The Effects of Resistance Training on Aged Skeletal Muscle and Mitochondrial Function

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

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January 14, 2014

Blacksburg, Virginia

Keywords: Resistance Training, Mitochondrial Function, Aging

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ABSTRACT

With the aging of the baby boom population and an increased life expectancy, individuals aged 65 years and older are the fastest growing segment of our population. Aging brings about changes in skeletal muscle such as reduced muscle strength and mass, as well as cellular deficits such as increased production of reactive oxygen species (ROS), and mitochondrial DNA (MtDNA) deletions and mutations. Muscle mass declines at a rate of 1-2% each year after the age of 50, leading to muscle weakness, functional impairments, loss of independence, and an increase in falls. Additional declines in muscle mass and reduced muscle strength may result in a lower resting metabolic rate, reduced lipid oxidative capacity, increased adiposity, and insulin resistance. The rising number of individuals aged 65+ will increase demands on health care and health care costs, possibly leading to inadequate public resources and less care for the aged. This large societal impact, coupled with the aging of our population, suggests a clear need for methods that will improve the aging phenotype to enhance functionality, quality of life, and overall health for our aging population. This investigation aspires to delve into a relatively unexplored area of aging research and evaluate potential means that could help improve the aging phenotype.

The associated mitochondrial impairments, mitochondrial mediated apoptosis, and mitochondrial DNA (MtDNA) deletions and mutations that accompany aging lead to a decline in

physical fitness and oxidative capacity, and exercise has been shown to reverse or help prevent

many of these disturbances. Resistance exercise training (RT) is currently the most effective

known strategy to stimulate skeletal muscle hypertrophy and increase strength. Strength gains

after RT lead to an improvement in activities of daily living and quality of life. There is some

evidence suggesting that RT may lead to increased antioxidant enzyme capacity, decreased ROS

production and increased electron transport chain (ETC) function in older individuals. The

present study will lay a foundation for future research and further developments in the area of

RT, mitochondrial function and aging.

ACKNOWLEDGEMENTS

First and foremost I would like to express my deepest gratitude to Dr. Brenda Davy for the opportunity to be a part of the Laboratory of Eating Behaviors and Weight Management during my tenure at Virginia Tech. The guidance and support I have received has been paramount to my accomplishments and growth as a graduate student and will serve me well as I move forward in my career.

I would also like to extend my appreciation to all of those who played a role in the completion of the present dissertation research study, "The Effects of Resistance Training on Aged Skeletal Muscle and Mitochondrial Function." The efforts of Dr. Kevin Davy and his students in carrying out the muscle biopsies as well as Dr. Matt Hulver, his students and post-doctoral fellows carrying out the in the metabolic measurements were vital to the successful completion of the study.

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Chapter 1: INTRODUCTION

With the aging of the baby boom population and an increased life expectancy, individuals aged 65 years or older are the fastest growing segment of our population[1]. Aging brings about functional changes in skeletal muscle such as reduced muscle strength and mass[2, 3], as well as metabolic alterations such as increased production of reactive oxygen species (ROS) [4], and mitochondrial DNA (MtDNA) deletions and mutations leading to reduced electron transport chain function (ETC)[5-7]. Muscle mass declines at a rate of 1-2% each year after the age of 50[2, 8], leading to muscle weakness, functional impairments, loss of personal independence and an increase in falls[2, 4]. Additional declines in muscle mass and reduced muscle strength may result in a lower resting metabolic rate, reduced lipid oxidative capacity, increased adiposity, and insulin resistance[9-11]. These age-related impairments cost the US healthcare system over 18 billion dollars in the year 2000, although these figures have likely increased[12]. The rising number of individuals aged 65+ will increase demands on health care and health care costs, possibly leading to inadequate public resources and less care for the aged[1]. This large societal impact, coupled with the aging of our population, suggests a clear need for strategies that will improve the aging phenotype with the goals of enhancing functionality, quality of life, and overall health for our aging population. To address this need we conducted a literature review to investigate the potential resistance training may have in preventing or reversing some of these age-related changes (Appendix D). Reversing the deleterious effects the process of aging has on mitochondrial health is our primary focus. By laying a nessessary foundation for future research, this investigation has the potential to result in improved health and well being for the aging population.

Background

Mitochondria are double membrane cellular organelles with their own genome whose primary role is to produce energy for the cell in the form of Adenosine triphosphate (ATP)[13]. They are the primary producers of ROS, therefore contributing to oxidative stress/ damage and participating in numerous other cellular processes such as signal transduction, cell cycle regulation, thermogenesis, and apoptosis. To accomplish these roles mitochondrion are highly dynamic organelles that are continuously remodeling through biogenesis, fission fusion, and autophagy[14-16]. Mitochondrion are found in most eukaryotic cells, however we will focus on skeletal muscle mitochondrion as these mitochondrion have been implicated in the aging process[4, 6, 7, 17-23].

Mitochondria meet skeletal muscle's energy demands through the process of oxidative phosphorylation. This is accomplished primarily through the ETC, consisting of multipolypeptide complexes (I-V) located in the inner mitochondrial membrane (IMM). Reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$), generated in the tricarboxylic acid cycle (TCA cycle), or the Krebs cycle, provide electrons that are transferred along the complexes in a stepwise fashion until they finally reduce oxygen to form water[13]. Electrons are passed from one complex to other complexes with more positive oxidative potentials where the release of energy during the electron transfer is used to pump protons across the IMM[13]. Complexes I, III and IV function as H+ pumps and are responsible for creating a proton gradient and membrane potential (i.e. proton motive force) that functions to drive the synthesis of ATP when protons flow back into the matrix through the final complex in the ETC, ATP synthase[24-26]. This process depends on oxygen. When oxygen is

limited, glycolytic products are metabolized directly in the cytosol by the less efficient anaerobic respiration that is independent of mitochondria.

Mitochondria alter their organization, shape and size, depending on intracellular and extracellular signals, undergoing a continuous cycle of fusion, fission, and autophagy[15, 16]. The balance between these events determines the morphology of the organelle[14]. Mitochondria frequently encounter each other and rapidly fuse, typically end to end while there are normally an equal number of fission events that split a single mitochondrial tubule into two. By regulating the rates of fusion and fission the morphology of the mitochondrial population can be dramatically altered, which influences the functionality of the organelle[14, 27]. Fusion is a brief event (~100 s) and is followed by fission[16], resulting in mitochondria remaining in their post-fission state as single units for the majority of their life cycle before entering a subsequent fusion. Therefore the life cycle of mitochondria can be divided into two periods, the pre-fusion period (solitary period) and the post-fusion period when mitochondria are connected to each other[15, 16]. There have been several suggestions as to why mitochondria behave in this manner and what the benefits are to these dynamics. Fusion is needed for the inheritance of mtDNA, as preventing fusion leads to a loss of mtDNA[28]. Fusion also allows inter-mitochondrial compartmentalization of mtDNA, rRNA, tRNAs, and proteins. Allowing these gene products to mix presumably reduces the risk of losing, or increases the risk of maintaining mitochondrial functionality[29]. In line with this thought, the term "mitochondrial functional complementation" has been coined, suggesting that mitochondria fuse together to prevent individual mitochondrion from expressing mutant or damaged mtDNAs and regulate respiratory function[30, 31]. It appears that the mixing of the mitochondrial matrix and inner membrane organelles also improves respiratory function, as fused mitochondria networks are preferred when optimal mitochondrial

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function is needed and found in respiratory active cells[32]. However, mitochondrial fusion can only take place between organelles with sufficient membrane potential, therefore mitochondria with decreased ECT activity have no means to grow by fusion and mix gene products with more functional organelles[33, 34]. It has been observed that the mitochondrial network undergoes dramatic remodeling in atrophied muscle trending towards a more fragmented population[35]. Fusion allows for the diffusion and mixing of gene products between mitochondria, leading to an interconnected network that has superior respiratory capacity compared to solitary mitochondria; however, this fusion process cannot rescue mitochondria that are bioenergetically compromised[15, 32].

Fission is a necessary step in mitochondrial-dependent apoptosis, aiding in disposing dysfunctional mitochondria[15, 34, 36]. After mitochondria fuse and mix contents, fission produces two functionally divergent daughters with one possessing greater amounts of mtDNA mutations and dysfunctional proteins. Thus, fission often generates depolarized mitochondria that are separated from the mitochondrial network. These fragmented, depolarized mitochondria are often found in resting cells where respiratory activity is not required. The process of fission contributes to the maintenance of the healthy mitochondrial population as it permits the elimination of damaged and fragmented mitochondria to be degraded and removed by autophagy[15, 32, 36]. Therefore, the process of fission and the following autophagy is important to maintaining a healthy mitochondrial population and function. This idea is underscored in the "mitochondrial-lysosomal axis theory of aging" where depressed autophagy observed in aged cells causes impairments in mitochondrial turnover. This creates a prolonged life span of dysfunctional mitochondria resulting in a decline in ATP production, increased ROS, and increases in mtDNA mutations/deletions[37].

