;17(3):485–96. doi:10.1007/s10522-015-9627-3

484. Brown WF. A method for estimating the number of motor units in thenar muscles and the changes in motor unit count with ageing. J Neurol Neurosurg Psychiatry. 1972;35(6):845–52. doi:10.1136/jnnp.35.6.845

485. de Carvalho M, Barkhaus PE, Nandedkar SD, Swash M. Motor unit number estimation (MUNE): Where are we now? Clin Neurophysiol. 2018;129(8):1507–16. doi:10.1016/j.clinph.2018.04.748

486. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr. 2006;84(3):475–82.

487. DeFronzo R. Glucose intolerance and aging. Diabetes Care. 1981;4(4):493–501. doi:10.2337/diacare.4.4.493

488. Weisberg H. Evaluation of nutritional status. Ann Clin Lab Sci. 1983;13(2):95–106.

489. Lukaski H. Sarcopenia: assessment of muscle mass. J Nutr. 1997;127(5):994S-997S.

490. Azagury DE, Ris F, Pichard C, Volonté F, Karsegard L, Huber O. Does perioperative nutrition and oral carbohydrate load sustainably preserve muscle mass after bariatric surgery? A randomized control trial. Surg Obes Relat Dis. 2015 Jul;11(4):920–6. doi:10.1016/j.soard.2014.10.016

491. Bhasin S, Travison TG, Storer TW, Lakshman K, Kaushik M, Mazer NA, et al. Effect of testosterone supplementation with and without a dual 5α-reductase inhibitor on fat-free mass in men with suppressed testosterone production: a randomized controlled trial. Jama. 2012;307(9):931–9.

492. Martins WR, Safons MP, Bottaro M, Blasczyk JC, Diniz LR, Fonseca RMC, et al. Effects of short term elastic resistance training on muscle mass and strength in

untrained older adults: a randomized clinical trial. BMC Geriatr [Internet]. 2015 Dec [cited 2017 Nov 17];15(1). doi:10.1186/s12877-015-0101-5

493. Amano S, Ludin AFM, Clift R, Nakazawa M, Law TD, Rush LJ, et al. Effectiveness of blood flow restricted exercise compared with standard exercise in patients with recurrent low back pain: study protocol for a randomized controlled trial. Trials [Internet]. 2016 Dec [cited 2017 Nov 13];17(1). doi:10.1186/s13063-016-1214-7

494. Ampomah K, Amano S, Wages N, Volz L, Clift R, Mat Ludin A, et al. Blood flow restricted exercise does not induce a cross-transfer of effect: and RCT. Med Sci Sports Exerc. 2019;In Press.

495. Segal NA, Glass NA, Baker JL, Torner JC. Correcting for fat mass improves DXA quantification of quadriceps specific strength in obese adults aged 50–59 years. J Clin Densitom. 2009 Jul;12(3):299–305. doi:10.1016/j.jocd.2008.11.003

496. Maden-Wilkinson TM, Degens H, Jones DA, McPhee JS. Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles. J Musculoskelet Neuronal Interact. 2013;13(3):320–8.

497. Hangartner TN, Warner S, Braillon P, Jankowski L, Shepherd J. The official positions of the International Society for Clinical Densitometry: Acquisition of dual-energy X-ray absorptiometry body composition and considerations regarding analysis and repeatability of measures. J Clin Densitom. 2013;16(4):520–36. doi:10.1016/j.jocd.2013.08.007

498. Bland J, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. Int J Nurs Stud. 2010 Aug;47(8):931–6.

499. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med. 1998;26(4):217–38.

500. Coin A, Sarti S, Ruggiero E, Giannini S, Pedrazzoni M, Minisola S, et al. Prevalence of sarcopenia based on different diagnostic criteria using DEXA and appendicular skeletal muscle mass reference values in an Italian population aged 20 to 80. J Am Med Dir Assoc. 2013 Jul;14(7):507–12. doi:10.1016/j.jamda.2013.02.010

501. Reijnierse EM, de van der Schueren MAE, Trappenburg MC, Doves M, Meskers CGM, Maier AB. Lack of knowledge and availability of diagnostic equipment could hinder the diagnosis of sarcopenia and its management. Mogi M, editor. PLoS ONE. 2017 Oct 2;12(10):e0185837. doi:10.1371/journal.pone.0185837

502. Bechshøft RL, Reitelseder S, Højfeldt G, Castro-Mejía JL, Khakimov B, Ahmad HFB, et al. Counteracting age-related loss of skeletal muscle mass: a clinical and ethnological trial on the role of protein supplementation and training load (CALM Intervention Study): study protocol for a randomized controlled trial. Trials [Internet]. 2016 Dec [cited 2018 Mar 20];17(1). doi:10.1186/s13063-016-1512-0

503. Zembron-Lacny A, Dziubek W, Rogowski L, Skorupka E, Dabrowska G. Sarcopenia: Monitoring, molecular mechanisms, and physical intervention. Physiol Res. 2014;63:683–91.

504. Pietrobelli A, Formica C, Wang Z, Heymsfield SB. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. Am J Physiol-Endocrinol Metab. 1996 Dec;271(6):E941–51. doi:10.1152/ajpendo.1996.271.6.E941

505. Friedl KE, Moore RJ, Martinez-Lopez LE, Vogel JA, Askew EW, Marchitelli LJ, et al. Lower limit of body fat in healthy active men. J Appl Physiol. 1994 Aug;77(2):933–40. doi:10.1152/jappl.1994.77.2.933

506. Isidori AM, Giannetta E, Greco EA, Gianfrilli D, Bonifacio V, Isidori A, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol (Oxf). 2005;63(3):280–93. doi:10.1111/j.1365-2265.2005.02339.x

507. Bhasin S, Bagatell CJ, Bremner WJ, Plymate SR, Tenover JL, Korenman SG, et al. Issues in testosterone replacement in older men. J Endocrinol Metab. 1998;83(10):3435–48.

508. Tracy BL, Ivey FM, Jeffrey Metter E, Fleg JL, Siegel EL, Hurley BF. A more efficient magnetic resonance imaging-based strategy for measuring quadriceps

muscle volume. Med Sci Sports Exerc. 2003 Mar;35(3):425–33. doi:10.1249/01.MSS.0000053722.53302.D6

509. Martí-Bonmatí L, Kormano M. MR equipment acquisition strategies: low-field or high-field scanners: Eur Radiol. 1997;7(S5):S263–8. doi:10.1007/PL00006906

510. Merton PA. Voluntary strength and fatigue. J Physiol. 1954;123(3):553–64. doi:10.1113/jphysiol.1954.sp005070

511. Babault N, Desbrosses K, Fabre M-S, Michaut A, Pousson M. Neuromuscular fatigue development during maximal concentric and isometric knee extensions. J Appl Physiol. 2006;100(3):780–5. doi:10.1152/japplphysiol.00737.2005

512. Behm D, Power K, Drinkwater E. Comparison of interpolation and central activation ratios as measures of muscle inactivation. Muscle Nerve. 2001;24(7):925–34. doi:10.1002/mus.1090

513. Behrens M, Mau-Moeller A, Mueller K, Heise S, Gube M, Beuster N, et al. Plyometric training improves voluntary activation and strength during isometric, concentric and eccentric contractions. J Sci Med Sport. 2016;19(2):170–6. doi:10.1016/j.jsams.2015.01.011

514. Clark BC, Mahato NK, Nakazawa M, Law TD, Thomas JS. The power of the mind: the cortex as a critical determinant of muscle strength/weakness. J Neurophysiol. 2014;112(12):3219–26. doi:10.1152/jn.00386.2014

515. Clark BC, Manini TM, Wages NP, Simon JE, Clark LA. Voluntary vs electrically stimulated activation in age-related muscle weakness. JAMA Netw Open. 2019;2(9):e1912052. doi:10.1001/jamanetworkopen.2019.12052

516. Clark BC, Taylor JL, Hong SL, Law TD, Russ DW. Weaker seniors exhibit motor cortex hypoexcitability and impairments in voluntary activation. J Gerontol A Biol Sci Med Sci. 2015;70(9):1112–9. doi:10.1093/gerona/glv030

517. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001;81(4):1725–89. doi:10.1152/physrev.2001.81.4.1725

518. Hvid LG, Strotmeyer ES, Skjødt M, Magnussen LV, Andersen M, Caserotti P. Voluntary muscle activation improves with power training and is associated with changes in gait speed in mobility-limited older adults — A randomized controlled trial. Exp Gerontol. 2016;80:51–6. doi:10.1016/j.exger.2016.03.018

519. Jensen L, Djurtoft JB, Bech RD, Nielsen JL, Jørgensen LH, Schrøder HD, et al. Influence of resistance training on neuromuscular function and physical capacity in ALS patients. J Neurodegener Dis. 2017;2017:1436519. doi:10.1155/2017/1436519

520. Kent-Braun JA, Ng AV. Specific strength and voluntary muscle activation in young and elderly women and men. J Appl Physiol. 1999;87(1):22–9. doi:10.1152/jappl.1999.87.1.22

521. Mizner RL, Petterson SC, Stevens JE, Snyder-Mackler L. Early quadriceps strength loss after total knee arthroplasty; The contributions of muscle atrophy and failure of

voluntary muscle activation. J Bone Jt Surg Am. 2005;87(5):1047–53. doi:10.2106/JBJS.D.01992

522. Stackhouse SK, Stevens JE, Lee SC, Pearce KM, Snyder-Mackler L, Binder-Macleod SA. Maximum voluntary activation in nonfatigued and fatigued muscle of young and elderly individuals. Phys Ther. 2001;81(5):1102–9. doi:10.1093/ptj/81.5.1102

523. Steens A, Heersema DJ, Maurits NM, Renken RJ, Zijdewind I. Mechanisms underlying muscle fatigue differ between multiple sclerosis patients and controls: A combined electrophysiological and neuroimaging study. NeuroImage. 2012;59(4):3110–8. doi:10.1016/j.neuroimage.2011.11.038

524. de Haan A, de Ruiter C, van der Woude L, Jongen P. Contractile properties and fatigue of quadriceps muscles in multiple sclerosis. Muscle Nerve. 2000;23(10):1534–41. doi:10.1002/1097-4598(200010)23:10<1534::aid-mus9>3.0.co;2-d

525. Del Vecchio A, Negro F, Falla D, Bazzucchi I, Farina D, Felici F. Higher muscle fiber conduction velocity and early rate of torque development in chronically strength-trained individuals. J Appl Physiol. 2018;125(4):1218–26. doi:10.1152/japplphysiol.00025.2018

526. Tillin NA, Pain MTG, Folland J. Explosive force production during isometric squats correlates with athletic performance in rugby union players. J Sports Sci. 2013 Jan;31(1):66–76. doi:10.1080/02640414.2012.720704

527. West DJ, Owen NJ, Jones MR, Bracken RM, Cook CJ, Cunningham DJ, et al. Relationships between force–time characteristics of the isometric midthigh pull and dynamic performance in professional rugby league players. J Strength Cond Res. 2011;25(11):3070–5. doi:10.1519/JSC.0b013e318212dcd5

528. Maffiuletti NA, Bizzini M, Widler K, Munzinger U. Asymmetry in quadriceps rate of force development as a functional outcome measure in TKA. Clin Orthop Relat Res. 2010;468(1):191–8. doi:10.1007/s11999-009-0978-4

529. Del Vecchio A, Negro F, Holobar A, Casolo A, Folland JP, Felici F, et al. You are as fast as your motor neurons: Speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. J Physiol. 2019;597(9):2445–56. doi:10.1113/JP277396

530. Dideriksen JL, Del Vecchio A, Farina D. Neural and muscular determinants of maximal rate of force development. J Neurophysiol. 2020;123(1):149–57. doi:10.1152/jn.00330.2019

531. Lin DC, McGowan CP, Blum KP, Ting LH. Yank: The time derivative of force is an important biomechanical variable in sensorimotor systems. J Exp Biol. 2019;222(18):jeb180414. doi:10.1242/jeb.180414

532. Clark LA, Manini TM, Wages NP, Simon JE, Russ DW, Clark BC. Reduced neural excitability and activation contribute to clinically meaningful weakness in older adults. Newman AB, editor. J Gerontol Ser A. 2020;Online ahead of print:glaa157. doi:10.1093/gerona/glaa157

533. Russ DW, Clark BC, Krause J, Hagerman FC. Development of a neuromuscular electrical stimulation protocol for sprint training. Med Sci Sports Exerc. 2012 Sep;44(9):1810–9. doi:10.1249/MSS.0b013e31825423f1

534. Thomas S, Reading J, Shepard R. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Can J Spt Sci. 1992;17(4):338–45.

535. Eid M, Gollwitzer M, Schmitt M. Statistik und Forschungsmethoden. 5th ed. Psychologie Verlagsunion; 2015.

536. Lenhard W, Lenhard A. Hypothesis tests for comparing correlations. [Internet]. Psychometrica. 2014. URL https://www.psychometrica.de/correlation.html.

537. Hinkle D, Wiersma W, Jurs S. Applied statistics for the behavioral sciences. 5th ed. Boston, MA: Houghton Mifflin; 2003.

538. Clark BC, Issac LC, Lane JL, Damron LA, Hoffman RL. Neuromuscular plasticity during and following 3 wk of human forearm cast immobilization. J Appl Physiol. 2008;105:11. doi:10.1152/japplphysiol.90530.2008

539. Clark BC, Fernhall B, Ploutz-Snyder LL. Adaptations in human neuromuscular function following prolonged unweighting: I. Skeletal muscle contractile properties and applied ischemia efficacy. J Appl Physiol. 2006;101(1):256–63. doi:10.1152/japplphysiol.01402.2005

540. Clark BC, Manini TM, Bolanowski SJ, Ploutz-Snyder LL. Adaptations in human neuromuscular function following prolonged unweighting: II. Neurological properties and motor imagery efficacy. J Appl Physiol. 2006;101(1):264–72. doi:10.1152/japplphysiol.01404.2005

541. Tesarz J, Schuster AK, Hartmann M, Gerhardt A, Eich W. Pain perception in athletes compared to normally active controls: A systematic review with meta-analysis. Pain. 2012;153(6):1253–62. doi:10.1016/j.pain.2012.03.005

542. Tavoian D, Russ DW, Law TD, Simon JE, Chase PJ, Guseman EH, et al. A randomized clinical trial comparing three different exercise strategies for optimizing aerobic capacity and skeletal muscle performance in older adults: Protocol for the DART study. Front Med. 2019;6:236. doi:10.3389/fmed.2019.00236

543. Seeman TE, Merkin SS, Crimmins EM, Karlamangla AS. Disability trends among older Americans: National Health and Nutrition Examination Surveys, 1988–1994 and 1999–2004. Am J Public Health. 2010;100(1):100–7. doi:10.2105/AJPH.2008.157388

544. Matos MA de, Vieira DV, Pinhal KC, Lopes JF, Dias-Peixoto MF, Pauli JR, et al. High-intensity interval training improves markers of oxidative metabolism in skeletal muscle of individuals with obesity and insulin resistance. Front Physiol. 2018;9:1451. doi:10.3389/fphys.2018.01451

545. Perry CGR, Heigenhauser GJF, Bonen A, Spriet LL. High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. Appl Physiol Nutr Metab. 2008;33(6):1112–23. doi:10.1139/H08-097

546. Lusardi MM, Pellecchia GL, Schulman M. Functional performance in community living older adults. J Geriatr Phys Ther. 2003;26(3):14–22. doi:10.1519/00139143-200312000-00003

547. Steffen TM, Hacker TA, Mollinger L. Age- and gender-related test performance in community-dwelling elderly people: six-minute walk test, Berg balance scale, timed up & go test, and gait speeds. Phys Ther. 2002;82(2):128–37.

548. Camarri B, Eastwood PR, Cecins NM, Thompson PJ, Jenkins S. Six minute walk distance in healthy subjects aged 55–75 years. Respir Med. 2006;100(4):658–65. doi:10.1016/j.rmed.2005.08.003

549. Troosters T, Gosselink R, Decramer M. Six minute walking distance in healthy elderly subjects. Eur Respir J. 1999;14(2):270–4. doi:10.1034/j.1399-3003.1999.14b06.x

550. Khan I, Sarker S-J, Hackshaw A. Smaller sample sizes for phase II trials based on exact tests with actual error rates by trading-off their nominal levels of significance and power. Br J Cancer. 2012;107(11):1801–9. doi:10.1038/bjc.2012.444

551. Osawa Y, Tabata S, Katsukawa F, Ishida H, Oguma Y, Kawai T, et al. Effects of 16-week high-intensity interval training using upper and lower body ergometers on aerobic fitness and morphological changes in healthy men: a preliminary study. Open Access J Sports Med. 2014;5:257–65. doi:10.2147/OAJSM.S68932

552. Abe T, Fujita S, Nakajima T, Sakamaki M, Ozaki H, Oga R. Effects of low-intensity cycle training with restricted leg blood flow on thigh muscle volume and VO2max in young men. J Sports Sci Med. 2010;9:452–8.