The events of fusion, fission, and autophagy ultimately play a major role in the mitochondrial content of skeletal muscle (i.e. density of number of mitochondria). It appears that some reductions in mitochondrial function are due to reduced mitochondrial content with studies associating decreases in ATP synthesis flux with lower mitochondrial density[38, 39]. Increasing mitochondrial density, or number of mitochondria, could result in enhanced fat-oxidative capacity and reduced fatty acid-metabolite accumulation in skeletal muscle[40-44]. The processes of mitochondrial biogenesis is the other event that determines mitochondrial content that diminishes as we age[27]. This process is largely dependent on peroxisome proliferator-activated receptor-γ co-activator (PGC-1α). PGC-1α interacts with and coactivates a variety of transcription factors and nuclear receptors leading to the regulation of nuclear- and mitochondrial-encoded genes required for contractile and metabolic adaptations in skeletal muscle[45-47]. The up-regulation of PGC-1α has been shown to increase mitochondrial biogenesis and content, leading to greater fat oxidation and ATP production[48-52]. PGC-1α is up-regulated by exercise training through a variety of pathways including calmodulin-dependent signaling, reactive nitrogen and oxygen species-dependent signaling, AMP activated protein kinase (AMPK) signaling, and mitogen-activated protein kinase (p38 MAPK) signaling[48, 53-56].

Mitochondria and Aging

Skeletal muscle mitochondria are thought to play a major role in the age-related declines in skeletal muscle function. Mitochondria are the main producers of both cellular energy and free radicals, as 90% of ROS are generated within the mitochondria[13]. Alterations in mitochondria have been noted in aging, including decreased total volume, increased oxidative damage, and reduced oxidative capacity potentially due to decreased mitochondrial biogenesis, and increases in mitochondrial mediated apoptosis and MtDNA deletions and mutations[6, 19, 57-62]. Some have

referred to these changes in mitochondria as "mitochondrial dysfunction" [5, 18, 40, 61, 63-66];

however, it is not certain if these changes in mitochondria are a physiological adaptation,

pathological maladaptation, or a normal cellular adaptive response. The term mitochondrial

dysfunction is also vague and does not tell the whole story, as there are many mitochondrial

functions in the cell (i.e., ATP production, substrate oxidation, intracellular calcium buffering,

andinduction of apoptosis). In addition, the total mitochondrial oxidative capacity of a cell

depends on the total mitochondrial content as well as the functional capacity of each

mitochondrion, or ''intrinsic mitochondrial function''. When referring to mitochondrial

''function'' or ''dysfunction'', it may not be always obvious whether these terms are referring to

a deficit in total oxidative capacity or to the intrinsic function of each mitochondrion. It is

therefore best to avoid such terminology and focus on the specific mitochondrial alterations in

aging populations and their consequences.

Mitochondria are the only organelles in animal cells aside from the nucleus that contain

their own DNA. The human mitochondrial DNA is a circular, double-stranded molecule that

encodes 13 protein subunits, all of which are respiratory chain subunits, and 24 RNA

components (22 tRNAs and two rRNAs) necessary for mitochondrial protein synthesis and

therefore determine the efficiency of, oxidative phosphorylation[23]. MtDNA deletions,

mutations, and content have all been shown to be negatively affected by aging[4-6, 20, 22, 58]. Some

suggest that mtDNA deletions and mutations may lead to a decrease in total number of

mitochondria which may cause impaired fatty acid oxidation and fatty acid-metabolite

accumulation in skeletal muscle[40-44], which has potential to negatively impact insulin resistance

and further promote the generation of ROS[61, 67-69]. Aging mitochondria exhibit a less efficient

transfer of electrons, resulting in a greater proportion of electrons partially reducing oxygen,

producing amplified levels of ROS [18]. In 1956, Harman proposed a theory of aging centered around how ROS lead to senescence[70]. This thinking has evolved into the "mitochondrial theory of aging", where ROS damage mtDNA causing more ROS to be produced leading to further damage[4, 19]. The lack of protective histones and close proximity to the ETC make mtDNA extremely vulnerable to oxidative stress, which may cause alterations in genes encoding subunits of the ETC as well as other mutations effecting ETC flux[7]. In support of this, aged individuals have displayed greater amounts of mtDNA point mutations and deletions in skeletal muscle and more fibers defective for cytochrome c oxixidase (a mitochondrial enzyme encoded by mtDNA)[71]. Any error in mtDNA that encodes polypeptides of ETC and components required for their synthesis has the potential to affect ETC activity as a whole, which could affect both the assembly and functionality of the products of numerous genes, leading to ETC defects. These errors affect cellular energetics as a whole[7], and may bring about age-related phenotypes such as alopecia, kyphosis, osteoporosis, and cardiac hypertrophy[6]. For these reasons ROS have been implicated as one of the causative factors of aging, being a central aspect of the declines in lean body mass, strength, fatty acid oxidation, and glucose tolerance older adults may experience[4, 18, 40]. Skeletal muscle mass and strength are impacted, as skeletal muscle fibers that lose ETC activity display significant atrophy[59, 60]. Despite the evidence linking ROS, mitochondrial damage and aging, controversy exists about whether these changes are causal in the aging process. Some suggest that the respiratory decline in old individuals is largely due to physical inactivity, rather than chronological age[72, 73]. Also the functional significance of mtDNA alterations is unclear as the levels of mtDNA mutations found in most studies have been deemed too low to affect respiratory function possibly due to the fact that the vast majority of mitochondrial proteins (about 900) are synthesized by nuclear DNA[26, 71]. Mouse studies have

added controversy to the mitochondrial theory of aging, as mutator mice that show a 3- to 8-fold increase in mtDNA point mutations have reduced life spans and additional features that could be interpreted as accelerated aging, displaying an early onset of weight loss, reduction in subcutaneous fat, hair loss, curvature of the spine, and osteoporosis. However, not all mice with increased mtDNA mutations display this aging phenotype[74] and in spite of the widespread mtDNA mutations, these mice do not appear to have any change in the levels of ROS and there is no evidence for increased oxidative damage to proteins, lipids, or DNA[75]. Despite the controversy around oxidative stress on the mitochondrial genome, decreased mitochondrial capacity has been accepted as a constant theme in the aging process, with oxidative capacity[22, 61, 76], ATP synthesis[22], mitochondrial protein synthesis[77], and mitochondrial protein content[22] all being negatively affected. Additional work with mtDNA mutator mice have revealed that these mice display a progressive reduction of both respiratory chain enzyme activities and mitochondrial ATP production rates as well as a general reduction in all inducible respiratory states[78], suggesting that a casual relationship between mitochondrial functionality and aging indeed exists. The conflicting nature of the present aging research on mitochondrial function is not easy to reconcile in a unifying theory. Certainly more experiments are needed to clarify the role of mitochondrial biogenesis, mitochondrial respiration rate and ROS production in different aspects of aging. However, mitochondria are now in the scientific spotlight, and by evaluating mitochondria's roles in the aging process we may gain knowledge on improving the aging phenotype.

Current literature strongly suggests that the lower mitochondrial capacity associated with aging is not irreparable. Increasing physical activity improves many aspects of mitochondrial metabolism including electron-transport chain oxidative capacity, mitochondrial content,

biogenesis, and a remodeling of mitochondrial morphology[79-82]. These changes have been observed apart from weight loss, suggesting that exercise training is the stimulus for mitochondrial improvements rather than the reduction in body weight individuals may experience with exercise even in obesity[79, 83]. The vast majority of this literature indicates improvements at the mitochondrial level with exercise have focused on aerobic exercise interventions. This is logical as mitochondria function aerobically and inducing improvements in this process would likely come from focusing a training intervention on the same energy pathway. However, this mode of exercise does little to attenuate the loss in muscle mass and strength exhibited in the elderly. In addition, factors such as obesity, arthritis, lower back pain, and physical disabilities affecting many older adults may preclude this population from regularly performing aerobic exercise[84-86].

Resistance Training

Resistance exercise training (RT) is currently the most effective strategy to stimulate skeletal muscle hypertrophy and increase strength[3, 87, 88]. This mode of exercise has been shown to be a safe and effective intervention to increase lean body mass and strength in older adults, with many clinical studies reporting improvements in strength and lean body mass (reviewed in[89]). These strength gains after RT also improve physical function and lead to improvements in activities of daily living and quality of life[2, 3, 88]. The cellular adaptations that RT may induce in older individuals that combat skeletal muscle aging remains to be fully understood; however, there are speculations that skeletal muscle mitochondria may be targeted in older adults who RT [90-93]. One study investigating endurance training and RT in elderly adults (average age 69 years), showed greater improvements in oxidative capacity with RT vs endurance training (57% improvement vs. 31% respectively). In addition, only the RT group exhibited increases in

mitochondrial volume density and muscle size, suggesting that the aerobic pathway may be particularly sensitive to exercise training in elderly muscle and may be unregulated by RT[93]. This is in contrast to younger subjects who display lower oxidative enzyme activity and mitochondrial oxidative capacity with RT possibly due to the dilution of the mitochondrial structure with increases in muscle size[94]. A plausible explanation for these changes in older adults may lie in the possibility that aging results in damage to the myosin isoforms and training induces a replacement of the damaged myosin with undamaged protein[93]. Additional evidence points to the potential for ETC proteins becoming upregulated from the inflammatory response, membrane damage, and loss of calcium homeostasis that peaks 24-48 hours after RT[95, 96] causing an increased oxidant status and forcing mitochondrial adaption[19]. In support of this, studies exist demonstrating that RT may lead to increased antioxidant enzyme activity[97], oxidative capacity[93], ETC function and decreased ROS production[4] in older individuals. The attenuation of these mtDNA deletions and ROS production may be invoked by a phenomenon termed "gene shifting"[98, 99]. The gene shifting model is based on two assumptions. First, that muscle stem cell mtDNA acquire deletions and mutations at a much slower rate than mtDNA of generated skeletal muscle. And second, when these muscle stem cells proliferate they donate their mitochondria (and other organelles) to functional, post–mitotic skeletal muscle fibers[99]. This proliferation and differentiation of muscle satellite cells (SC) into mature skeletal muscle causes a shift in the mitochondrial heteroplasmy towards a more wild-type mtDNA population in skeletal muscle. Fewer mutated mitochondria and greater amounts of functional wild-type mitochondria lead to improved mitochondrial function[98, 99]. SC activation and proliferation generally appear following exercise training[100, 101], and it has been shown that patients with mitochondrial myopathy exhibit a remarkable increase in the ratio of wild-type to mutant mtDNAs, the

proportion of muscle fibers with normal ETC activity, improvements in muscle oxidative capacity and muscle fiber cross-sectional area after a RT program[99, 102]. It has also been observed that mtDNA deletions, although absent in younger individuals, can be improved with RT in older adults[91]. It is therefore believed that in addition to being effective at increasing lean body mass and strength, RT may evoke changes in the mitochondrion of older adults[4, 19, 90, 91, 93, 103]. However this postulation is only in its infancy, as there are many dimensions that are unknown. The duration, intensity, and volume of RT required for mitochondrial improvement is uncertain. We also do not know the associations, if any, between strength, lean mass accretion, and mitochondrial adaption. Therefore this potential avenue to improve the growing aged population's health and quality of life is worthy of further investigation.

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Chapter 2: RESISTANCE TRAINING AND MITOCHONDRIAL METABOLISM IN OLDER ADULTS: IMPLICATIONS FOR ROS PRODUCTION, CITRATE SYNTHASE ACTIVITY, AND THE IMPACT OF STATIN USAGE

Abstract

During aging, a general decline in cellular function occurs due to accumulation of damaged macromolecules and organelles which are not readily removed. This brings about a progressive loss in skeletal muscle mass and strength which is considered part of the normal aging process. This muscle weakness leads to functional impairments, loss of personal independence and a decrease in quality of life as well as chronic conditions that exert a profound economic impact on our nation. Aged mitochondria exhibit a less efficient transfer of electrons, resulting in a greater proportion of electrons partially reducing oxygen, producing amplified levels of reactive oxygen species (ROS). This oxidative stress may cause alterations in genes encoding subunits of the electron transport chain (ETC) as well as other mutations effecting ETC flux. Exercise (predominantly aerobic exercise training) has shown to induce positive adaptations at the muscular and mitochondrial level. However, the impact of resistance exercise on mitochondrial function remains unclear. The current study employed a 12-week resistance training (RT) intervention to stimulate hypertrophy and increase strength, with the goal to target both the progressive loss in skeletal muscle and age-associated mitochondrial alterations. Nineteen males aged ≥60 years were randomized into an intervention group that performed RT three days per week under the supervision of a personal trainer or to a control group that remained sedentary for 12 weeks. Muscle biopsies were obtained at baseline, 3 weeks and 12 weeks. Isolated mitochondria as well as whole muscle homogenates (substrate oxidation), mRNA (qRT pcr) and protein samples (Western blot) were analyzed. Changes in mitochondrial biogenesis were assessed by mRNA levels of peroxisome proliferator-activated receptors (PPAR) delta, peroxisome proliferator-activated receptor-γ co-activator (PGC-1α), and transcription factor A (TFAM). Protein levels of mitochondrial uncoupling protein three (UCP3)

and lipid peroxidation by-product 4-hydroxy-2-nonenal (4-HNE) were assessed, along with enzyme activities of citrate synthase (CS), 3-hydroxyacyl-CoA dehydrogenase (β-HAD). Pyruvate and Fatty acid oxidation measures were also performed. Clinical measures included a complete lipid panel, plasma free fatty acids (FFA), glucose and insulin concentrations which were used to perform the homeostasis model assessment (HOMA) to estimate beta cell function and insulin sensitivity. Strength (3 repetition maximum), body composition (DEXA), waist circumference, blood pressure, physical activity outside the RT program, and dietary intake were assessed. Secondary analysis of statin users vs. non-statin users was also performed.

No significant changes in mRNA levels of the targeted genes, pyruvate and fatty acid oxidation, and β-HAD activity were observed. CS activity was not changed in whole muscle homogenates, indicating that mitochondrial content did not change; however, CS activity when measured in isolated mitochondria was increased after 12-weeks of RT, indicating a qualitative adaption with RT. Complex I ROS production decreased with both 3 and 12 weeks of RT, while decreases in complex III ROS occurred after 3 weeks and approached significance (p=.069) after 12. Waist circumference and strength significantly improved with RT. These changes occurred without any affects on substrate metabolism.

Introduction

Aging is associated with a progressive loss in skeletal muscle mass and strength which is considered part of the normal aging process[1, 2]. Muscle strength has been proposed as the single best measure of age-related muscle change and its decline is associated with physical disabilities and functional limitations[3]. It has been observed that the majority of this age-related skeletal muscle loss is caused by the selective atrophy of type II fibers[4-6]. This shift towards a more slow-twitch (Type I) phenotype results in an overall decrease in force-generating capacity as type II muscle fiber composition is an important determinant of strength and peak force generating capacity[4, 6]. This muscle weakness leads to functional impairments, loss of personal independence and a decrease in quality of life[7, 8]. Activities of daily living (ADL) such as walking, standing, bathing, and dressing require sufficient strength and are more difficult with these age-related declines in strength [4]. The risk of falls, incidence of fractures, and survival rates following illness are all negatively impacted by reductions in muscle mass and strength[9-11]. A loss of muscle mass also increases risk of developing glucose intolerance and diabetes due to the fact that muscle tissue is the primary site of glucose disposal[12, 13], which relates to the findings that diabetes prevalence (undiagnosed and diagnosed) is highest in those aged ≥65 years[14]. These chronic conditions exert a profound economic impact on our nation being a major cause of morbidity and mortality in older individuals[14, 15]. An increase in the population of those aged ≥65 years will place a greater burden on health care and elevate health care costs, which could lead to inadequate public resources and less care for the aged[16]. The large societal and individual impact of age-related losses in muscle mass, coupled with the aging of our population, suggests a clear need for effective strategies that will lessen the impact of aging on skeletal muscle.

Mitochondrial Aging

Skeletal muscle mitochondria are thought to play a major role in the age-related declines in skeletal muscle function as the mitochondria are the main producers of both cellular energy and free radicals, as 90% of ROS are generated within the mitochondria[17]. Decreased mitochondrial function is believed to be a constant theme in the aging process, with oxidative capacity[18, 19], ATP synthesis[18], mitochondrial protein synthesis[20], mitochondrial protein content[18], and mtDNA deletions, mutations, and content all being negatively affected[4, 18, 21-24]. Aging mitochondria exhibit a less efficient transfer of electrons, resulting in a greater proportion of electrons partially reducing oxygen, producing amplified levels of ROS[25]. The lack of protective histones and close proximity to the ETC make mtDNA extremely vulnerable to oxidative stress, which may cause alterations in genes encoding subunits of the ETC as well as other mutations effecting ETC flux[26]. MtDNA encodes polypeptides of ETC and components required for their synthesis. Therefore, any coding mutation in mtDNA will affect ETC activity as a whole, which could affect both the assembly and functionality of the products of numerous genes, leading to ETC defects. These errors affect cellular energetics as a whole[26], and may bring about age-related phenotypes such as alopecia, kyphosis, osteoporosis, and cardiac hypertrophy[23]. Skeletal muscle mass and strength are also negatively impacted, as regions of skeletal muscle fibers that lose ETC activity display significant atrophy[27, 28]. Thus improvements in the aging phenotype may involve restoring mitochondrial function.

Improving mitochondrial function

Mitochondrial adaptation has been traditionally achieved through aerobic exercise training (AT)[29-34]. This occurs through a variety of pathways including calmodulin-dependent

signaling, reactive nitrogen and oxygen species-dependent signaling, AMP-activated protein kinase (AMPK) signaling, and p38 mitogen activated protein kinase (MAPK) signaling, leading to enhanced mitochondrial content and function (reviewed in[35])[29-33]. However, this mode of exercise does little to attenuate the loss in muscle mass exhibited in the elderly. In addition, factors such as obesity, arthritis, lower back pain, and physical disabilities affecting many older adults may preclude this population from regularly performing AT[36-38]. RT is an alternative mode of exercise that has been shown to be safe and effective at increasing lean body mass (primarily muscle mass) in older and even frail adults[39-43]. RT is the most effective strategy for stimulating hypertrophy and increasing strength[8, 44, 45], leading to improvements in ADL and a better quality of life[46]. In addition to being effective at increasing elderly individuals' lean body mass and quality of life, RT may invoke improvements at the cellular level including increased ETC complex IV activity and decreased oxidative stress[47], increased antioxidant capacity [48], and increased oxidative capacity and mitochondrial volume[49]. Improvements at the transcriptional level have also been noted, targeting genes affected by age[50]. One study investigating endurance training and resistance training in elderly adults (average age 69 years), showed greater improvements in oxidative capacity with RT vs endurance training (57% improvement vs. 31% respectively). In additionally, only the RT group exhibited increases in mitochondrial volume density and muscle size, concluding that the aerobic pathway is particularly sensitive to exercise training in elderly muscle and may be unregulated by RT[49]. This is in contrast to younger subjects who display lower oxidative enzyme activity and mitochondrial oxidative capacity with RT possibly due to the dilution of the mitochondrial structure with increases in muscle size[51]. This leads us to believe that RT may represent a potential avenue to improve the growing aged population's health and quality of life.