553. Schjerve IE, Tyldum GA, Tjønna AE, Stølen T, Loennechen JP, Hansen HEM, et al. Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. Clin Sci. 2008;115(9):283–93. doi:10.1042/CS20070332

554. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: Association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol. 1994;49(2):M85-94. doi:10.1093/geronj/49.2.M85

555. Mason RE, Likar I. A new system of multiple-lead exercise electrocardiography. Am Heart J. 1966;71(2):196–205.

556. Kligfield P, Gettes LS, Bailey JJ, Childers R, Deal BJ, Hancock EW, et al. Recommendations for the standardization and interpretation of the electrocardiogram: Part I: The electrocardiogram and its technology. Circulation. 2007;115(10):1306–24. doi:10.1161/CIRCULATIONAHA.106.180200

557. Borg G. Perceived exertion as an indicator of somatic stress. J Rehabil Med. 1970;2:92–8.

558. Moore M, Barker K. The validity and reliability of the four square step test in different adult populations: a systematic review. Syst Rev. 2017;6(187). doi:10.1186/s13643-017-0577-5

559. Dite W, Temple VA. A clinical test of stepping and change of direction to identify multiple falling older adults. Arch Phys Med Rehabil. 2002;83(11):1566–71. doi:10.1053/apmr.2002.35469

560. Shiratori AP, Iop R da R, Júnior NGB, Domenech SC, Gevaerd M da S. Evaluation protocols of hand grip strength in individuals with rheumatoid arthritis: a systematic review. Rev Bras Reumatol Engl Ed. 2014;54(2):140–7. doi:10.1016/j.rbre.2014.03.009

561. Mathiowetz V, Weber K, Volland G, Kashman N. Reliability and validity of grip and pinch strength evaluations. J Hand Surg. 1984;9(2):222–6. doi:10.1016/S0363-5023(84)80146-X

562. Huang G, Wang R, Chen P, Huang SC, Donnelly JE, Mehlferber JP. Dose–response relationship of cardiorespiratory fitness adaptation to controlled endurance training in sedentary older adults. Eur J Prev Cardiol. 2016;23(5):518–29. doi:10.1177/2047487315582322

563. Ratamess N, Alvar B, Evetoch T, Housh T, Kibler W, Kraemer W, et al. Progression models in resistance training for healthy adults. Med Sci Sports Exerc. 2009;41(3):687–708. doi:10.1249/MSS.0b013e3181915670

564. Hedges LV. Distribution theory for Glass's estimator of effect size and related estimators. J Educ Stat. 1981;6(2):107–28.

565. Sawilowsky SS. New effect size rules of thumb. J Mod Appl Stat Methods. 2009;8(2):597–9. doi:10.22237/jmasm/1257035100

566. Keating CJ, Párraga Montilla JÁ, Latorre Román PÁ, Moreno del Castillo R. Comparison of high-intensity interval training to moderate-intensity continuous training in older adults: A systematic review. J Aging Phys Act. 2020;28(5):798–807. doi:10.1123/japa.2019-0111

567. Consitt LA, Saxena G, Saneda A, Houmard JA. Age-related impairments in skeletal muscle PDH phosphorylation and plasma lactate are indicative of metabolic inflexibility and the effects of exercise training. Am J Physiol-Endocrinol Metab. 2016;311(1):E145–56. doi:10.1152/ajpendo.00452.2015

568. Consitt LA, Dudley C, Saxena G. Impact of endurance and resistance training on skeletal muscle glucose metabolism in older adults. Nutrients. 2019;11(11):2636. doi:10.3390/nu11112636

569. Ploug T, van Deurs B, Ai H, Cushman SW, Ralston E. Analysis of GLUT4 distribution in whole skeletal muscle fibers: Identification of distinct storage compartments that are recruited by insulin and muscle contractions. J Cell Biol. 1998;142(6):1429–46. doi:10.1083/jcb.142.6.1429

570. Coker RH, Hays NP, Williams RH, Brown AD, Freeling SA, Kortebein PM, et al. Exercise-induced changes in insulin action and glycogen metabolism in elderly adults. Med Sci Sports Exerc. 2006;38(3):433–8. doi:10.1249/01.mss.0000191417.48710.11

571. Mandrup C, Egelund J, Nyberg M, Enevoldsen L, Kjær A, Clemmensen A, et al. Effects of menopause and high-intensity training on insulin sensitivity and muscle metabolism. Menopause. 2018;25(2):165–75. doi:10.1097/GME.0000000000000981

572. Röhling M, Herder C, Stemper T, Müssig K. Influence of acute and chronic exercise on glucose uptake. J Diabetes Res. 2016;2016:1–33. doi:10.1155/2016/2868652

573. Torma F, Gombos Z, Jokai M, Takeda M, Mimura T, Radak Z. High intensity interval training and molecular adaptive response of skeletal muscle. Sports Med Health Sci. 2019;1(1):24–32. doi:10.1016/j.smhs.2019.08.003

574. Altenburg TM, Degens H, van Mechelen W, Sargeant AJ, de Haan A. Recruitment of single muscle fibers during submaximal cycling exercise. J Appl Physiol. 2007;103(5):1752–6. doi:10.1152/japplphysiol.00496.2007

575. Morton RW, Sonne MW, Zuniga AF, Mohammad IYZ, Jones A, McGlory C, et al. Muscle fibre activation is unaffected by load and repetition duration when resistance exercise is performed to task failure. J Physiol. 2019;13.

576. Bouaziz W, Schmitt E, Kaltenbach G, Geny B, Vogel T. Health benefits of cycle ergometer training for older adults over 70: A review. Eur Rev Aging Phys Act. 2015;12(1):8. doi:10.1186/s11556-015-0152-9

577. Hansen EA, Rønnestad BR. Effects of cycling training at imposed low cadences: A systematic review. Int J Sports Physiol Perform. 2017;12(9):1127–36. doi:10.1123/ijspp.2016-0574

578. Kristoffersen M, Gundersen H, Leirdal S, Iversen VV. Low cadence interval training at moderate intensity does not improve cycling performance in highly trained veteran cyclists. Front Physiol. 2014;5:34. doi:10.3389/fphys.2014.00034

579. Koninckx E, Leemputte MV, Hespel P. Effect of isokinetic cycling versus weight training on maximal power output and endurance performance in cycling. Eur J Appl Physiol. 2010;109(4):699–708. doi:10.1007/s00421-010-1407-9

580. Bohannon RW, Crouch R. Minimal clinically important difference for change in 6-minute walk test distance of adults with pathology: A systematic review. J Eval Clin Pract. 2017;23(2):377–81. doi:10.1111/jep.12629

581. De Brandt J, Spruit MA, Hansen D, Franssen FM, Derave W, Sillen MJ, et al. Changes in lower limb muscle function and muscle mass following exercise-based interventions in patients with chronic obstructive pulmonary disease: A review of the English-language literature. Chron Respir Dis. 2018;15(2):182–219. doi:10.1177/1479972317709642

582. Yazar T, Yazar H. Prevalance of sarcopenia according to decade. Clin Nutr ESPEN. 2019;29:137–41. doi:10.1016/j.clnesp.2018.11.005

583. Rasinaho M, Hirvensalo M, Leinonen R, Lintunen T, Rantanen T. Motives for and barriers to physical activity among older adults with mobility limitations. J Aging Phys Act. 2007;15(1):90–102. doi:10.1123/japa.15.1.90

584. Aily JB, Carnaz L, Farche ACS, Takahashi AC de M. Perception of barriers to physical exercise in women population over 60. Mot Rev Educ Física. 2017;23(2):e101653. doi:10.1590/s1980-6574201700020012

585. Burton E, Farrier K, Lewin G, Pettigrew S, Hill A-M, Airey P, et al. Motivators and barriers for older people participating in resistance training: A systematic review. J Aging Phys Act. 2017;25(2):311–24. doi:10.1123/japa.2015-0289

586. Burton E, Lewin G, Pettigrew S, Hill A-M, Bainbridge L, Farrier K, et al. Identifying motivators and barriers to older community-dwelling people participating in resistance training: A cross-sectional study. J Sports Sci. 2017;35(15):1523–32. doi:10.1080/02640414.2016.1223334

587. Kimura T, Kobayashi H, Nakayama E, Kakihana W. Seasonality in physical activity and walking of healthy older adults. J Physiol Anthropol. 2015;34(1):33. doi:10.1186/s40101-015-0071-5

588. Peçanha T, Goessler KF, Roschel H, Gualano B. Social isolation during the COVID-19 pandemic can increase physical inactivity and the global burden of cardiovascular disease. Am J Physiol-Heart Circ Physiol. 2020;318(6):H1441–6. doi:10.1152/ajpheart.00268.2020

589. Hammami A, Harrabi B, Mohr M, Krustrup P. Physical activity and coronavirus disease 2019 (COVID-19): Specific recommendations for home-based physical training. Manag Sport Leis [Internet]. 2020 [cited 2020 May 26]; doi:10.1080/23750472.2020.1757494

590. Craighead DH, Heinbockel TC, Hamilton MN, Bailey EF, MacDonald MJ, Gibala MJ, et al. Time-efficient physical training for enhancing cardiovascular function in midlife and older adults: promise and current research gaps. J Appl Physiol. 2019;127(5):1427–40. doi:10.1152/japplphysiol.00381.2019

Tables

Table 2.1. Clinically relevant physical function assessments in gerontological research

Task	Description
6-minute walk	Total distance walked in six minutes (m)
400-meter walk	Time to walk 400 meters (s)
Timed up-and-go	Time to rise from a chair, walk three meters, turn around and walk back to the chair and sit (s)
4-square step test	Time to complete an agility task consisting of stepping into subsequent quadrants of a square (s)
Stair climb	Time to climb a set number of stairs with or without weights (s)
Sit-to-stand	Time to rise from a chair 10 times (s)
SPPB	
4-meter gait speed	Self-selected walking speed (m/s)
Balance	Ability to maintain balance for 10 seconds in three different standing positions (y/n)
Chair rise	Time to rise from a chair five times (s)

Table 2.2. Physiological and non-physiological factors that influence the surface EMG signal

Factors that Influence the Surface EMG	
Non-physiological	
Anatomic	Shape of the volume conductor
	Thickness of the subcutaneous tissue layers
	Tissue inhomogeneities
	Distribution of the motor unit territories in the muscle
	Size of the motor unit territories
	Distribution and number of fibers in the motor unit territories
	Length of the fibers
	Spread of the endplates and tendon junctions within the motor units
	Spread of the innervation zones and tendon regions among motor units
Detection system	Presence of more than one pinnation angle
	Skin-electrode contact (impedance, noise)
	Spatial filter for signal detection
	Interelectrode distance
	Electrode size and shape
	Inclination of the detection system relative to muscle fiber orientation
	Location of electrodes over the muscle
Geometrical	Muscle fiber shortening
	Shift of the muscle relative to the detection system
Physical	Conductivity of the tissues
	Amount of crosstalk from nearby muscles
Physiological	
Fiber membrane properties	Average muscle fiber conduction velocity
	Distribution of motor unit conduction velocities
	Distribution of conduction velocities of the fibers within the motor units
	Shape of the intracellular action potentials
Motor unit properties	Number of recruited motor units
	Distribution of motor unit discharge rates
	Statistics and coefficient of variation for discharge rate
	Motor unit synchronization

EMG, electromyogram. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. J Appl Physiol. 2004;96(4):1486–95. doi:10.1152/japplphysiol.01070.200. Reprinted with permission (License#: 1095451-2)

Table 3.1. Participant characteristics before and after 10 weeks of low-load resistance exercise

		BFR	Control	*p*-values
	Participants (N)	12	14	
	% Female	75	50	
	Age (yrs)	28.0 ± 9.9	30.1 ± 9.4	0.58
	Body Mass (kg)	72.7 ± 15.6	72.9 ± 14.1	0.97
	Height (cm)	168.2 ± 9.4	171.3 ± 9.1	0.40
	BMI	25.6 ± 5.1	24.6 ± 3.2	0.57
MRI	Baseline Thigh Volume (cm^3)	988.5 ± 171.5	1124.8 ± 224.6	0.02*
MRI	Post Thigh Volume (cm^3)	999.6 ± 160.4	1125.4 ± 225.0	0.03*
MRI	Thigh Volume Change (%)	1.49 ± 5.83	0.28 ± 6.18	0.48
DXA	Baseline Thigh Lean Mass (kg)	4.98 ± 0.81	5.43 ± 1.15	0.11
DXA	Post Thigh Lean Mass (kg)	4.98 ± 0.80	5.53 ± 1.18	0.06
DXA	Thigh Lean Mass Change (%)	0.20 ± 3.98	1.79 ± 3.71	0.14

BFR, blood flow restricted experimental group; BMI, body mass index; DXA, dual-energy X-ray absorptiometer; MRI, magnetic resonance imager; *significant difference between BFR and control group.

Table originally published in Tavoian D, Ampomah K, Amano S, Law T, Clark B. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. Sci Rep. 2019;9:10028. doi:10.1038/s41598-019-46428-w

Table 4.1. Baseline characteristics of young and older adults and post-intervention characteristics of older adults

	Young Adults (N = 20)	Older Adults (N = 16)	Older Adults (Post) (N = 15)	Young vs Older *p*-value	Pre-Post *p*-value
Descriptives and Anthropometrics					
Female (N (%))	13 (65.0)	10 (62.5)	10 (66.7)	-	-
Age (yrs)	22.0 ± 1.7	72.3 ± 7.5	72.3 ± 7.7	-	-
Mass (kg)	70.7 ± 12.3	71.8 ± 13.9	73.8 ± 16.0	0.801	0.196
BMI (kg/m^2)	24.2 ± 2.9	26.4 ± 4.5	27.3 ± 5.3	0.112	0.100
Body Fat (%)	30.6 ± 7.5[d]	33.9 ± 9.8	34.2 ± 10.0	0.273	0.963
ALM (kg/m^2)	7.18 ± 1.66[d]	6.83 ± 1.29	6.66 ± 1.04	0.503	0.763
SPPB (0-12)	-	11.4 ± 1.0	11.9 ± 0.5[b]	-	**0.048**
Gait Speed (m/s)	-	1.01 ± 0.14	1.15 ± 0.18[b]	-	**0.004**
Voluntary Strength					
MVC (N-m)	201.6 ± 85.3	113.1 ± 48.5[a]	127.4 ± 53.8[b]	**< 0.001**	**0.012**
VA (%)	94.5 ± 3.9[c]	92.5 ± 4.7[e]	89.4 ± 6.8[f]	0.192	0.168
Contractile Properties					
Doublet Force (N-m)	58.3 ± 28.3	37.2 ± 12.9[a]	40.5 ± 13.2	**0.006**	0.116
$Yank_{EVOKED}$ (N-m/s)	1485.5 ± 537.8	910.6 ± 293.4[a]	928.5 ± 265.0	**<0.001**	0.872
Rate of Force Development					
$Yank_{VOLUNTARY}$ (N-m/s)	1082.2 ± 474.5	611.5 ± 241.1[a]	869.6 ± 308.1	**0.001**	0.216
$RFD_{0\text{-}50}$ (N-m/s)	846.6 ± 265.8	443.6 ± 206.3[a]	504.2 ± 230.3	**< 0.001**	0.309
$RFD_{50\text{-}100}$ (N-m/s)	958.7 ± 540.1	589.0 ± 257.7[a]	580.9 ± 259.1	**0.012**	0.563
$RFD_{100\text{-}150}$ (N-m/s)	612.8 ± 305.5	297.2 ± 166.2[a]	326.1 ± 199.3	**< 0.001**	0.695
$RFD_{150\text{-}200}$ (N-m/s)	435.9 ± 293.2	287.8 ± 136.5	335.1 ± 225.3	0.055	0.163