The purpose of the present study was to evaluate the effects of RT on older adult's mitochondrial function in terms of substrate oxidative capacity, ROS production, enzyme activities, and content. We employed a 12-week resistance training program and performed assessments at baseline, weeks 3 and 12. Performing mitochondrial measures at week three allowed us to determine if there are any changes taking place at the mitochondrial level early in the RT program before significant strength and lean mass accrue, while the 12 week assessment allowed us to capture the longer-term effects a RT program has at the mitochondrial level. We hypothesized that RT would lead to improvements in mitochondrial metabolism indicative by an increased substrate oxidative capacity, increased enzyme activities, and mitochondrial content. We further hypothesized that these changes would coincide with declines in ROS production at complexes I and III.

Materials and Methods

Design

This investigation used a randomized controlled trial design (see figure 1), employing one treatment group and one wait-list control group. The treatment group was enrolled in a 12-week RT program supervised by an ACSM certified personal trainer carried out at an on-campus exercise facility three days per week. During this 12-week period, muscle biopsies were obtained at week 0, 3, and 12. The wait-list control group remained sedentary for a 12-week period and had muscle biopsies at week 0, 3, and 12. The control group was offered the RT intervention after study completion. Using a randomized controlled design with a control group gave us the ability to attribute any changes assessed during the three and 12-week periods to the

RT program, and a wait-list control group was employed to reduce attrition among control group

participants.

Figure 1- Study Design
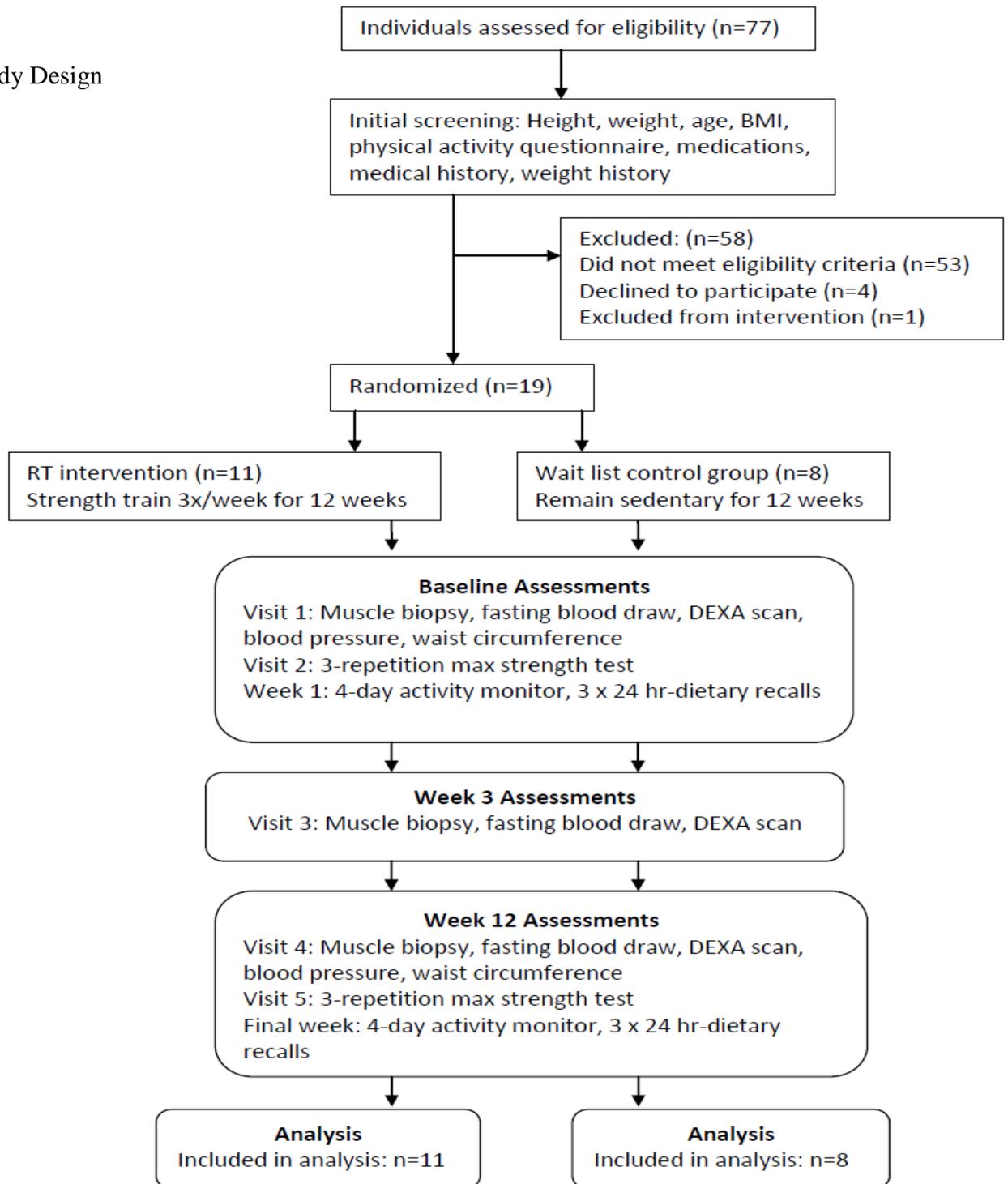
Participants

We recruited 20 male participants, aged ≥60 years. We focused on this age group because those aged 60+ have shown the greatest reductions in lean body mass, with muscle strength decreasing by 1.5% annually between ages 50 and 60 and by 3% thereafter[52]. Women were not included for the small sample size warranted a homogeneous group to best explore effects of the RT intervention on the mitochondrial outcomes. Participants were recruited using newspaper and e-mail advertisements from the surrounding area. Nineteen total participants completed the intervention as one participant had to be excluded due to an unexpected need for steroid medication after enrollment. Participants were asked to maintain their current weight and health behaviors as to minimize possible confounding factors. Only non-obese individuals were included (BMI<30) as this would eliminate the possible confounding effects obesity may have on mitochondrial function, (reviewed in[53]). Other inclusion criteria required participants to be sedentary to recreationally active (not engaging in weight training or more than 30 minutes of moderate intensity physical activity five days a week, or more than 20 minutes of vigorous physical activity three days a week). They had to be free of orthopedic injuries that would hinder training, and not allergic to lidocane used during the biopsy procedure. Due to the risks in beginning a new exercise regimen, those who smoke, have cardiovascular disease (uncontrolled hypertension, coronary artery disease, past myocardial infarction), liver, pulmonary disease, or a terminal illness such as cancer were excluded. Other exclusion criteria included taking medications for < one year that may influence energy metabolism and body composition (e.g, beta blockers, anti depressants and statins). Hypoglycemic agents such as metformin and thiazolidinediones (TZD's) along with thyroid replacements were not allowed. Participants taking over the counter anti-oxidant supplements such as multi-vitamins or a daily aspirin went

through a two-week wash-out period before their initial biopsy. We required all interested individuals obtain documentation of medical clearance from their personal physician prior to study enrollment.

RT intervention

The current investigation employed a 12-week RT intervention performed on three non-consecutive days per week using machine exercises (Life Fitness). The protocol included three upper body exercises: chest press, shoulder press, and lateral pulldown, and four lower body exercises: leg press, leg curl, leg extension, and plate-loaded leg press. Greater focus was placed on lower body exercises since muscle biopsies were taken from the vastus lateralis (explained below). Subjects performed one set of each exercise to volitional fatigue/failure (i.e. could not do another repetition) with the goal of performing 8 to 12 repetitions at a slow and controlled pace. The goal of this RT intervention was to improve muscle strength and hypertrophy while not changing aerobic performance, as increases in aerobic fitness may explain any improvements in mitochondrial function and confound results. Low volume RT protocols using low (3-5) to moderate (9-11) number of repetitions do not improve aerobic performance[54-56]. Similar single set protocols have proven effective at increasing lean body mass in this population while minimizing energy expended relative to multiple set protocols[57], possibly minimizing any adaptations in aerobic/oxidative metabolism. Employing a single set protocol where participants reached muscle fatigue after 8-12 repetitions has shown to be more affective at increasing muscle strength, muscle volume, static peak torque and dynamic peak torque compared to multiple set protocols or protocols using lighter weight where muscle fatigue was reached at 80-100 repetitions in older adults[58]. The caveat of training to muscle fatigue (or failure) has also been

emphasized as a key modulator for improving muscle strength and mass with RT. The degree of

effort determines the activation level of motor units and the resulting force output as a maximal

level of effort exerts a maximal activation of motor units[59]. Therefoe we anticipated this RT

intervention to maximize motor unit recruitment and the hypertrophic response, leading to

strength gains as seen previously[60].