$NYank_{VOLUNTARY}$ (%MVC/s)	737.2 ± 125.6	760.8 ± 182.1	722.2 ± 192.0	0.650	0.374
$NRFD_{0\text{-}50}$ (%MVC/s)	437.5 ± 142.1	400.6 ± 140.9	426.7 ± 176.2	0.443	0.538
$NRFD_{50\text{-}100}$ (%MVC/s)	460.6 ± 98.2	538.5 ± 149.4	462.8 ± 130.8	0.068	0.073
$NRFD_{100\text{-}150}$ (%MVC/s)	304.7 ± 85.0	258.9 ± 111.0	247.2 ± 108.0	0.170	0.443
$NRFD_{150\text{-}200}$ (%MVC/s)	209.8 ± 92.0	256.6 ± 53.4	246.8 ± 57.6	0.081	0.682
Electromyography					
EMG_{RR} (%EMG_{MAX}/s)	1696.9 ± 1249.0	1652.3 ± 760.4	1639.2 ± 903.1	0.901	0.841
$EMG_{AVG0\text{-}50}$ (%EMG_{MAX})	41.90 ± 30.0	55.6 ± 18.4	66.2 ± 32.3	0.102	0.317
$EMG_{AVG50\text{-}100}$ (%EMG_{MAX})	126.1 ± 67.8	106.3 ± 37.3	106.7 ± 41.6	0.304	.0889
$EMG_{AVG100\text{-}150}$ (%EMG_{MAX})	119.9 ± 43.8	121.3 ± 42.9	105.4 ± 36.5	0.924	0.202
$EMG_{AVG150\text{-}200}$ (%EMG_{MAX})	107.6 ± 40.5	116.6 ± 43.0	101.6 ± 30.9	0.523	0.182
Central Activation Ballistic Force Ratio					
CAB_{YANK} (%$Yank_{EVOKED}$)	97.7 ± 18.5	92.9 ± 24.8	97.3 ± 31.9	0.511	0.621
$CAB_{0\text{-}50}$ (%$Yank_{EVOKED}$)	57.9 ± 18.3	48.4 ± 16.4	57.4 ± 26.8	0.116	0.195
$CAB_{50\text{-}100}$ (%$Yank_{EVOKED}$)	61.8 ± 17.1	66.2 ± 20.9	63.7 ± 22.9	0.488	0.621
$CAB_{100\text{-}150}$ (%$Yank_{EVOKED}$)	40.9 ±13.5	31.4 ± 12.5*	34.1 ± 17.5	**0.037**	0.787
$CAB_{150\text{-}200}$ (%$Yank_{EVOKED}$)	28.9 ± 15.1	31.9 ± 9.9	34.5 ± 13.1	0.487	0.558

Mean ± SD. Males and females are combined. Differences between young and older adults assessed with unpaired t-tests; [a]older adults significantly different from young adults, $p < 0.05$. Differences between pre- and post-intervention assessed with paired t-tests; [b]significant change from baseline in older adults, $p < 0.05$. [c]N=19; [d]N=18; [e]N=15; [f]N=14. ALM, appendicular lean mass; BMI, body mass index; CAB, central activation ballistic force ratio; EMG_{AVG}, average amplitude of the electromyograph signal at specific timepoint of the ballistic force-time curve; EMG_{MAX}, average amplitude of the electromyograph signal at peak force of maximal voluntary contractions; EMG_{RR}, rate of rise of the electromyograph signal; MVC, maximal voluntary contraction; NRFD, normalized rate of force development; $NYank_{VOLUNTARY}$, normalized voluntary peak rate of force development; SPPB, short physical performance

battery; RFD, rate of force development; VA, voluntary activation; $Yank_{EVOKED}$, peak rate of force development during the doublet-evoked contraction; $Yank_{VOLUNTARY}$, voluntary peak rate of force development.

Table 4.2. Pearson's bivariate correlation coefficients between baseline voluntary force, contractile properties, electromyography, and central activation ballistic force ratio variables and absolute and normalized rate of force development variables in young (N = 20) and older adults (N = 16).

		RFD				NRFD			
Time (ms):		0-50	50-100	100-150	150-200	0-50	50-100	100-150	150-200
Voluntary Force									
MVC	Young	0.73**	0.93**	0.80**	0.76**	-	-	-	-
	Old	0.69**	0.83**	0.75**	0.90**	-	-	-	-
	Combined	0.80**	0.92**	0.85**	0.79**	-	-	-	-
Contractile Properties									
Doublet Force	Young	0.67**	0.93**	0.80**	0.74**	-0.38	0.42	-0.01	0.17
	Old	0.39	0.68**§	0.81**	0.64**	-0.23	-0.12	0.47	-0.05
	Combined	0.70**	0.91**	0.84**	0.75**	-0.23	0.04	0.21	-0.01
$Yank_{EVOKED}$	Young	0.66**	0.89**	0.69**	0.54*	-0.25	0.42	-0.06	-0.01
	Old	0.66**	0.68**	0.77**	0.77**	0.10	-0.23‡	0.36	0.07
	Combined	0.77**	0.87**	0.79**	0.62**	-0.04	-0.05	0.20	-0.16
Electromyography									
EMG_{RR}	Young	0.57**	0.10	0.06	.054	0.48*	0.05	-.014	-0.12
	Old	-0.03§	0.14	-0.34	-0.34	0.23	0.47	-0.15	-0.09
	Combined	0.38*	0.06	-0.01	-0.01	0.39*	0.19	-0.13	-0.11
$EMG_{AVG0-50}$	Young	0.46*	-	-	-	0.68**	-	-	-
	Old	0.54*	-	-	-	0.74**	-	-	-
	Combined	0.23	-	-	-	0.61**	-	-	-
$EMG_{AVG50-100}$	Young	-	0.22	-	-	-	0.14	-	-
	Old	-	-0.11	-	-	-	0.46	-	-
	Combined	-	0.21	-	-	-	0.17	-	-
$EMG_{AVG100-150}$	Young	-	-	0.19	-	-	-	-0.07	-

	Old	-	-	0.30	-	-	-	0.34	-
	Combined	-	-	0.17	-	-	-	0.13	-
	Young	-	-	-	0.45*	-	-	-	0.43
$EMG_{AVG150-200}$	Old	-	-	-	0.18	-	-	-	-0.13
	Combined	-	-	-	0.30	-	-	-	0.26
Central Activation Ballistic Force Ratio									
	Young	0.60**	-	-	-	0.75**	-	-	-
CAB_{0-50}	Old	0.73**	-	-	-	0.60*	-	-	-
	Combined	0.65**	-	-	-	0.69**	-	-	-
	Young	-	0.79**	-	-	-	0.69**	-	-
CAB_{50-100}	Old	-	0.50*	-	-	-	0.66**	-	-
	Combined	-	0.53**	-	-	-	0.67**	-	-
	Young	-	-	0.73**	-	-	-	0.80**	-
$CAB_{100-150}$	Old	-	-	0.81**	-	-	-	0.74**	-
	Combined	-	-	0.77**	-	-	-	0.77**	-
	Young	-	-	-	0.86**	-	-	-	0.90**
$CAB_{150-200}$	Old	-	-	-	0.50*[§]	-	-	-	0.43[§]
	Combined	-	-	-	0.70**	-	-	-	0.79**

*$p \leq 0.05$; **$p \leq 0.01$; [§]Correlation coefficient of older adults significantly different than correlation coefficient of young adults.CAB, central activation ballistic force ratio; EMG_{AVG}, average amplitude of the electromyograph signal at specific timepoint of the ballistic force-time curve; EMG_{RR}, rate of rise of the electromyograph signal from 0-50 ms; MVC, maximal voluntary contraction; NRFD, normalized rate of force development; RFD, rate of force development; $Yank_{EVOKED}$, peak rate of force development during the doublet-evoked contraction.

Table 4.3. Pearson's bivariate correlation coefficients between exercise induced changes in voluntary force, contractile properties, electromyography, and central activation ballistic force ratio variables and absolute and normalized rate of force development variables in older adults (N=15).

	RFD				NRFD			
Time (ms):	0-50	50-100	100-150	150-200	0-50	50-100	100-150	150-200
Voluntary Force								
MVC	0.05	-0.19	0.53*	0.50	-	-	-	-
Contractile Properties								
Doublet Force	-0.23	0.25	0.46	0.05	-0.24	0.24	0.34	-0.10
$Yank_{EVOKED}$	0.42	-0.41	0.05	0.04	0.49	-0.37	0.16	-0.05
Electromyography								
EMG_{RR}	0.40	0.06	-0.67**	0.04	0.37	0.32	-0.58**	0.11
$EMG_{AVG0-50}$	0.65**	-	-	-	0.77**	-	-	-
$EMG_{AVG50-100}$	-	0.13	-	-	-	0.28	-	-
$EMG_{AVG100-150}$	-	-	0.10	-	-	-	0.24	-
$EMG_{AVG150-200}$	-	-	-	0.02	-	-	-	-0.05
Central Activation Ballistic Force Ratio								
CAB_{0-50}	0.74**	-	-	-	0.73**	-	-	-
CAB_{50-100}	-	0.80**	-	-	-	0.77**	-	-
$CAB_{100-150}$	-	-	0.80**	-	-	-	0.75**	-
$CAB_{150-200}$	-	-	-	0.71**	-	-	-	0.76**

*$p \leq 0.05$; **$p \leq 0.01$. CAB, central activation ballistic force ratio; EMG_{AVG}, average amplitude of the electromyograph signal at specific timepoint of the ballistic force-time curve; EMG_{RR}, rate of rise of the electromyograph signal; MVC, maximal voluntary contraction; NRFD, normalized rate of force development; RFD, rate of force development; $Yank_{EVOKED}$, peak rate of force development during the doublet-evoked contraction.

Table 4.4. Pearson's bivariate correlation coefficients between baseline voluntary activation, electromyography, and central activation ballistic force ratio variables in young (N = 20) and older adults (N = 16).

		CAB Force Ratio			
Time (ms):		0-50	50-100	100-150	150-200
VA	Young†	0.36	0.09	-0.10	-0.18
	Old‡	0.10	-0.15	0.12	-0.39
	Combined	0.28	-0.11	0.12	-0.28
EMG_{RR}	Young	0.58**	0.19	-0.01	0.02
	Old	0.00§	0.14	-0.35	-0.36
	Combined	0.39*	0.16	-0.10	-0.07
$EMG_{AVG0-50}$	Young	0.69**	-	-	-
	Old	0.31	-	-	-
	Combined	0.46**	-	-	-
$EMG_{AVG50-100}$	Young	-	0.37	-	-
	Old	-	-0.03	-	-
	Combined	-	0.19	-	-
$EMG_{AVG100-150}$	Young	-	-	0.25	-
	Old	-	-	0.24	-
	Combined	-	-	0.23	-
$EMG_{AVG150-200}$	Young	-	-	-	0.44
	Old	-	-	-	-0.02
	Combined	-	-	-	0.28

*$p \leq 0.05$; **$p \leq 0.01$; §Correlation coefficient of older adults significantly different than correlation coefficient of young adults. †N=19; ‡N=15. CAB, central activation ballistic force ratio; EMG_{AVG}, average amplitude of the electromyograph signal at specific timepoint of the ballistic force-time curve; EMG_{RR}, rate of rise of the electromyograph signal from 0-50 ms; VA, voluntary activation.

Table 4.5. Pearson's bivariate correlation coefficients between exercise induced changes in voluntary activation, electromyography, and central activation ballistic force ratio variables in older adults (N=15).

	CAB Force Ratio			
Time (ms):	0-50	50-100	100-150	150-200
VA	-0.34†	0.03†	-0.16†	-0.22†
EMG_{RR}	0.41	0.19	-0.61**	0.05
$EMG_{AVG0-50}$	0.65**	-	-	-
$EMG_{AVG50-100}$	-	0.06	-	-
$EMG_{AVG100-150}$	-	-	0.37	-
$EMG_{AVG150-200}$	-	-	-	0.05

*$p \leq 0.05$; **$p \leq 0.01$. †N=14. CAB, central activation ballistic force ratio; EMG_{AVG}, average amplitude of the electromyograph signal at specific timepoint of the ballistic force-time curve; EMG_{RR}, rate of rise of the electromyograph signal from 0-50 ms; VA, voluntary activation.

Table 5.1. Inclusion and exclusion criteria for the DART study

Inclusion Criteria:

- Age 60-75 years with no significant health issues or conditions that, in the investigators' opinion, would limit the subject's ability to complete the study per protocol or that would impact the capability to get an accurate measurement of study endpoints.
- Body mass index between 18 and 40 kg/m^2.
- Willingness to maintain current diet and adhere to the intervention programs described for the study and willing to undergo all testing procedures.
- Able to read, understand, and complete study-related questionnaires
- Able to read and understand, and willing to sign the informed consent form (ICF).
- 6-minute walk distance of 450-725 meters for men and 400-675 meters for women.

Exclusion Criteria:

- Short physical performance batter (SPPB) score < 8
- Any activities of daily living disability (difficulty feeding, dressing, continence, bathing, toileting, and transferring).
- Lives in a nursing home or assisted living facility
- Known neuromuscular or neurological conditions affecting somatosensory or motor function or control (e.g., hemiplegia, multiple sclerosis, peripheral neuropathy, Parkinson's disease, Myasthenia Gravis, Ataxia, Apraxia, post-polio syndrome, mitochondrial myopathy, etc.).

- Unable to communicate because of severe hearing loss or speech disorder.
- Severe visual impairment, which would preclude completion of the assessments.
- Cancer requiring treatment currently or in the past 2 years (except primary non-melanoma skin cancer or in situ cervical cancer)
- Hospitalization (medical confinement for 24 hours), or immobilization, or major surgical procedure requiring general anesthesia within 12 weeks prior to screening, or any planned surgical procedures during the study period.
- Chronic or relapsing/remitting gastrointestinal disorders such as inflammatory bowel disease and irritable bowel syndrome.
- Known history of human immunodeficiency virus (HIV) antibody at screening.
- Use of systemic glucocorticoids.
- Any history of angina pectoris
- Any history of heart failure
- Any history of myocardial infarction
- Any coronary artery bypass graft or percutaneous coronary intervention
- Heart disease that limits exercise (valvular, congenital, ischemic and hypertrophic cardiomyopathy)
- Complex ventricular arrhythmias or heart block
- Chronic obstructive pulmonary disease, cerebrovascular disease, or peripheral vascular disease
- Diabetes mellitus
- Severe neuropathy

- Mini-mental state exam score below 19
- Psychiatric conditions that warrant acute or chronic therapeutic intervention (e.g., major depressive disorder, bipolar disorder, panic disorder, schizophrenia) that in the investigators' opinion may interfere with the conduct of study procedures
- Unable to undergo magnetic resonance imaging (MRI) (e. g. body containing any metallic medical devices or equipment, including heart pacemakers, metal prostheses, implants or surgical clips, any prior injury from shrapnel or grinding metal, exposure to metallic dusts, metallic shavings or having tattoos containing metallic dyes).
- Unable to reliably undergo exercise or strength tests described for this study.
- Participation in progressive resistance exercise 2 or more days/week for most weeks over the 24 weeks prior to screening, OR 150+ minutes of accumulated aerobic exercise each week for most weeks over the 24 weeks prior to screening.
- Current self-reported activity level that, in the investigators' opinion, is considered highly active for older adults
- Participation in any clinical trial within 12 weeks prior to screening.
- Limb amputation (except for toes)
- Bone fracture within 24 weeks prior to screening
- Any disorder that will not allow completion of the motions required for resistance or aerobic exercise
- Conditions (such as myasthenia gravis, myositis, muscular dystrophy or myopathy, including drug-induced myopathy) leading to muscle loss, muscle weakness, muscle cramps or myalgia.
- Acute viral or bacterial upper or lower respiratory infection at screening

- Abnormal or uncontrolled blood pressure (BP) at the screening visit defined as BP > 170/100 mmHg. If taking anti-hypertensive medication, have to be on stable doses of medication for more than 3 months.
- Current or recent history (within 1 year of screen) of heavy alcohol consumption or drug abuse that in the investigators' opinion may interfere with the conduct of study procedures.
- Reports being pregnant, lactating, or that they anticipate becoming pregnant in the next 3-months. If a woman becomes pregnant while on study protocol, they will be withdrawn from the study

Prohibited Medications: Medications that, in the PIs opinion, would confound study integrity by interacting with study outcomes. For instance:

- Anti-obesity drugs, nutraceuticals, and dietary supplements that may affect body mass and body composition.
- Any drug or supplement known to influence muscle mass or performance including but not limited to anabolic steroids, insulin-like growth factor 1, growth hormone, replacement androgen therapy, anti-androgen therapy.