Assessments

Muscle Biopsy: A muscle biopsy obtained in the fasted state from the vastus lateralis using the

Bergström needle suction technique[61] was performed to analyze targets of mitochondrial

function (explained below) at baseline, week 3 and week 12 for both the intervention and control

groups. Muscle tissue samples were used to assess fatty acid and pyruvate oxidation, enzyme

activities, ROS production, gene expression, and protein levels of selected targets. Samples were

immediately placed in SET buffer (0.25M Sucrose, 1mM EDTA, 0.01M Tris-HCl and 2mM

ATP) and stored on ice until homogenization (~25 min). Muscle used for mitochondrial

isolation was placed in isolation buffer (67mM sucrose, 50mM Tris/HCl, 50mM KCl, 10mM

EDTA/ Tris, and 10% bovine serum albumin; all from Sigma-Aldrich, St. Louis, MO) and stored

on ice until isolation. Muscle used for quantitative real-time polymerase chain reaction (qRT-

PCR) was placed in Trizol (Invitrogen, Carlsbad, CA) and snap-frozen in liquid nitrogen.

Mitochondrial Assessment

Isolated mitochondria as well as whole muscle homogenates were analyzed. Changes in

mitochondrial biogenesis were assessed by mRNA levels of peroxisome proliferator-activated

receptors (PPAR) delta[62], peroxisome proliferator-activated receptor-γ co-activator (PGC-1α)[63-]

[65], and transcription factor A (TFAM)[66] measured whole muscle homogenate samples. Mitochondrial content was determined by citrate synthase (CS) activity in whole muscle homogenates and in isolated mitochondria[67]. Substrate oxidation rates of pyruvate and fatty acids were performed in both whole muscle homogenates and isolated mitochondria offering insight into mitochondrial metabolism of glucose and fatty acids[68]. ROS production was also assessed at complexes I and III in isolated mitochondria. 3-hydroxyacyl-CoA dehydrogenase (β-HAD) was assessed in whole muscle homogenates to assess beta oxidation activity.

Skeletal muscle whole homogenate preparation: Each sample was minced 200 times with scissors and transferred to a glass homogenization tube and homogenized on ice using a Teflon pestle (12 passes at 150 RPM). The sample was rested on ice for ~30 seconds and the homogenization steps were repeated. The homogenate was transferred to an Eppendorf tube and fresh sample was used to measure pyruvate and fatty acid oxidation. Enzyme activity, protein expression, and mRNA levels were measured at a later date. Homogenate protein concentrations were determined spectrophotometrically using the bicinchoninic acid (BCA) assay (Thermo Scientific, Pittsburg, PA).

Mitochondrial isolation: Mitochondria were isolated and prepared as previously described with modifications[69]. Briefly, the biopsy sample was placed in 900μl of solution I (Final concentration in mmol/L: 100 KCL, 40 Tris-Cl, 10 Tris-base, 5 MgCl2, 1 EDTA, 1 ATP). 5mg protease Subtilisin A (Sigma, St. Louis, MO) in 1ml Solution I was added and muscle sample was minced for 1 minute and incubated for 6 minutes on ice. Sample was homogenized with loose #A homogenizer for 5 rotations and centrifuged at 700g for 10 minutes at 4°C.

Supernatant was removed and centrifuged at 14,000g for 10 minutes at 4°C. Pellet was

resuspended in 1ml of Solution II (Final concentration in mmol/L: 100 KCL, 40 Tris-Cl, 10 Tris-

base, 1 MgCl2, 0.1 EDTA, 0.2 ATP, 1.5% BSA) and centrifuged at 7000g for 10 minutes at 4°C.

Pellet was resuspended in 500µl of Solution III (Final concentration in mmol/L: 100 KCL, 40

Tris-Cl, 10 Tris-base, 1 MgCl2, 0.1 EDTA, 0.2 ATP) and centrifuged at 3500g for 10 minutes at

4°C. Isolated mitochondria were resuspended in 75µl of mannitol-sucrose buffer (Final

concentration in mmol/L: 220 Mannitol, 70 Sucrose, 10 Tris-HCL, 1 EGTA). Mitochondrial

protein concentrations were determined spectrophotometrically using the BCA assay (Thermo

Scientific, Pittsburg, PA). Isolated mitochondria were used fresh for analysis of fatty acid and

pyruvate oxidation as well as ROS production. All samples were run in triplicate.

Fatty acid and pyruvate oxidation: Palmitate and pyruvate oxidation rates were determined in

the fresh muscle homogenates prepared as described above. The oxidation rate in muscle

homogenates were measured by counting the $^{14}CO_2$ produced from [1-^{14}C] palmitic acid or [U-

^{14}C] pyruvate during incubation as previously described[70]. Briefly, 80 µl of a 20-fold (wt:vol)

diluted muscle homogenates were incubated with 320 µl of reaction media (pH 7.4). Final

concentrations of the reaction media were in mmol per liter: sucrose, 100; Tris-HCl, 10;

potassium phosphate, 5; potassium chloride, 80; magnesium chloride, 1; L-carnitine, 2; malate,

0.1; ATP, 2; coenzyme A, 0.05; dithiothreitol, 1; EDTA, 0.2; and bovine serum albumin, 0.3%.

After 60 minutes of incubation at 37°C, 200 µl of 70% perchloric acid were injected to stop the

reaction and evolve $^{14}CO_2$ from the reaction media. $^{14}CO_2$ produced during the 60-minute

incubation was trapped with 400 µl of 1M sodium hydroxide. Trapped $^{14}CO_2$ was determined by

liquid scintillation counting by use of 5 ml EcoLite liquid scintillation cocktail (MP Biomedicals,

Santa Ana, CA) in the LS 6500 scintillation counter (Beckman Coulter, Pasadena, CA). Total

fatty acid oxidation was determined by measuring and summing the production of ^{14}C-labeled

CO_2 and ^{14}C-labeled acid soluble metabolites. All samples were run in triplicate and data was

normalized to protein content.

ROS production: Amplex Red Hydrogen Peroxide/Peroxidase assay Kit was used for measures

of ROS production. Immediately following isolation and protein quantification, mitochondria

were plated on 96-well black plate at a concentration of 5ug/well in the presence of pyruvate

(20mM)/ malate (10mM)/oligomycin (2μM)/rotenone (200nM), pyruvate (20mM)/ malate

(10mM)/oligomycin (2μM)/ SOD (400U/ml)/antimycin A (2μM), or succinate (20mM).

Experiments were conducted in sucrose/ mannitol solution in order to maintain the integrity of

the mitochondria. Amplex Red working solution was loaded into the plate to begin the reactions.

Experiments consisted of 1 minute delay and 1 minute reading cycles, followed by a 5 second

mixing cycle performed every third reading. All experiments were performed at 37°C. Measures

for ROS levels were conducted on a microplate reader (Biotek synergy 2, Winooski, VT).

Fluorescence of Amplex Red was measured using a 530nm excitation filter and a 560nm

emission filter.

Enzyme activities: β-HAD and CS activities were determined spectrophotometrically from

muscle homogenates (β-HAD) and both muscle homogenates and isolated mitochondria (CS). β-

HAD activity was determined by measuring the oxidation of NADH to NAD.   Measurements

were conducted at 37 °C in assay buffer containing 0.1 M triethanolamine-HCl, 5 mM EDTA,

and 0.45 mM NADH (pH 7.0). After an initial 1-minute absorbance reading at 340 nm, the

reaction was initiated with the addition of 0.1 mM acetoacetyl-CoA, and the change in absorbance was measured every 10 seconds for 5 minutes. CS catalyzes the formation of citrate and CoASH from acetyl-CoA and oxaloacetate. CoASH reduces DTNB (Ellman's Reagent, 5,5'-Dithiobis-(2-Nitrobenzoic Acid) and CS activity was determined from the reduction of DTMB over time. CS activity was measured at 37 °C in 0.1 M Tris-HCl (pH 8.3) assay buffer containing 0.12 mM 5, 5′-dithio-bis (2-nitrobenzoic acid) and 0.6 mM oxaloacetate. After an initial 2-minute absorbance reading taken at 412 nm, the reaction was initiated with the addition of 3.0 mM acetyl-CoA, and the change in absorbance was measured every 10 seconds for 7 minutes.

RNA extraction and qRT-PCR: RNA was extracted using an RNeasy Mini Kit (Qiagen) and DNase I treatment (Qiagen, Valencia, CA), according to the manufacturer's instructions. qRT-PCR was performed using an ABI PRISM 7900 Sequence Detection System instrument and TaqMan Universal PCR Master Mix used according to the manufacturer's specifications (Applied Biosystems, Foster City, CA). Target gene expression in skeletal muscle was normalized to cyclophilin B RNA levels. Primers and 5# FAM-labeled TaqMan probes were purchased as prevalidated assays (ABI). Relative quantification of target genes was calculated using the $2^{-\Delta C_T}$ method. Derivation of the $2^{-\Delta C_T}$ equation has been described in Applied Biosystems User Bulletin no. 2 (P/N 4303859). All samples were run in triplicate.