Table originally published in Tavoian D, Russ DW, Law TD, Simon JE, Chase PJ, Guseman EH, et al. A randomized clinical trial comparing three different exercise strategies for optimizing aerobic capacity and skeletal muscle performance in older adults: Protocol for the DART study. Front Med. 2019;6:236. doi:10.3389/fmed.2019.00236

Table 5.2. Schedule of events for the DART study

	Baseline Period			Exercise Intervention		Follow-up	
	Visit 1[c]	Visit 2[c]	Visit 3[c]	Sessions 1-35[a]	Session 36 (Visit 4)[b]	Visit 5[d]	Visit 6[d]
Day (window)	-21 to -5	-19 to -3	-17 to -1	1 to 79	80-83	84 to 92	86 to 94
Screening/Baseline:							
Informed Consent	X						
Medical History	X						
Inclusion/Exclusion	X						
Demographics	X						
Vitals	X						
Height and Weight	X						
Surveys:							
PASE	X						
MMSE	X						
SEE	X						
Exercise Sessions:							
Exercise				X	X		
Functional Tasks:							
4SST	X				X		
6-minute walk	X				X		
SPPB	X				X		
Stair climb	X				X		
Grip Strength	X				X		
Medical Imaging:							
MRI		X				X	
DXA		X				X	
Cardiorespiratory:							

GXT	X		X	
Muscular Testing:				
MVC		X		X
Ballistic		X		X
Twitch force		X		X
VA		X		X
Isokinetic power		X		X
Isokinetic fatigue		X		X
Randomization		X		

[a]Exercise training sessions will be performed three times per week with at least 1 day between sessions, and no more than two exercise sessions on consecutive days in the same week. [b]Post-intervention functional task testing will be performed immediately prior to the final exercise training session (session 36). [c]Baseline testing will be completed within 21 days of Visit 1, with at least two days between testing sessions. [d]Post-intervention testing will be completed within 10 days of the final exercise session, with at least two days between testing sessions. 4SST, 4-square step test; DXA, dual-energy X-ray Absorptiometry; GXT, graded exercise test; MMSE, mini-mental state examination; MRI, magnetic resonance imaging; MVC, maximal voluntary contraction; PASE, physical activity scale for the elderly; SEE, self-efficacy for exercise scale; SPPB, short physical performance battery; VA, voluntary activation.
Table originally published in Tavoian D, Russ DW, Law TD, Simon JE, Chase PJ, Guseman EH, et al. A randomized clinical trial comparing three different exercise strategies for optimizing aerobic capacity and skeletal muscle performance in older adults: Protocol for the DART study. Front Med. 2019;6:236. doi:10.3389/fmed.2019.00236

Table 5.3. Criteria for test termination during graded exercise testing

Criteria for maximal effort-related test termination	Criteria for health concern-related test termination
• Subject requests to stop • Physical or verbal manifestations of severe fatigue • Failure of heart rate to increase with increased exercise intensity • Unable to maintain a cycle frequency of 50 RPMs for greater than five seconds Or 2 of the 4 following criteria: • RPE of 17 or greater (Borg 6-20 scale) • Peak heart rate that is 85% of age-predicted maximal heart rate (220-age) • Plateau of VO2 • Respiratory exchange ratio (RER) of 1.1 or higher	• Angina or angina-like symptoms (subjective score of 2 or greater on ACSM angina scale) • Shortness of breath, wheezing, leg cramps, or claudication (subjective score of 3 or greater on ACSM claudication scale) • Signs of poor perfusion: light-headedness, confusion, ataxia, pallor, cyanosis, nausea, or cold and clammy skin • Drop in systolic blood pressure of >10 mm Hg, despite an increase in workload • Central nervous system symptoms (e.g., ataxia, dizziness, or near syncope) • Sustained ventricular tachycardia or other arrhythmia, including second- or third-degree atrioventricular block, that interferes with normal maintenance of cardiac output during exercise • Exaggerated hypertensive response (systolic blood pressure >250 mm Hg or diastolic blood pressure >115 mm Hg) • Abnormal changes in sinus rhythm • Exercise induced conduction blocks

ACSM, American College of Sports Medicine; RPE, rating of perceived exertion; RPM, rotations per minute; VO2, volume of oxygen consumption.

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Table 5.4. MICT and HIIT exercise groups cycling duration

Session #	1	2	3	4	5	6	7	8	9	10	11	12
	Week 1			Week 2			Week 3			Week 4		
MICT	20	20	30	30	20	30	30	30	30	30	30	30
HIIT	20	20	30	30	20	30	15	15	15	15	15	15
Session #	13	14	15	16	17	18	19	29	21	22	23	24
	Week 5			Week 6			Week 7			Week 8		
MICT	30	30	30	30	45	30	30	45	30	45	30	45
HIIT	15	20	15	15	20	15	20	15	20	20	15	20
Session #	25	26	27	28	29	30	31	32	33	34	35	36
	Week 9			Week 10			Week 11			Week 12		
MICT	45	45	45	45	45	45	45	45	45	45	45	45
HIIT	20	20	20	20	30	20	20	30	20	20	30	20

Duration (in minutes) of cycling for the moderate-intensity continuous training (MICT) and the high-intensity interval training (HIIT) groups for exercise sessions 1-36.

Table originally published in Tavoian D, Russ DW, Law TD, Simon JE, Chase PJ, Guseman EH, et al. A randomized clinical trial comparing three different exercise strategies for optimizing aerobic capacity and skeletal muscle performance in older adults: Protocol for the DART study. Front Med. 2019;6:236. doi:10.3389/fmed.2019.00236

Table 5.5. Resistance training group exercises

Daily Exercises	Rotating Exercises
Leg Press	Lunges
Knee Extensions	Step-Ups (weighted or unweighted)
Leg Curls	Hip Abduction
Calf Raises	Hip Bridge (single- or double-leg)
Chest Press	Box Squat
	Sumo Squat
	Planks (knee and elbows or knees and toes)
	Biceps Curls
	Push-ups (incline or flat)
	Seated Cable Pull-Down
	Seated Cable Row
	Triceps Extensions
	Shoulder Overhead Press
	Lateral Arm Raises

The five *Daily Exercises* are performed during each exercise session. The remaining five exercises each session will be chosen by the exercise supervisor from the list of *Rotating Exercises*.

Table originally published in Tavoian D, Russ DW, Law TD, Simon JE, Chase PJ, Guseman EH, et al. A randomized clinical trial comparing three different exercise strategies for optimizing aerobic capacity and skeletal muscle performance in older adults: Protocol for the DART study. Front Med. 2019;6:236. doi:10.3389/fmed.2019.00236

Table 5.6. Baseline and post-intervention characteristics

	HIIT		MICT		RT	
	Pre	Post	Pre	Post	Pre	Post
Age (years)	66.0 ± 3.3	-	65.3 ± 4.5	-	67.8 ± 4.5	-
N (% Female)	5 (80)	-	4 (75)	-	5 (80)	-
Body Mass (kg)	76.1 ± 19.7	75.9 ± 18.4	86.7 ± 30.6	88.0 ± 31.8	72.5 ± 15.7	71.2 ± 16.3
BMI (kg/m^2)	28.3 ± 5.5	28.2 ± 5.0	30.4 ± 5.1	30.8 ± 5.4	27.6 ± 4.2	27.1 ± 4.0
KE Power (N-m)	99.4 ± 23.0	99.5 ± 24.1	105.9 ± 58.2	113.9 ± 64.4	94.5 ± 6.5	106.2 ± 25.0
Absolute VO_2max (L/min)	1.46 ± 0.35	1.61 ± 0.27	1.91 ± 0.68	2.04 ± 0.68	1.41 ± 0.28	1.55 ± 0.33
Relative VO_2max (mL/kg/min)	19.4 ± 1.6	21.7 ± 3.2	22.3 ± 3.2	24.0 ± 5.9	19.7 ± 2.7	22.2 ± 4.1
Muscle Volume (cm^3)	411.4 ± 82.4	429.2 ± 86.3	432.3 ± 194.5	478.9 ± 192.3	425.9 ± 89.0	456.9 ± 107.0
KE Strength (N-m)	129.8 ± 45.7	122.8 ± 32.6	129.4 ± 58.4	127.0 ± 51.5	117.5 ± 23.0	148.7 ± 32.4
KE Fatigue Resistance (% of maximal)	48.0 ± 9.7	57.6 ± 5.0	43.3 ± 8.0	54.0 ± 12.2	50.0 ± 10.6	55.6 ± 15.0
Fat Mass (kg)	29.6 ± 8.7	29.4 ± 8.3	31.3 ± 13.3	31.1 ± 13.6	25.9 ± 8.0	24.7 ± 7.1
6MW (m)	568.0 ± 34.2	611.2 ± 38.2	587.3 ± 56.1	600.5 ± 60.1	557.2 ± 60.3	585.0 ± 67.2
4SST (s)	6.41 ± 0.71	6.43 ± 0.42	6.34 ± 0.79	5.60 ± 1.15	7.34 ± 2.16	6.60 ± 2.00
Grip Strength (kg)	26.2 ± 3.7	28.2 ± 3.4	31.9 ± 5.7	33.3 ± 8.7	26.2 ± 8.9	26.5 ± 10.3

Data are means ± SD. 4SST, four-square step test; 6MW, six-minute walk; BMI, body mass index; HIIT, high-intensity interval training; KE, knee extensor; MICT, moderate-intensity continuous training; RT, resistance training; VO_2max, maximal oxygen consumption.

Table 5.7. 95% Confidence Intervals for baseline and post-intervention means

	HIIT (N = 5)		MICT (N = 4)		RT (N = 5)	
	Pre	Post	Pre	Post	Pre	Post
Body Mass (kg)	51.6 to 100.5	53.1 to 98.7	38.0 to 135.4	37.4 to 138.5	53.0 to 92.1	51.1 to 91.5
BMI (kg/m^2)	21.5 to 35.1	22.1 to 34.4	22.2 to 38.5	22.2 to 39.4	22.4 to 32.9	22.1 to 32.1
KE Power (N-m)	71.1 to 128.4	69.5 to 129.5	13.2 to 199.1	11.5 to 216.3	86.7 to 102.9	75.1 to 137.2
Absolute VO_2max (L/min)	1.03 to 1.89	1.28 to 1.94	0.83 to 2.99	0.95 to 3.12	1.06 to 1.76	1.14 to 1.96
Relative VO_2max (mL/kg/min)	17.4 to 21.4	17.7 to 25.7	17.3 to 27.3	14.7 to 33.3	16.3 to 23.1	17.0 to 27.3
Muscle Volume (cm^3)	309.0 to 513.7	322.0 to 536.3	122.7 to 741.8	172.9 to 784.9	315.3 to 536.4	324.1 to 589.7
KE Strength (N-m)	73.3 to 187.1	82.4 to 163.3	36.6 to 223.0	45.1 to 208.9	89.2 to 146.5	108.5 to 189.0
KE Fatigue Resistance (% of maximal)	36.0 to 60.0	51.4 to 63.8	30.5 to 56.0	34.5 to 73.5	36.9 to 63.1	37.0 to 74.2
Fat Mass (kg)	18.8 to 40.4	19.1 to 39.7	10.2 to 52.5	9.5 to 52.7	16.0 to 35.8	15.9 to 33.5
6MW (m)	525.6 to 610.4	563.8 to 658.6	498.0 to 676.5	504.8 to 696.2	482.3 to 632.1	501.5 to 668.5
4SST (s)	5.53 to 7.29	5.91 to 6.94	5.09 to 7.59	3.77 to 7.43	4.66 to 10.02	4.11 to 9.09
Grip Strength (kg)	21.6 to 30.8	24.0 to 32.4	22.8 to 40.9	19.4 to 47.1	14.1 to 36.3	13.7 to 39.3

4SST, four-square step test; 6MW, six-minute walk; BMI, body mass index; HIIT, high-intensity interval training; KE, knee extensor; MICT, moderate-intensity continuous training; RT, resistance training; VO_2max, maximal oxygen consumption.

Figures

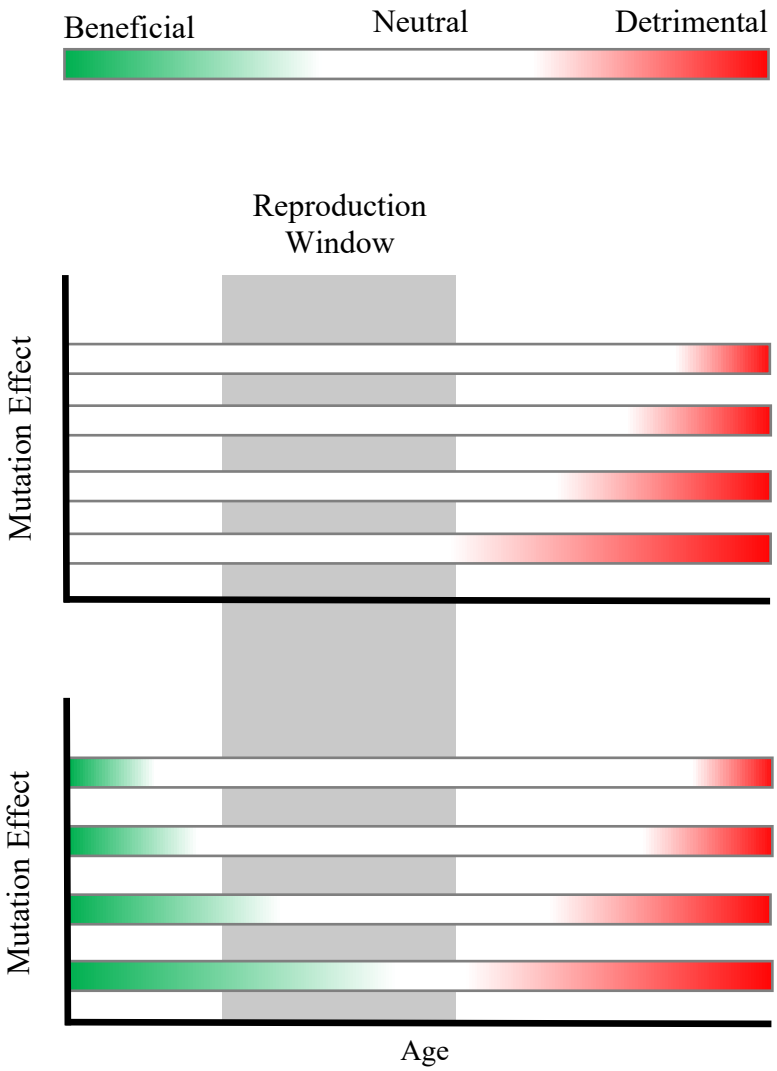

Figure 2.1. Graphical representation of the Mutation Accumulation and Antagonistic Pleiotropy theories. (A) The Mutation Accumulation theory states that deleterious mutations with negative effects that do not begin until late in life (i.e., they do not affect reproductive success) will likely already have been passed to the offspring, and natural selection would be ineffective at removing the mutation. **(B)** The Antagonistic Pleiotropy theory states that natural selection may favor genetic variants that are beneficial at younger ages, but detrimental in late life. Adapted from Fabian D, Flatt T. The evolution of aging. Nat Knowl Educ. 2011;3(10):9.

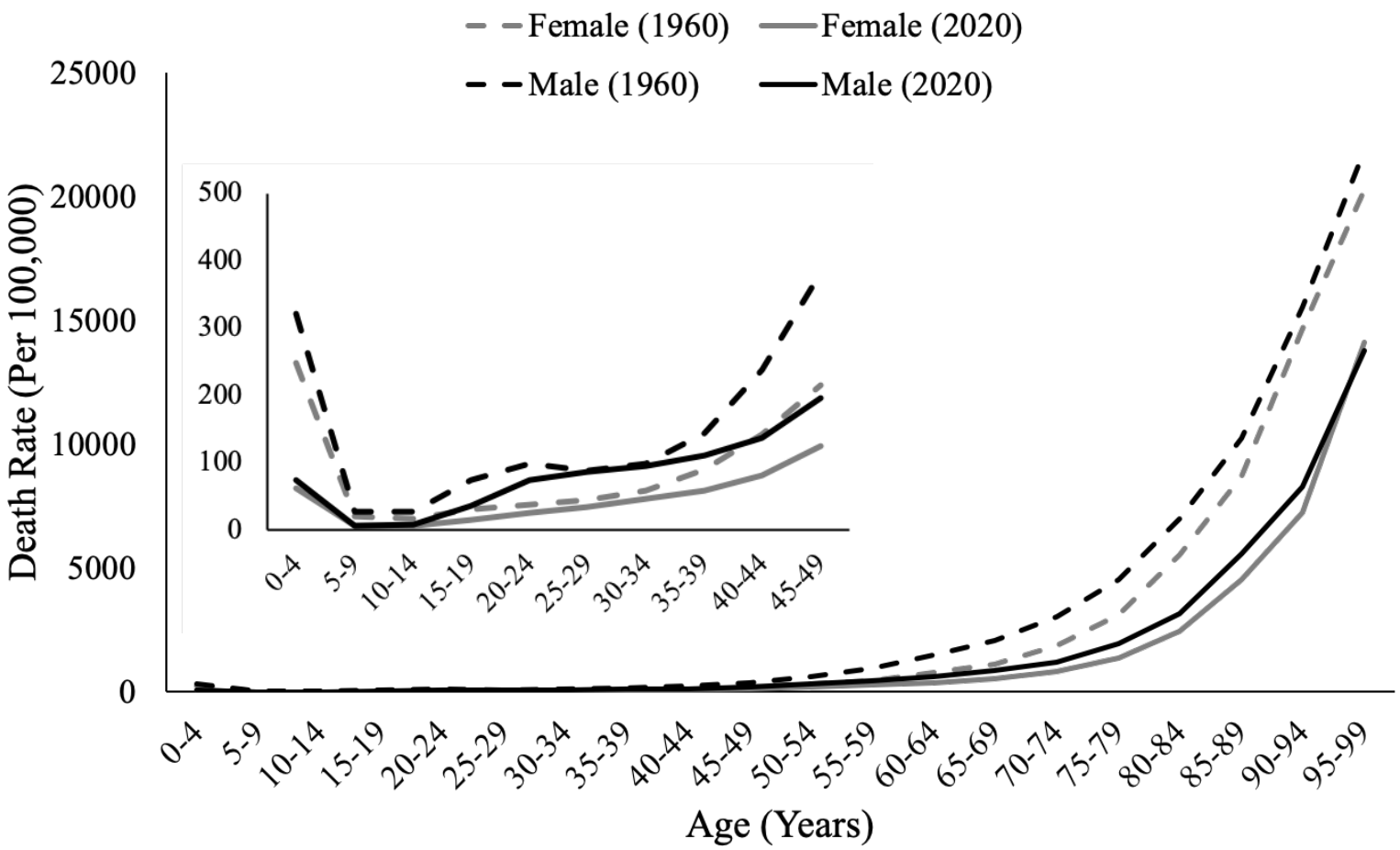

Figure 2.2. Death rates by age and gender in the US, 1960 and 2020. Current death rates increase linearly until approximately 50 years of age, increasing exponentially thereafter. This is a similar pattern to those from 1960, though decreased infant mortality rate and lower death rates at higher ages area apparent. Adapted from data from United Nations, Department of Economic and Social Affairs, Population Division. World population prospects, 2019, online edition. 2019. Report No.: Rev. 1. Under Creative Commons license CC BY 3.0 IGO: http://creativecommons.org/licenses/by/3.0/igo/

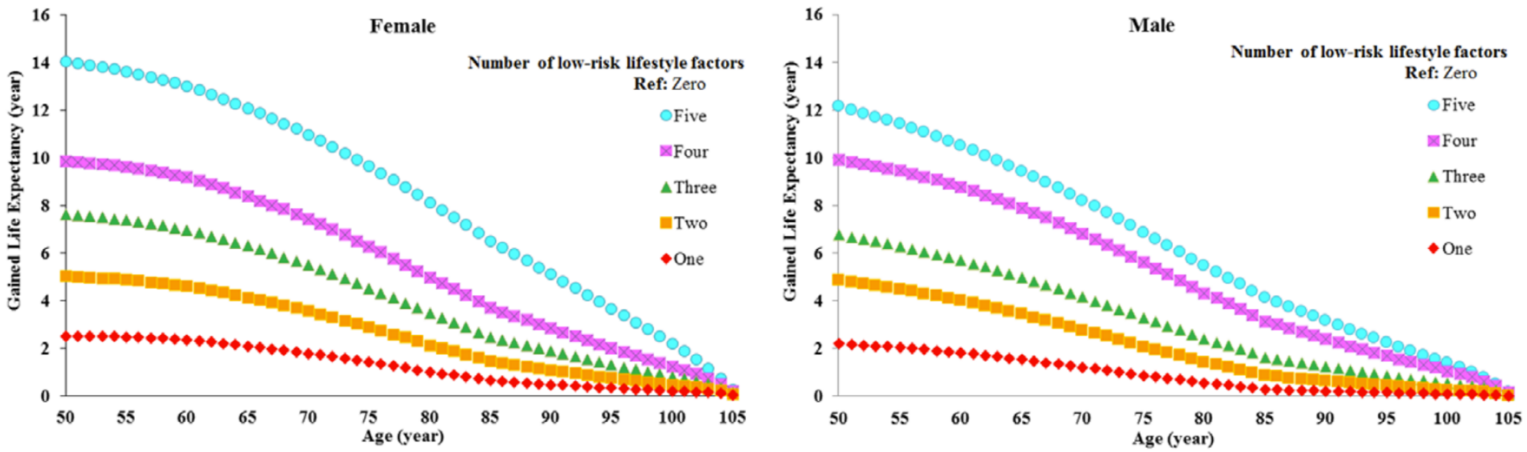

Figure 2.3. Gained life expectancy from adherence to low-risk lifestyle behaviors. Life expectancy can be increased by up to 14 years in women and up to 12 years in men by adhering to five low-risk lifestyle behaviors (never smoking, healthy body weight, healthy diet, regular physical activity, and moderate alcohol consumption). The more factors that are adopted, and the earlier in life they are adopted, the greater the increase in life expectancy. Li Y, Pan A, Wang DD, Liu X, Dhana K, Franco OH, et al. Impact of healthy lifestyle factors on life expectancies in the US population. Circulation. 2018 Jul 24;138(4):345–55. Figure reprinted with permission (Wolters Kluwer Health, Inc., License #: 4996070373734).

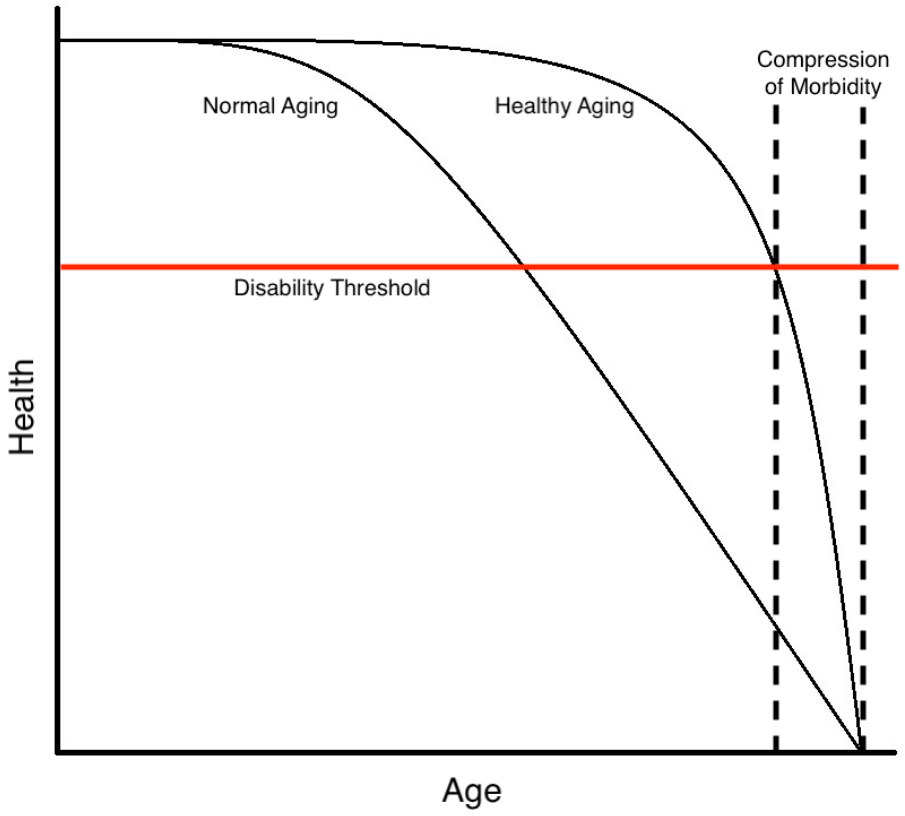

Figure 2.4. Conceptual model of compression of morbidity. Normal aging consists of a general decline in health beginning at or around middle age and continuing until death, spending a large portion of later life living with disability. Compressing morbidity and disability into a shorter time frame later in life is the driving force behind aging research.

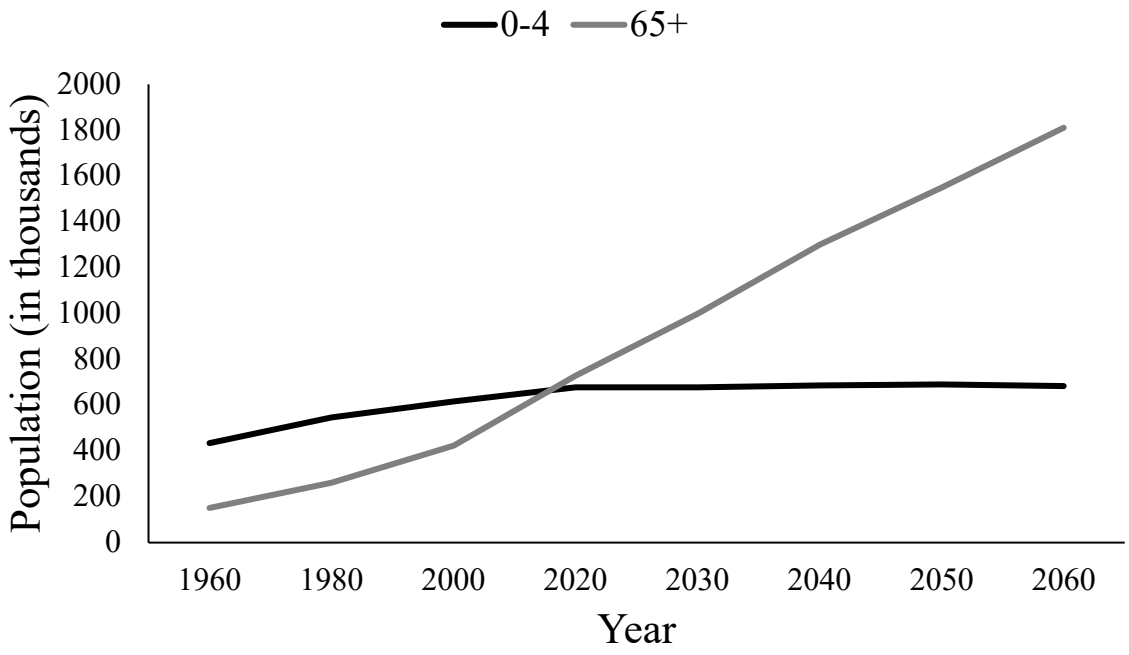

Figure 2.5. Changes in global population of children <5 and adults 65+ years. Historically, children under age five have outnumbered adults over the age of 65. However, while the number of children under the age of 5 will remain relatively stable over the next 30 years, the number of adults will continue to increase for the foreseeable future. Adapted from data from United Nations, Department of Economic and Social Affairs, Population Division. World population prospects, 2019, online edition. 2019. Report No.: Rev. 1. Under Creative Commons license CC BY 3.0 IGO: http://creativecommons.org/licenses/by/3.0/igo/

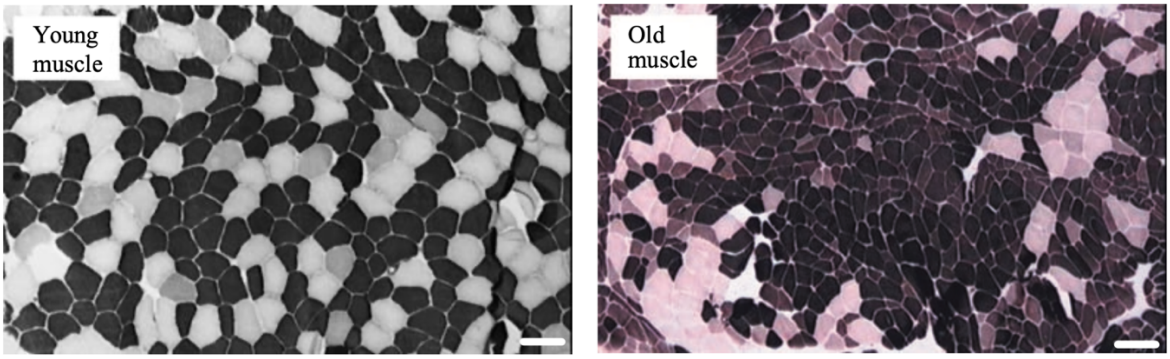

Figure 2.6. Fiber type distribution in young and old human muscle. Grouping of muscle fiber types in young and old human muscle. Dark fibers are type I, white fibers are type IIa, and gray fibers are type IIx. Andersen JL. Muscle fibre type adaptation in the elderly human muscle. Scand J Med Sci Sports. 2003;13(1):40–7. Reprinted with permission (License #1093314-1).

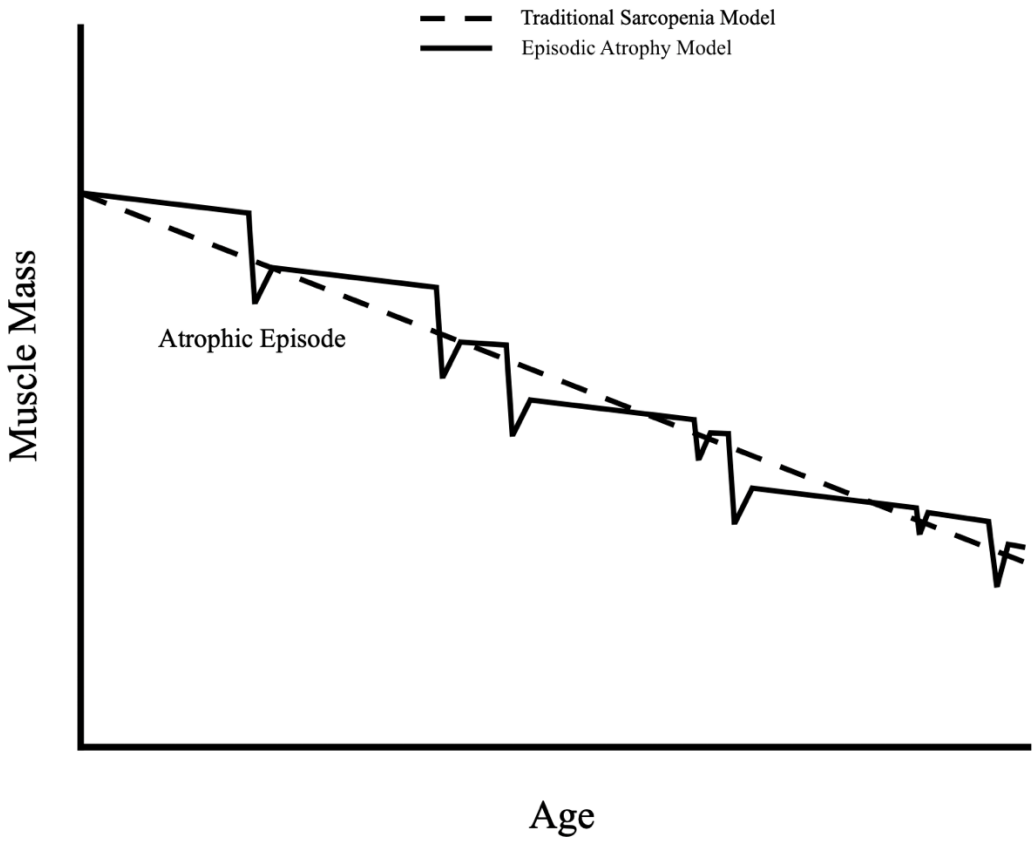

Figure 2.7. Conceptual model of episodic atrophy. Atrophic episodes, typically instigated by an injury or other medical concern that results in reduced physical activity, is characterized by a rapid decline in muscle mass, followed by an incomplete recovery once normal physical activity has resumed. Adapted from English KL, Paddon-Jones D. Protecting muscle mass and function in older adults during bed rest: Curr Opin Clin Nutr Metab Care. 2010;13(1):34–9.