Western blotting: Western analysis was performed using whole muscle homogenates as previously described. Proteins (30 μg) were separated using a 10% Criterion-Tris·HCl gel (Bio-Rad, Hercules, CA) and subsequently transferred to a polyvinylidene difluoride membrane (Bio-

Rad). Blots were probed with primary antibodies against 4-HNE and UCP3 (1:1,000; Cell

Signaling, Danvers, MA), followed by anti-rabbit, mouse, or goat secondary antibodies

(1:20,000; Jackson ImmunoResearch Laboratories, West Grove, PA). Proteins were visualized

using Super-Signal Chemiluminescent Substrate (Pierce, Rockville, IL) and a ChemiDoc XRS

Imaging System (Bio-Rad).

Anthropometrics and clinical measures

 Height was measured in meters without shoes using a wall-mounted stadiometer. Body weight

was measured to the nearest 0.1 kg using a digital scale with participants wearing light street

clothing and no shoes (Scale-Tronix model 5002, Wheaton, IL) before each muscle biopsy.

Waist circumference was measured to the nearest 0.5 cm at the umbilicus, using a Gulick tape

measure (Gulick, Country Technology, Inc, Gays Mill, WI).

Body composition: Before each muscle biopsy, lean and fat mass (measured in kg) was

determined by DEXA (GE Lunar Prodigy Advance, GE Healthcare, Madison, WI), which has

been shown to have excellent precision in determining lean body mass with root means squared

error values of 0.244 kg[71]. Bone mineral content was not included in the analysis as this was

measure was not of interest in the present study. Therefore the lean mass reported included only

the lean mass of soft tissues. DEXA results we were used to calculate skeletal muscle index

(SMI), a measure of sarcopenia expressed as appendicular lean mass / height (kg/m^2)[72]. Cut-

points have been set to diagnose sarcopenia in men at 7.23 kg/m^2 [73].

Physical activity: Prior to enrollment, subjects completed the Cooper Clinic PAQ to determine

their average weekly energy expenditure measured in METs[74, 75]. Each person was asked "For

the last 3 months which of the following activities have you performed regularly?" Individuals provided information about participation, frequency per week, the intensity level, and duration per activity. The activities participants reported were assigned MET values[76] and their activity level was determined in MET·hr·wk. Participants also wore an accelerometer (ActiGraph, Pensacola, FL) for a four-day period at baseline and again at 12 weeks to determine physical activity level outside of the training protocol measured in steps per day. This has been shown to be a sufficient time frame to assess habitual physical activity in adults[77], and was used to assess changes in PA.

Strength assessment: Upper body and leg strength were assessed at baseline and week 12 using a three-repetition max (3-RM) test for chest press and leg press. The 3-RM was defined as the maximal resistance that could be moved through the full range of motion for three repetitions. This test was used rather than a one-rep max test due to safety concerns, and a test using a higher number of repetitions may become a measure of muscle endurance rather than strength[78]. Subjects began each test with a warm-up set of three repetitions and resistance was progressively increased in subsequent sets to a point where the subjects could not perform three repetitions. Similar number of trials (4-8) and rest period between trials (30-60 sec.) were used to reach the 3-RM weight before and after training.

Clinical blood measures: Resting blood pressure was measured in the seated position using a mercury sphygmomanometer; the average of three measurements ±6 mmHg was used. Blood pressure and waist circumference were assessed at baseline and week 12. A fasting blood sample was taken to assess plasma glucose, insulin, and lipid/lipoprotein concentrations before each muscle biopsy (baseline, week 3, and week 12). This was used to calculate a HOMA-IR

and β-cell function as described in[79], using the equations HOMA-IR= glucose * insulin / 405 and % β-cell function= 360*insulin/glucose-63. Beta cell function is reported as the percent of normal weight, <35yrs old, male reference population. Glucose concentration (mg/dl) was determined via YSI 2300 STAT Plus™ Glucose & Lactate Analyzer (YSI Life Sciences Inc, Yellow Springs, Ohio). Insulin concentration (Mu/L) was determined via ELISA (ALPO Diagnostics, Salem, NH).

Dietary analysis: Subjects completed three 24-hour dietary recalls during the week of initial testing and again following their final biopsy to assess usual dietary intake. These were completed by a trained diet technician and included one weekend day and two weekdays. The dietary recalls were analyzed using NDS-R version 2011 (University of Minnesota, Minneapolis MN).

Statistical Analysis

Independent sample T-tests were performed to determine baseline group differences in height, weight, age, physical activity, strength, BMI, and body composition. A repeated measures ANOVA was performed to determine differences in lean body mass, fat mass, muscle strength, blood lipid, insulin, and glucose measures, along with the mitochondrial measures over time between the intervention and control groups. To gain further insight into changes occurring during the intervention, the value at baseline for each measure was subtracted from values at 3 and 12 weeks to yield change scores (change from baseline to 3 week and change from baseline to 12 week). Independent sample T tests were performed to assess changes between these two change scores offering insight to when changes were occurring. A one-tailed P-value was used for the T-test change scores for variables hypothesized to change with RT. Independent sample

T tests were also performed to analyze differences in statin users vs. non-users in the intervention group, and a second repeated measures ANOVA was carried out among all subjects who were not taking statin medication. An expectation maximization algorithm was used to complete missing data points due to laboratory or analytical errors for the mitochondrial measures. A total of 79 data points were filled, a total of 3.65% of all possible data points for mitochondrial related variables. All data is presented in means ± SD.

Results

Baseline subject characteristics are reported in Table 1, with no significant differences between groups.

Table 1. Baseline group characteristics (mean ± SD)

	RT Intervention Group, n=11	Waitlist Control Group, n=8	p-value
Race, white/nonwhite, n	11/0	8/0	
Age, years	64.8 ± 4.1	67.9 ± 5.8	0.177
Weight, kg	80.1 ± 10.1	85.1 ± 7.4	0.252
Height, m	1.76 ± 6.02	1.75 ± 5.77	0.675
BMI, kg/m2	25.8 ± 2.6	27.8 ± 2.0	0.089
Leg Press strength test, 3-RM	241.8 ± 62.3	268.8 ± 64.9	0.373
Chest Press strength test, 3-RM	115.5 ± 28.1	108.8 ± 34.0	0.644
Skeletal Muscle Index, ALM/m^2	9.58 ± 0.63	9.79 ± 0.83	0.528
Physical Activity, steps/day	7298 ± 3442	6861 ± 2428	0.763
Physical Activity, MET.hrs/week	35.8 ± 23.5	34.9 ± 17.0	0.934
% body fat	26.2 ± 7.0	28.32 ± 3.2	0.413
% lean mass	70.0 ± 6.5	68.0 ± 3.1	0.414

Mitochondrial Metabolism:

Changes in Substrate Oxidation

After both 3 and 12 weeks of RT, no changes were observed in PDH activity (CO_2 produced) or any of the FAO assessments as summarized in table 2 in either isolated mitochondria or whole muscle homogenates. FAO measurements included Total FAO, CO_2 produced, and acid soluble metabolites (ASM) produced. The CO_2 produced / ASM ratio was also calculated.

Changes in ROS production

ROS measured at Complex I significantly decreased with RT (p=0.016). Change scores indicate that most of this decrease occurred after the first 3 weeks (-65.787 au, p=0.004, although the change from baseline to 12 weeks (-17.982 au) remained significant (p=0.02). Similar reductions were observed for complex III ROS, although only approaching significance (p=0.069) when determined via ANOVA. Change scores yielded significant decreases from baseline to 3 weeks (-49.546, p=.036) but this improvement did not persist after 12 weeks (table 3). The control group increased ROS production at each time point for both complexes I and III. This is demonstrated in figure 2.

Changes in Enzyme Activity

The overall activity of β-HAD did not reach significance when assessed by ANOVA, but when further analyzing change scores, a significant change appears in the baseline to 12 week time point. Neither group experienced changes in CS activity measured in whole muscle homogenates during the intervention; however, CS activity in isolated mitochondria did show an

increase with RT compared to control (p=0.012, table 4). Further analysis assessing the change

scores from baseline indicated that this change requires a longer-term RT program as there was

no significant change after 3 weeks (figure 3).

Figure 2-
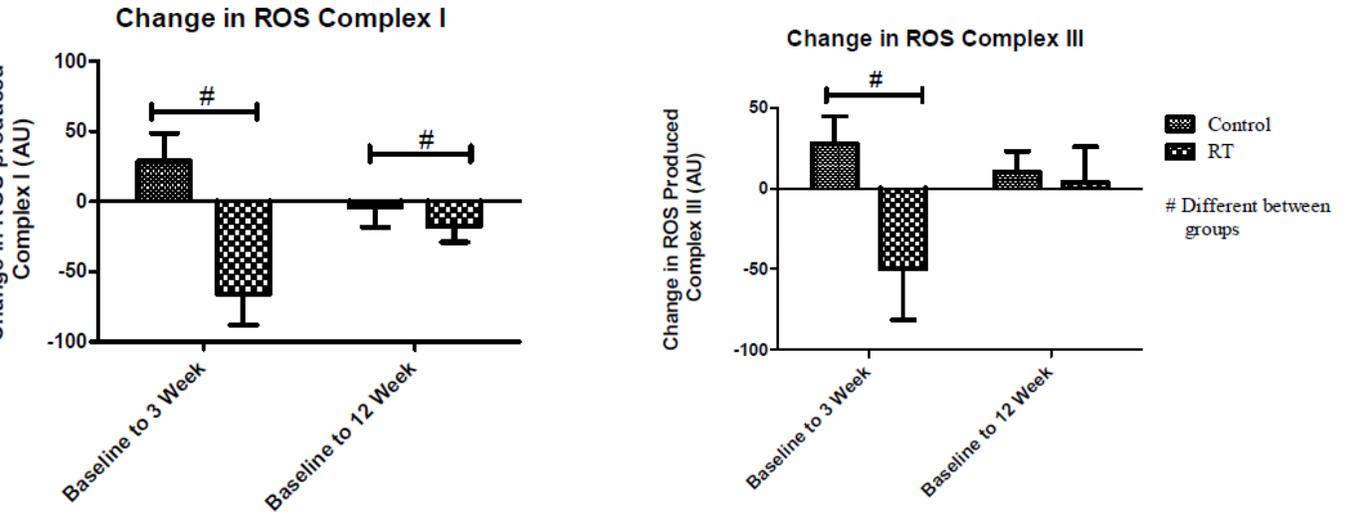Changes in
ROS in
intervention
and control
groups
(mean ± SD)

Figure 3-
Changes in
CS in
intervention
and control
groups
(mean ± SD)
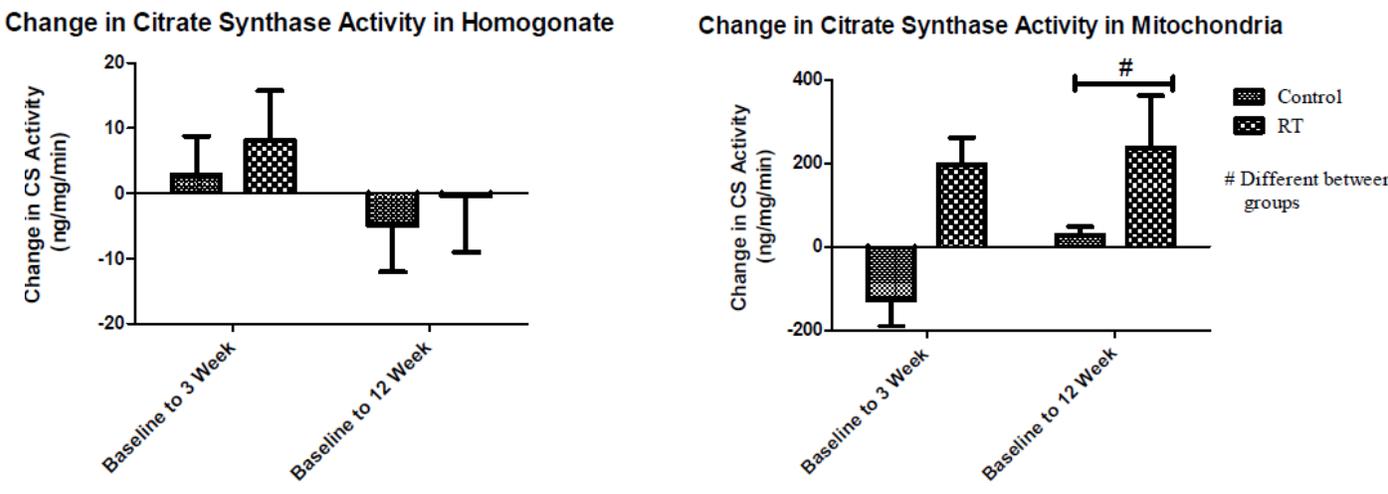
Changes in gene expression, mRNA levels

Similar to the substrate oxidation measures, no significant changes in mRNA levels were observed for the three target genes (PPAR δ, PGC-1α and TFAM, table 5) in either group during the intervention.

Western blot protein analysis

To glean insight into the significant decreases in ROS production with RT, western blots were performed to assess protein levels of mitochondrial uncoupling protein three (UCP3) and lipid peroxidation by-product 4-hydroxy-2-nonenal (4-HNE). 4-HNE is increased in periods of oxidative stress due to increased lipid peroxidation and UCP3 expression has been shown to decrease ROS production by lowering mitochondrial membrane potential[80]. The expression of these two proteins may coincide with the decreased ROS production with RT. As indicated in table 6 there were no changes in either parameter between groups, leading us to believe that other antioxidant defense mechanisms are involved.

Table 2- Changes in pyruvate and fatty acid oxidation in intervention and control groups after 3 and 12 weeks (mean ± SD)

	RT Intervention Group			Control Group			P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12				
PDH-CO2 produced in Homogenate	420.59 ± 179.04	352.85± 109.31	570.70 ± 271.74	398.43 ± 202.14	400.15 ± 291.53	442.71 ± 194.24	0.423	0.150		
Change		-67.74 ± 196.02	150.11 ± 291.76		10.715 ± 383.73	53.28 ± 302.44			0.283	0.246
PDH-CO2 produced in isolated mitos	1676.99 ± 702.82	1397.60 ± 364.05	2443.82 ± 1501.75	1536.87 ± 826.56	1660.52 ± 890.08	2131.88 ± 660.88	0.597	0.086		
Change		-279.39 ± 747.46	766.83 ± 1398.74		123.66 ± 1094.25	595.02 ± 996.62			0.176	0.386
FAO- CO2 produced in Homogenate	1.11 ± 0.42	0.79 ± 0.25	0.84 ± 0.40	0.98 ± 0.67	1.02 ± 1.02	1.01 ± 0.84	0.722	0.795		
Change		-0.32 ± 0.85	-0.27± 0.38		0.034± 1.44	0.03 ± 1.12			0.254	0.213
FAO- CO2 produced in Isolated mitos	1.70 ± 1.21	2.07 ± 2.77	1.43 ± 0.83	1.15 ± 1.38	1.53 ± 1.12	1.03 ± 0.35	0.873	0.705		
Change		0.370 ± 2.74	-0.27± 0.32		0.38 ± 1.21	-0.12± 1.20			0.495	0.383
FAO- Total FAO in Homogenate	12.52 ± 5.01	12.12 ± 3.30	13.76 ± 4.19	14.18 ± 5.44	14.43 ± 10.52	14.93 ± 5.21	0.942	0.742		
Change		-0.40 ± 4.72	1.23 ± 4.76		0.247 ± 12.39	0.64 ± 6.74			0.429	0.412
FAO- Total FAO in isolated mitos	36.61 ± 15.41	35.12 ± 7.86	40.15 ± 15.83	28.90 ± 23.18	45.70 ± 29.25	44.54 ± 39.13	0.182	0.113		
Change		-1.84 ± 15.34	3.49 ± 14.53		18.01 ± 36.68	17.32 ± 36.516			0.081	0.149
FAO- ASM in Homogenate	11.41± 4.60	11.24 ± 3.35	12.79 ± 3.95	13.21 ± 4.84	13.41 ± 9.87	13.80 ± 3.87	0.930	0.695		
Change		-0.18 ± 4.32	1.38 ± 4.51		0.21 ± 11.33	0.61± 5.69			0.459	0.373
FAO- ASM in isolated mitos	34.90 ± 14.82	33.36 ± 6.89	38.35 ± 14.74	27.77 ± 21.92	44.17 ± 28.53	41.74 ± 34.04	0.189	0.113		
Change		-1.54 ± 13.28	3.45 ± 13.79		16.41 ± 33.23	13.98 ± 26.48			0.061	0.137

	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12	P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
FAO- CO2:ASM in Homogenate	0.10 ± 0.05	0.08 ± 0.06	0.07 ± 0.04	0.07± 0.03	0.08 ± 0.04	0.07 ± 0.04	0.287	0.239		
Change		-0.02 ± 0.06	-0.03 ± 0.04		0.01± 0.06	0.00 ± 0.05			0.163	0.064
FAO- CO2:ASM in Isolated mitos	0.05 ± 0.04	0.06 ± 0.07	0.04 ± 0.02	0.03 ± 0.02	0.04 ± .02	0.03 ± 0.01	0.572	0.390		
Change		0.01 ± 0.7	-0.02 ± 0.04		0.00 ± 0.02	0.00 ± 0.02			0.449	0.182

Units: total FAO (nm CO_2 produced/hr); FAO CO_2 produced and ASM (nm CO_2/g protein/hr); PDH activity (nm CO_2/mg protein/hour)

Table 3- Changes in ROS production (au) in intervention and control groups after 3 and 12 weeks (mean ± SD)

	RT Intervention Group			Control Group			P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12				
Complex I	192.88 ± 71.52	127.09 ± 26.97	174.90± 87.37	127.43 ± 36.46	156.17 ± 65.82	144.02 ± 53.69	0.016	0.517		
Change		-65.79 ± 72.834	-17.982 ± 36.415		28.735 ± 56.043	16.59 ± 38.59			0.004	0.032
Complex II	188.33 ± 70.25	138.79 ± 53.131	192.24 ± 52.46	142.05± 38.25	169.54 ± 80.29	152.48 ± 60.98	0.069	0.384		
Change		-49.55± 105.04	3.907 ± 72.067		27.50 ± 48.24	10.44 ± 35.29			0.036	0.409
Reverse flow electrons	820.00 ± 275.86	636.88 ± 196.24	693.68 ± 159.98	619.20 ± 202.60	589.75 ± 273.60	656.69 ± 222.94	0.345	0.339		
Change		-183.12 ± 418.64	-126.31 ± 280.89		-29.45 ± 188.45	37.49 ± 186.80			0.175	0.086