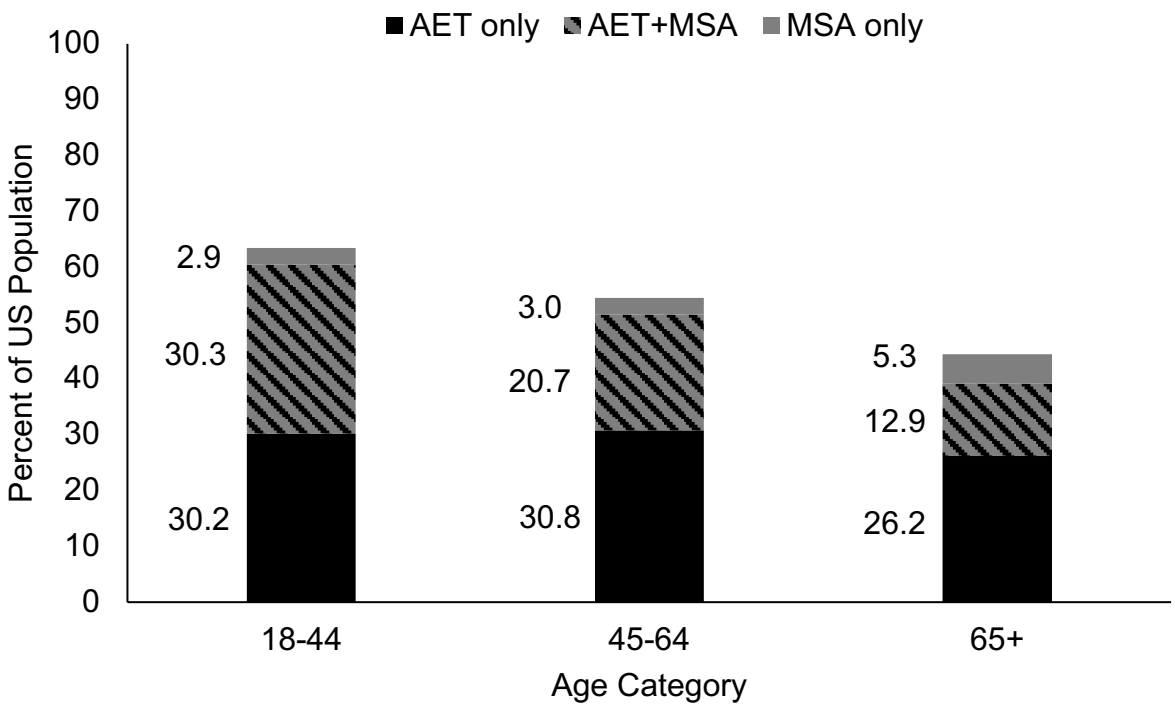

Figure 2.8. Proportion of adults in the US meeting the 2008 Physical Activity Guidelines for Americans. The proportion of young, middle-aged, and older adults who self-report meeting only the aerobic exercise guidelines of 150 min per week of moderate-intensity or 75 min per week of vigorous-intensity aerobic exercise, only the muscle strengthening guidelines of activities that strengthen the major muscle groups at least two times per week, or both aerobic and muscle strengthening guidelines concurrently. As age increases, self-reported adherence to exercise guidelines declines. A greater proportion of adults meet aerobic exercise guidelines than muscle strengthening guidelines in all age categories. AET, aerobic exercise training; RET, resistance exercise training. Adapted from public-use data provided by National Center for Health Statistics (2018).

Figure originally published in Tavoian D, Russ DW, Consitt LA, Clark BC. Perspective: Pragmatic exercise recommendations for older adults: The case for emphasizing resistance training. Front Physiol. 2020;11:799. doi:10.3389/fphys.2020.00799

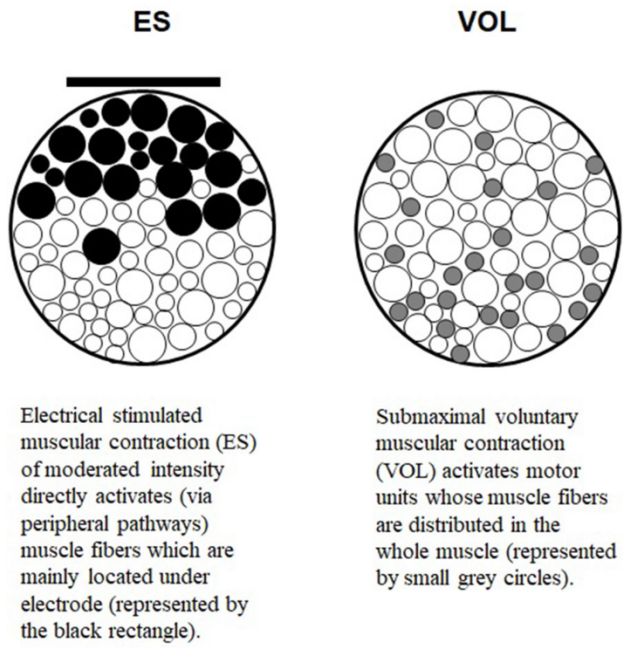

Figure 2.9. Voluntary and electrically stimulated muscle fiber recruitment distribution. Electrical stimulation activates preferentially activates large muscle fibers nearest the stimulating electrode, while voluntary muscular contraction preferentially activates small muscle fibers distributed throughout the muscle. Paillard T. Training based on electrical stimulation superimposed onto voluntary contraction would be relevant only as part of submaximal contractions in healthy subjects. Front Physiol. 2018;9:1428. doi:10.3389/fphys.2018.01428
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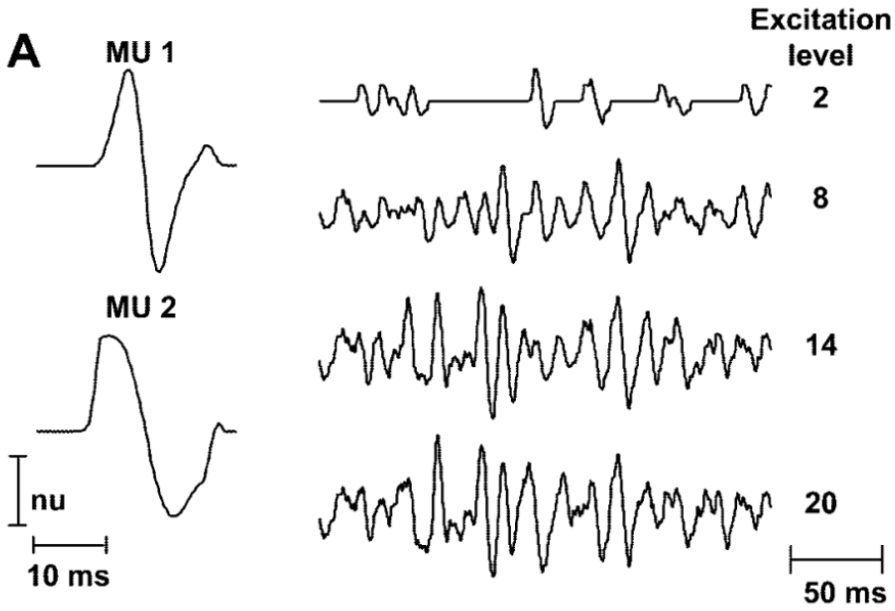

Figure 2.10. Two MU potentials and representative signals generated at different levels of excitation. Even at excitation level 2, examples of phase cancellation are clearly visible. MU, motor unit; nu, normalized units. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. J Appl Physiol. 2004;96(4):1486–95. doi:10.1152/japplphysiol.01070.2003. Reprinted with permission (License#: 109545-1)

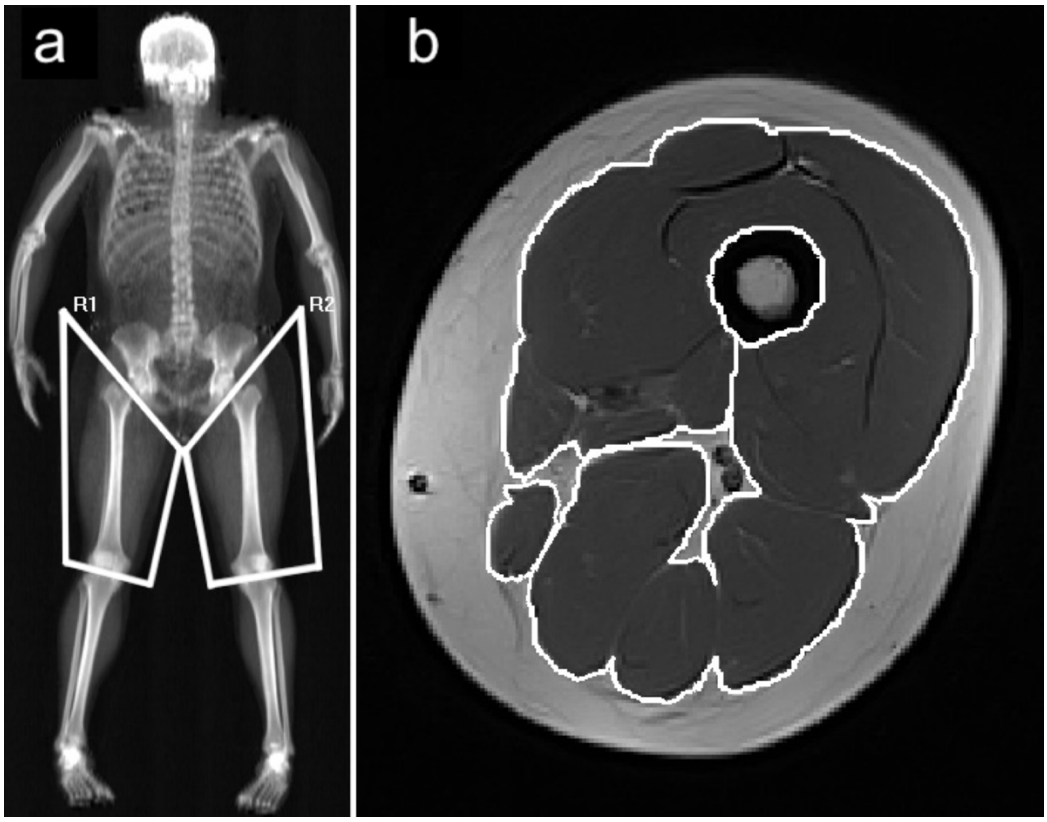

Figure 3.1. DXA and MRI regions used for analysis. (a) Diagram showing regions of interest in DXA scans. **(b)** Magnetic resonance image of the mid-thigh, muscles included for analysis traced in white.
Figure originally published in Tavoian D, Ampomah K, Amano S, Law T, Clark B. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. Sci Rep. 2019;9:10028. doi:10.1038/s41598-019-46428-w

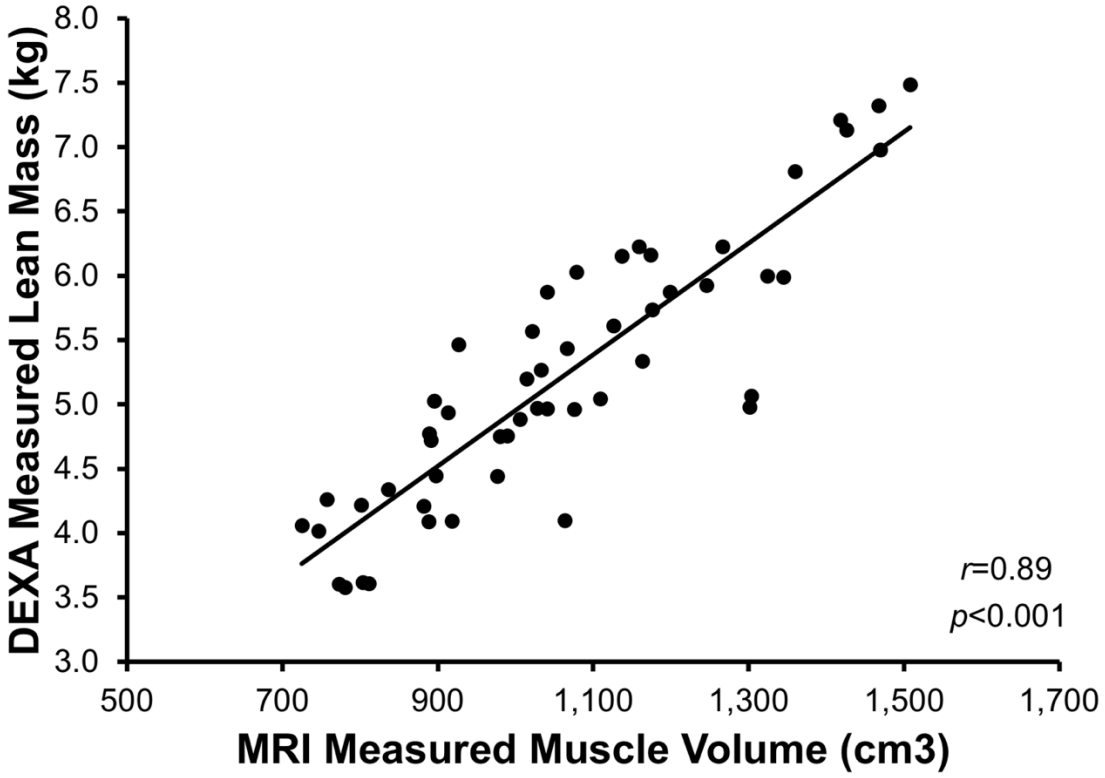

Figure 3.2. Correlation between MRI and DXA measures cross-sectionally. A strong positive association was observed between whole-thigh MRI- and DXA-derived measures at baseline (r=0.89, p<0.001)
Figure originally published in Tavoian D, Ampomah K, Amano S, Law T, Clark B. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. Sci Rep. 2019;9:10028. doi:10.1038/s41598-019-46428-w

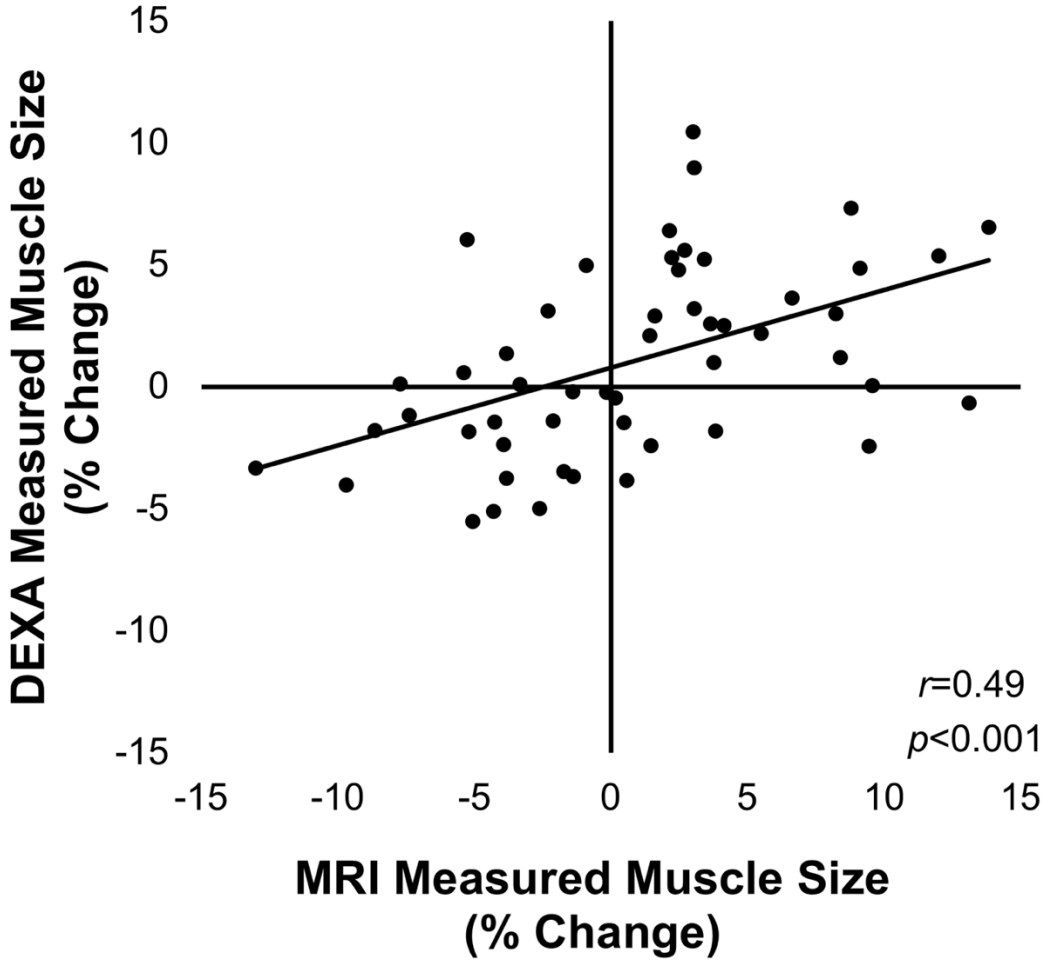

Figure 3.3. Correlation between MRI and DXA measures of percent change. There was only a modest positive association between the percentage change in estimates of muscle mass between the MRI and DXA techniques, such that explained variance was only 24% (r=0.49 p<0.01)

Figure originally published in Tavoian D, Ampomah K, Amano S, Law T, Clark B. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. Sci Rep. 2019;9:10028. doi:10.1038/s41598-019-46428-w

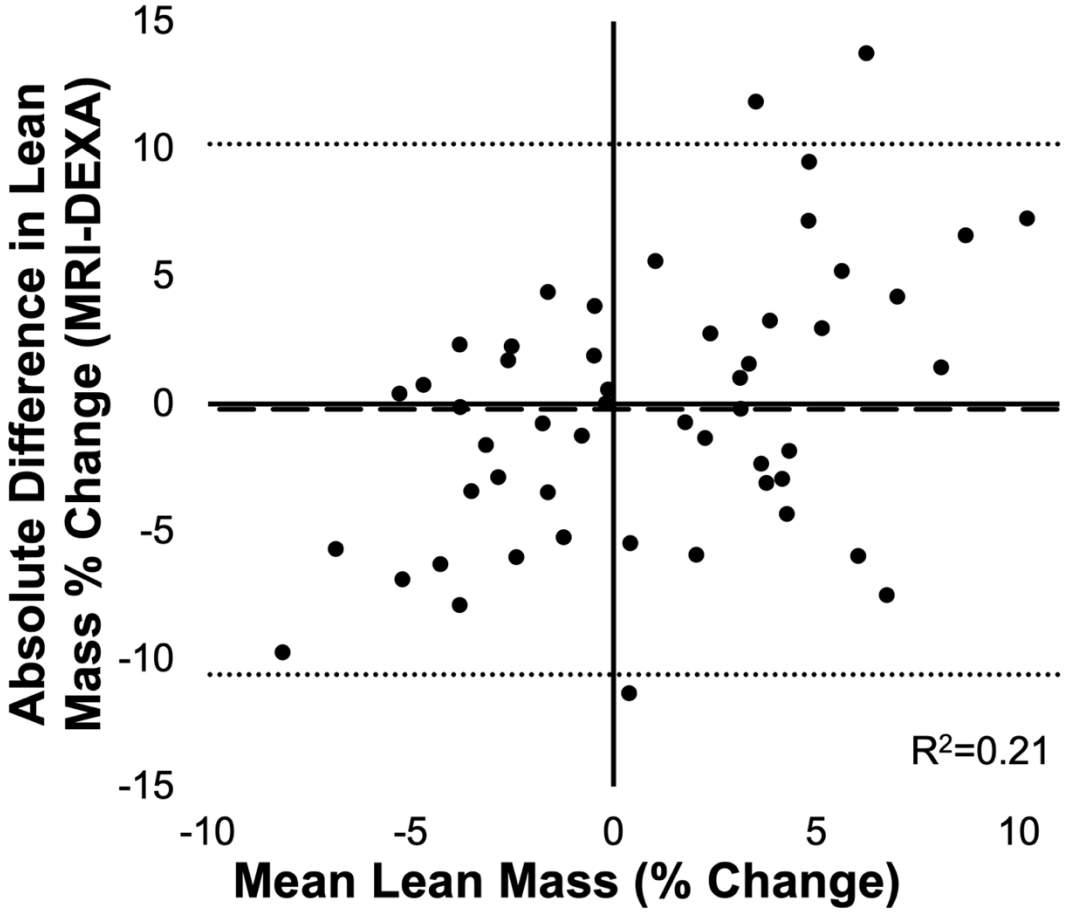

Figure 3.4. Bland-Altman plot of agreement between MRI- and DXA-derived measures of percent change in muscle size. There was a relationship between the error (difference between MRI and DXA) and the mean percent change in skeletal muscle size, indicating heteroscedacity. Upper LOA was 10.19% and lower LOA was -10.61% (dotted lines). DXA demonstrated a bias of -0.21% (dashed line)
Figure originally published in Tavoian D, Ampomah K, Amano S, Law T, Clark B. Changes in DXA-derived lean mass and MRI-derived cross-sectional area of the thigh are modestly associated. Sci Rep. 2019;9:10028. doi:10.1038/s41598-019-46428-w

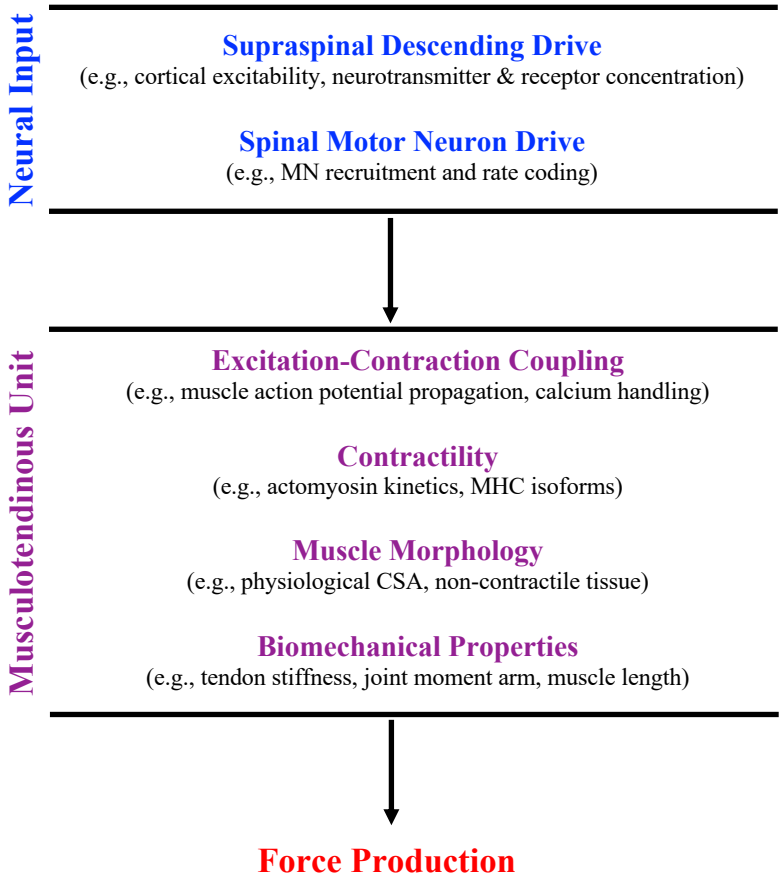

Figure 4.1. Potential sites along the neuromuscular pathway where voluntary force production can be affected. CSA, cross-sectional area; MHC, myosin heavy chain; MN, motor neuron.

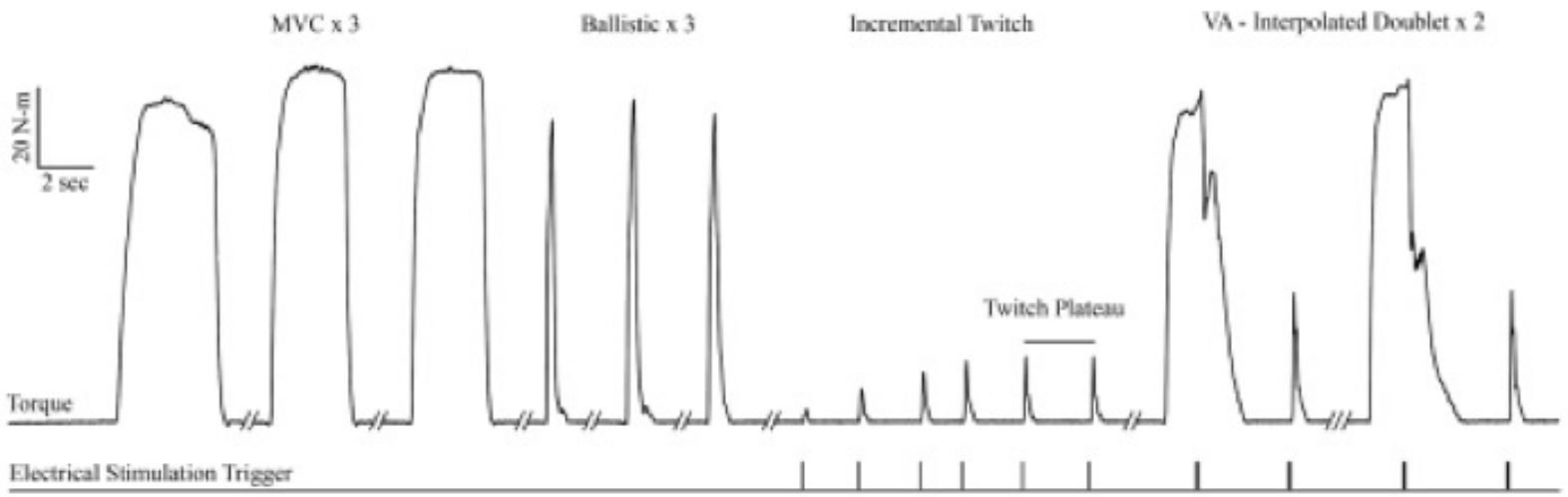

Figure 4.2. Testing order for assessing the CAB force ratio. Example trace illustrating the order of testing for assessing the CAB Force Ratio, including maximal voluntary efforts, electrical stimulation threshold, and voluntary activation. Breaks in the force trace indicate 60 seconds of rest between trials. MVC, maximal voluntary contraction; VA, voluntary activation.

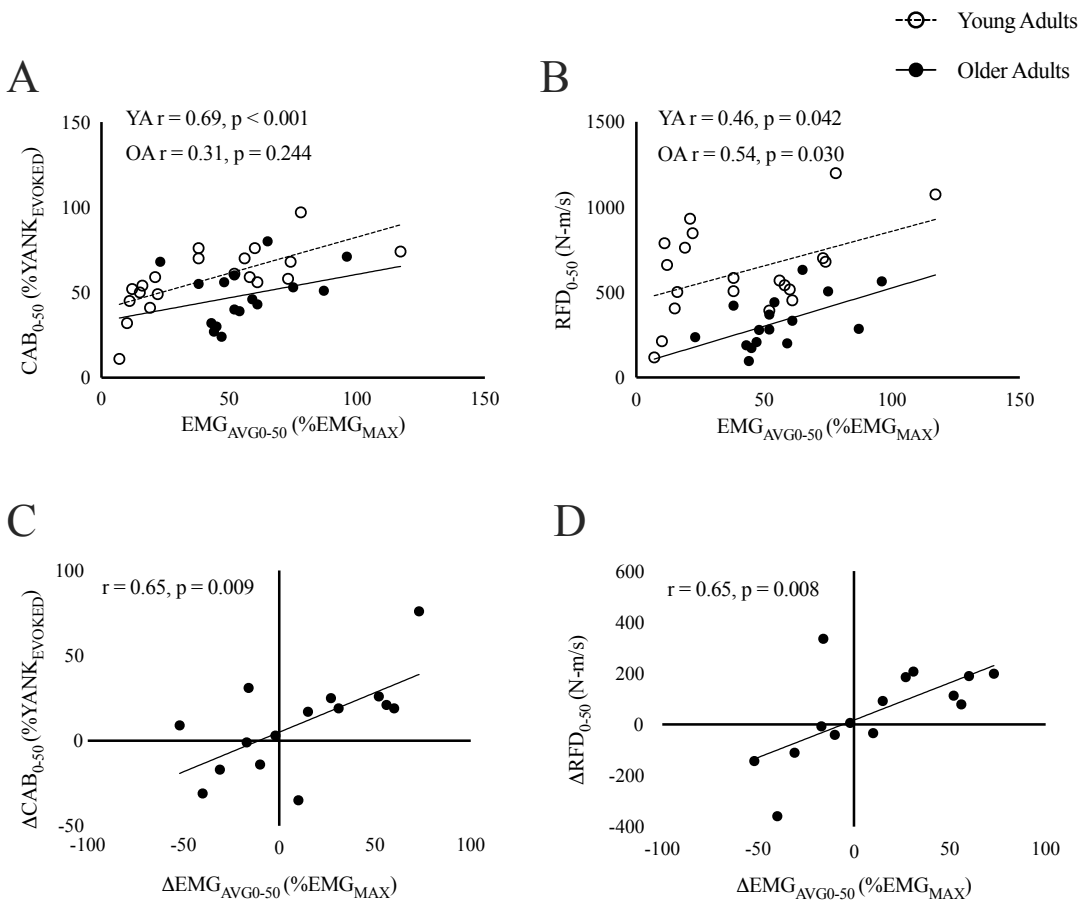

Figure 4.3. Correlation between EMG and CAB, and between EMG and RFD. Pearson bivariate correlations between **A)** $EMG_{AVG0\text{-}50}$ and $RFD_{0\text{-}50}$, and **B)** $EMG_{AVG0\text{-}50}$ $CAB_{0\text{-}50}$ in young (open circles; N=20) and older adults (closed circles; N=16), and exercise-induced changes in **C)** $EMG_{AVG0\text{-}50}$ and $RFD_{0\text{-}50}$, and **D)** $EMG_{AVG0\text{-}50}$ $CAB_{0\text{-}50}$ in older adults (N=15).

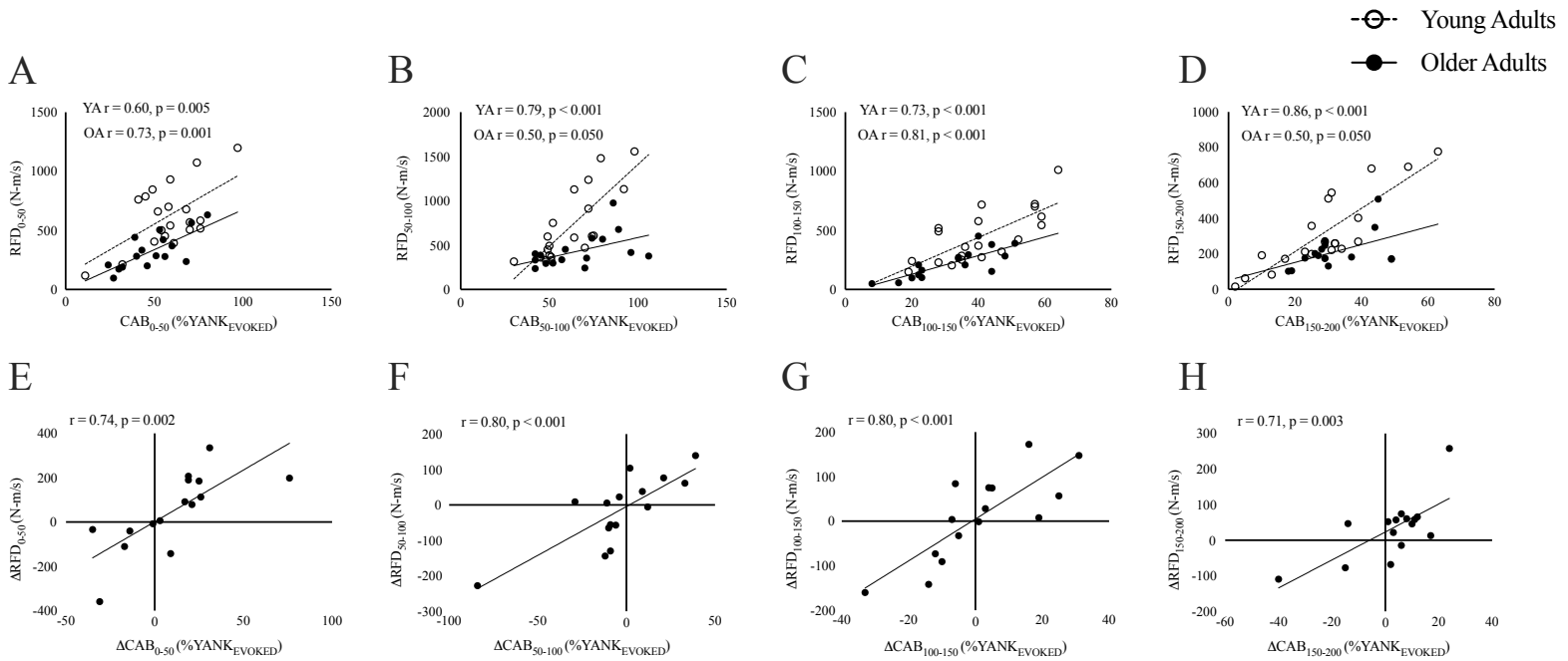

Figure 4.4. Correlation between CAB and RFD. Pearson bivariate correlations between CAB and RFD at **A)** 0-50 ms, **B)** 50-100 ms, **C)** 100-150 ms, and **D)** 150-200 ms in young (open circles; N=20) and older adults (closed circles; N=16), and between exercise-induced changes in CAB and RFD at **E)** 0-50 ms, **F)** 50-100 ms, **G)** 100-150 ms, and **H)** 150-200 ms in older adults (N=15).

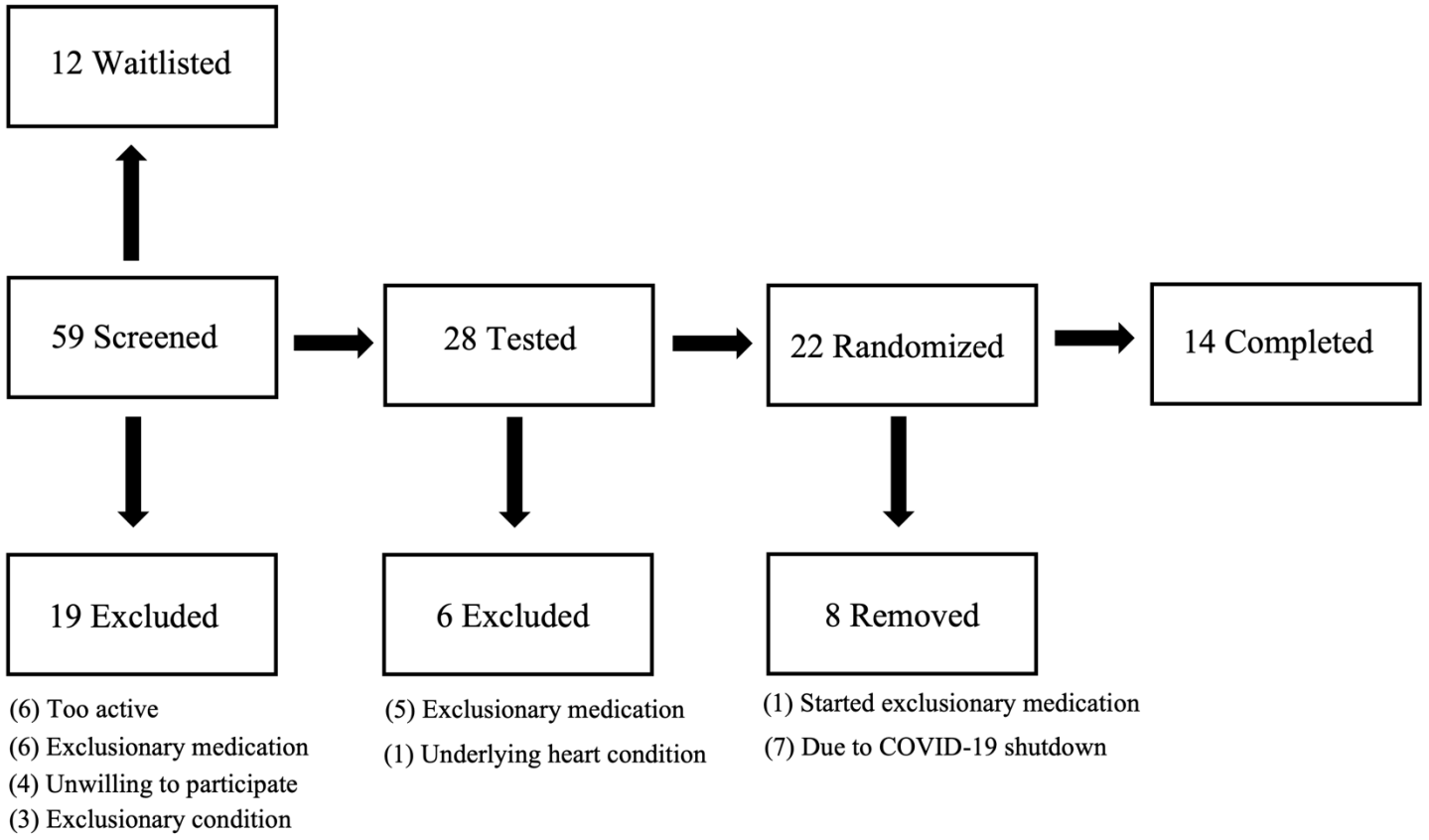

Figure 5.1. Recruitment flow chart for the DART study.

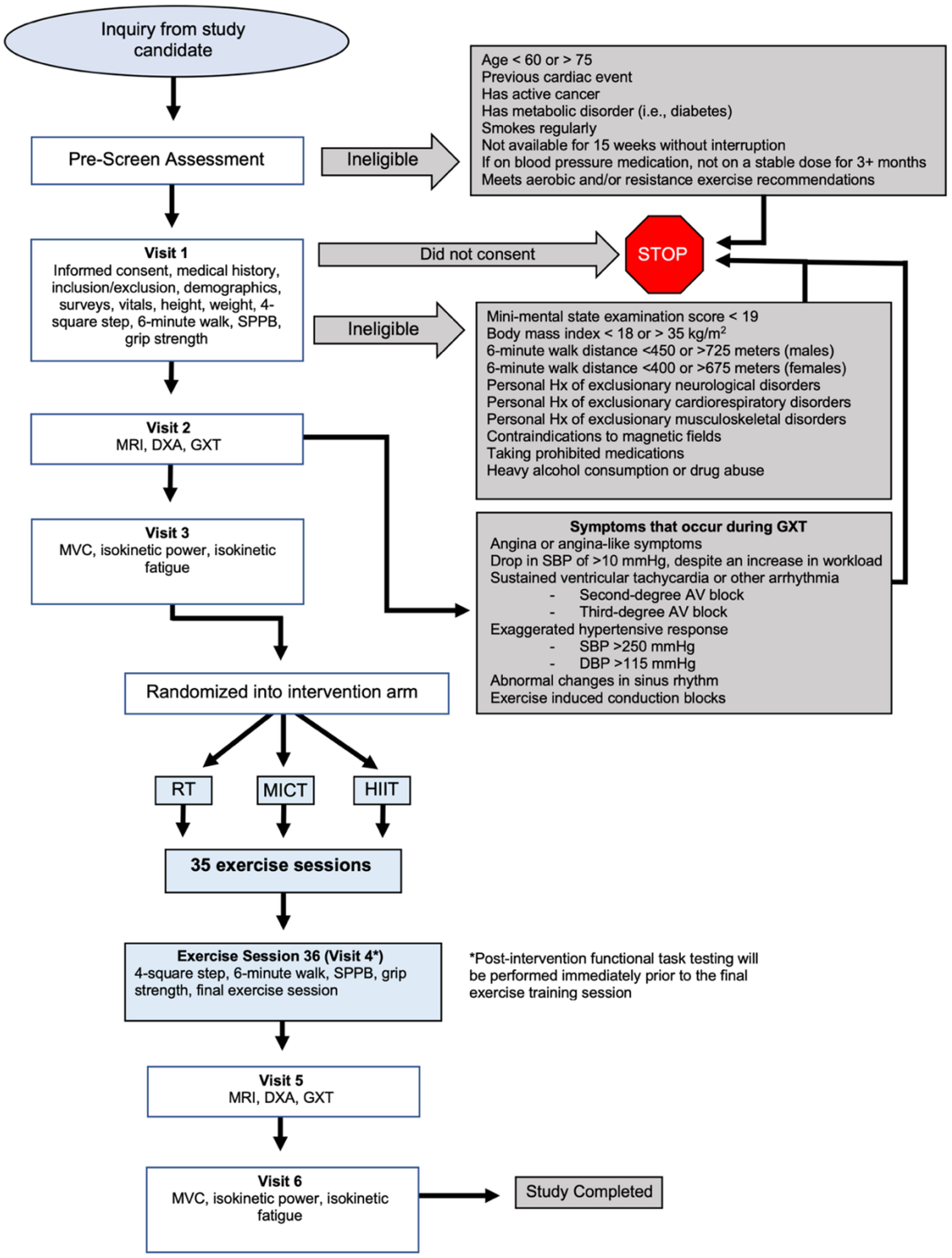

Figure 5.2. Detailed overview of DART study protocol. DBP, diastolic blood pressure; DXA, dual-energy X-ray absorptiometry; GXT, graded exercise test; HIIT, high-intensity interval training; Hx, history; MICT, moderate-intensity continuous training; MRI, magnetic resonance imaging; RT, resistance training; SBP, systolic blood pressure; SPPB, short physical performance battery.

Figure originally published in Tavoian D, Russ DW, Law TD, Simon JE, Chase PJ, Guseman EH, et al. A randomized clinical trial comparing three different exercise strategies for optimizing aerobic capacity and skeletal muscle performance in older adults: Protocol for the DART study. Front Med. 2019;6:236. doi:10.3389/fmed.2019.00236

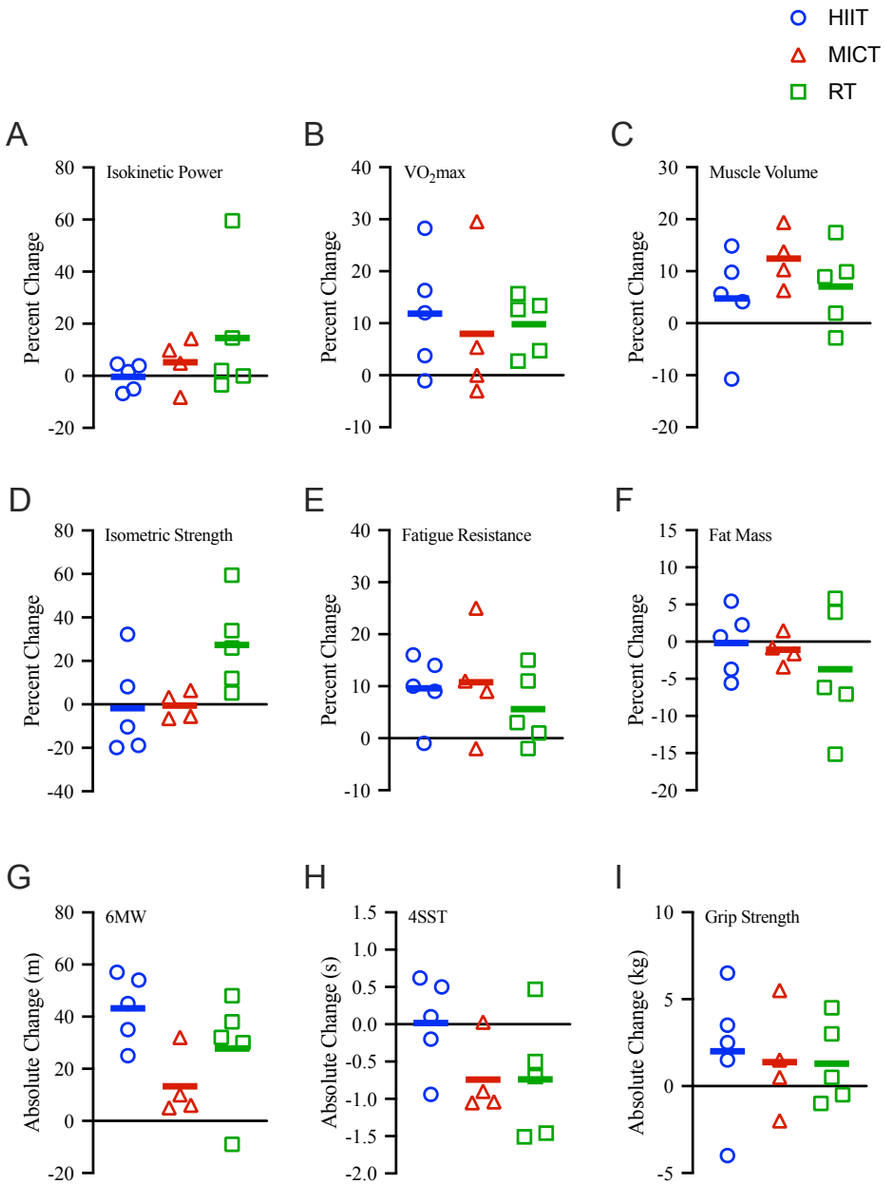

Fig. 5.3. Changes in primary (A-C), secondary (D-F) and physical function outcomes (G-I) after 12 weeks of HIIT, MICT, or RT. Open symbols are values for individual subjects and solid bars indicate group means. **A)** knee extensor power; **B)** absolute VO_2max; **C)** muscle volume; **D)** knee extensor strength; **E)** knee extensor fatigue resistance; **F)** total body fat mass; **G)** six-minute walk (6MW) distance; **H)** four-square step test (4SST) time; **I)** non-dominant hand grip strength.

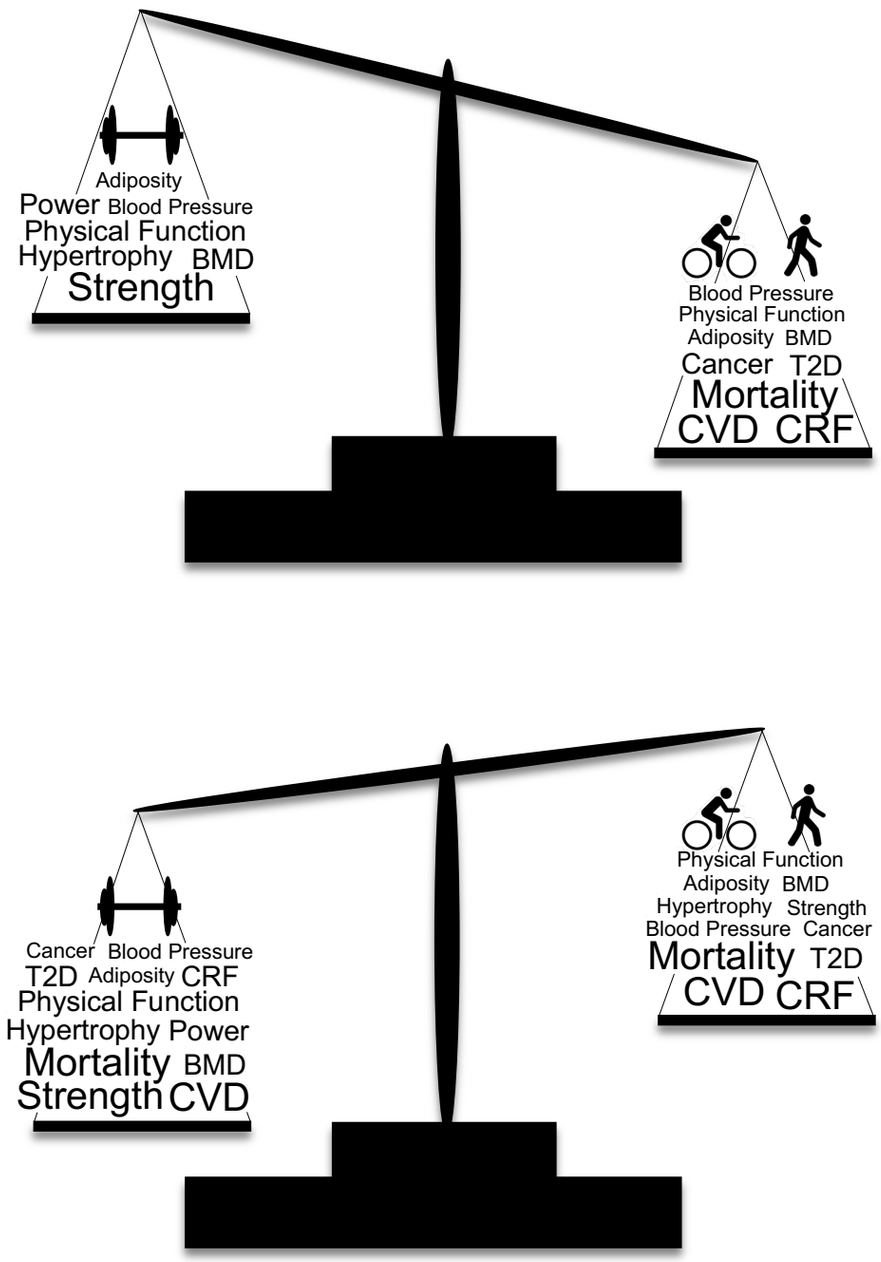

Figure 6.1. Traditional and modern depictions of the weighted importance of aerobic and muscle strengthening exercise and selected health benefits. (A) The traditional view of physical activity and health is based on the tenet that adaptations from AET and RET are largely independent of one another, with recommendations for AET given more weight for their beneficial effects on cardiovascular disease and mortality. **(B)** Our modern view of physical activity and health that includes the crossover benefits of AET and RET, indicating greater weight should be given to RET over AET. Larger font indicates a greater effect of the specific health benefit. AET, aerobic exercise training; BMD, bone mineral density; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; RT, resistance training; T2D, type 2 diabetes.
Figure originally published in Tavoian D, Russ DW, Consitt LA, Clark BC. Perspective: Pragmatic exercise recommendations for older adults: The case for emphasizing resistance training. Front Physiol. 2020;11:799. doi:10.3389/fphys.2020.00799

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