Table 4- Changes in CS and BHAD activity (ng/mg protein/min) in intervention and control groups after 3 and 12 weeks (mean ± SD)

	RT Intervention Group			Control Group			P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12				
BHAD activity	24.38 ± 7.42	26.69± 5.08	31.27± 4.71	24.09 ± 5.84	22.36± 5.52	23.83 ± 6.67	0.209	0.124		
Change		2.31 ± 5.19	6.89 ± 8.94		-1.73 ± 5.25	0.443 ± 6.45			0.114	0.101
CS activity in homogenate	74.84 ± 15.47	82.95 ± 30.70	74.38 ± 17.55	59.91 ± 18.33	62.71 ± 11.56	54.98 ± 12.11	0.848	0.506		
Change		8.11 ± 25.36	-0.46 ± 28.42		2.80 ± 17.08	-4.94 ± 20.10			0.615	0.708
CS activity in isolated mito)	608.89 ± 240.34	806.82 ± 353.72	846.31 ± 434.85	789.59 ± 349.97	839.78 ± 403.31	663.51 ± 228.17	0.012	0.089		
Change		197.92 ± 212.17	237.41 ± 411.83		50.19 ± 247.19	-126.09 ± 17.35			0.17	0.032

Table 5- Changes in mRNA expression of mitochondrial gene targets relative to cyclophilin B in intervention and control groups after 3 and 12 weeks (mean ± SD)

	RT Intervention Group			Control Group			P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12				
TFAM expression	1.03 ± 0.66	0.75 ± 0.45	2.08 ± 6.51	2.11 ± 3.71	1.78 ± 2.60	1.53 ± 1.78	0.626	0.759		
Change		-0.28 ± 0.77	1.78 ± 6.41		-0.33± 4.46	-0.84 ± 2.139			0.485	0.159
PGC1 alpha expression	1.06 ± 0.46	1.54 ± 0.74	0.94 ± 0.26	1.06 ± 0.38	1.20 ± 0.48	1.05 ± 0.65	0.405	0.100		
Change		0.48 ± 0.92	-0.11 ± 0.54		0.14± 0.33	-0.02 ± 0.52			0.170	0.361
PPAR delta expression	1.26 ± 0.70	0.85 ± 0.29	0.94± 0.71	1.05 ± 0.30	0.99± 0.21	0.86± 0.36	0.523	0.143		
Change		-0.42 ± 0.75	-0.33 ± 0.69		-0.06± 0.46	-0.15± 0.38			0.124	0.279

Table 6- Changes in protein expression of 4-HNE and UCP3 (au) in intervention and control groups after 3 and 12 weeks measured by western blot protein analysis (mean ± SD)

	RT Intervention Group			Control Group			P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12				
4-HNE	0.076 ± 0.199	0.082 ± 0.035	0.086 ± 0.039	0.061 ± 0.022	0.133 ± 0.089	0.082 ± 0.044	0.181	0.080		
Change		0.006 ± 0.021	0.010 ± 0.042		0.075 ± 0.088	0.022 ± 0.044			0.015	0.321
UCP3	48.46 ± 20.34	50.85 ± 16.61	47.60 ± 19.79	46.66 ± 10.79	41.52 ± 16.78	64.66 ± 40.72	0.343	0.528		
Change		2.39 ± 28.48	-0.86 ± 20.94		0.44 ± 24.43	18.0 ± 50.42			0.452	0.198

Clinical Measures:

Anthropometrics, strength, PA

As indicated in table 7, there was no significant change in LBM with the intervention in the RT or control group. Lean body mass only modestly increased, failing to reach significance at both the three week and 12 week time points with RT, increasing 0.302 kg after 3 weeks and 0.658 after 12 weeks (p= 0.1785 and 0.188 respectively). SMI (assessed pre-post) and lean mass of just the legs or arms independently also did not change significantly with RT. Kg body fat and % body fat showed similar trends as lean mass, only modestly improving with RT. Neither BMI nor body weight showed significant changes in either group. An interesting finding was that waist circumference (measured pre and post) significantly decreased with RT (p=0.006, table 7). As anticipated, strength (3-RM of chest and leg press, measured pre and post) was increased with RT (p=<0.001, table 7). Physical activity (4 day average step count) was not changed in either group (table 7).

HOMA-IR, beta cell function, fasting glucose

As indicated in Table 8, there were no changes in fasting glucose, insulin or HOMA-IR and % beta cell function in either group during the intervention. However, change scores for HOMA-IR and fasting insulin indicated trends approaching significance with RT after 3 weeks and 12 weeks (-0.731, p=.0565 and -0.458, p=.0575 for HOMA-IR respectively, and -2.130, p=0.097 and -1.687, p=0.085 respectively).

Blood lipoprotein and triglycerides

Table 8 also includes results of blood lipoproteins and triglycerides, indicating no significant changes in either group during the intervention when assessing both ANOVA between groups and change scores.

Dietary Intake

There was no group by time changes between groups for any of the dietary intake data, all $p > 0.05$. Kilocalories per day for the intervention group was 1916 ± 380 at baseline and 1928 ± 368 at 12 weeks; while control groups caloric intake was 2129 ± 359 at baseline and 2241 ± 473 at 12 weeks. Percent carbohydrates/ protein/and fat for the intervention group was 45/16/ 34 at baseline and 47/16/ 34 at 12 weeks respectively. The control group had similar dietary composition with baseline ratios of 42/16/ 33 for percent carbohydrate protein and fat respectively, and 45/16/32 at 12 weeks.

Table 7- Changes in Anthropometrics, Strength, and physical activity in intervention and control groups after 3 and 12 weeks (mean ± SD)

	RT Intervention Group			Control Group			P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12				
Body weight (kg)	80.0± 10.1	80.0 ± 9.6	80.4± 9.9	85.1 ± 7.4	84.7 ± 6.5	84.6 ± 6.9	0.642	0.651		
Change		-0.11 ± 1.1	0.28 ± 2.0		-0.39 ± 1.2	-0.54 ± 1.3			0.305	0.170
BMI, kg/m2	25.8 ± 2.6	25.8 ± 2.5	26.0± 2.4	27.8 ± 2.0	27.8 ± 2.0	27.8 ± 2.1	0.703	0.95		
Change		-0.02 ± 0.4	0.10 ± 0.58		-0.03 ± 0.28	-0.09 ± 0.39			0.485	0.221
Total body fat, kg	21.29 ± 7.56	20.93 ± 6.95	20.82 ± 6.69	23.82 ± 3.37	23.71 ± 3.46	23.32 ± 3.35	0.757	0.436		
Change		-0.37 ± 0.98	-0.469 ± 1.64		-0.11 ± 0.93	-0.49 ± 1.42			0.289	0.487
% body fat	26.18 ± 6.96	25.81 ± 6.45	25.64 ± 5.95	28.31 ± 3.21	28.19 ± 3.48	27.95 ± 3.41	0.906	0.486		
Change		-0.36 ± 1.07	-0.533 ± 1.63		-0.13 ± 1.16	-0.37 ±1.48			0.327	0.410
Total lean mass, kg	55.13 ± 5.06	55.43± 4.89	55.79 ± 5.33	57.35 ± 5.81	57.10 ± 5.75	57.46 ± 6.03	0.615	0.307		
Change		0.30 ± 1.21	0.66 ± 1.30		-0.25 ± 1.41	0.11 ± 1.13			0.188	0.179
% lean mass	70.03 ± 6.46	70.08 ± 5.98	70.25 ± 5.37	67.95 ± 3.14	68.00 ± 3.35	68.32 ± 3.32	0.970	0.710		
Change		0.05 ± 1.69	0.22 ± 2.08		0.05 ± 1.24	0.37 ± 1.56			0.499	0.432
Skeletal Muscle Index, ALM/m²	9.58 ± 0.63	-	9.83 ± 0.58	9.79 ± 0.83	-	10.03 ± 0.88	0.884	0.001		
Appendicular lean mass, kg	16.84 ± 1.25	-	17.29 ± 1.29	17.10 ± 1.59	-	17.52 ± 1.74	0.871	0.001		
Waist circumference, cm	97.36 ± 8.71	-	95.29 ± 8.23	100.50 ± 4.56	-	100.86 ± 4.06	0.006	0.036		

Physical Activity, steps/day	7298.1 ± 3442.2	-	7526.0 ± 3105.0	6860.7 ± 2427.7	-	7409.7 ± 2677.2	0.801	0.544
Leg Press strength test, 3-RM	241.82 ± 62.26	-	326.36 ± 51.44	268.75 ± 64.90	-	253.75 ± 57.80	<0.00	<0.00
Chest Press strength test, 3-RM	115.46 ± 28.06	-	153.67 ± 38.28	108.75 ± 33.99	-	106.25 ± 33.78	<0.00	<0.00

Table 8- Changes in clinical blood measures in intervention and control groups after 3 and 12 weeks (mean ± SD)

	RT Intervention Group			Control Group						
	Baseline	Week 3	Week 12	Baseline	Week 3	Week 12	P (group x time)	P (time)	p (Δ bsl to 3 wk)	P (Δ bsl to 12 wk)
Blood Glucose	104.6 ± 23.3	96.4± 22.5	103.2 ± 15.8	110.8 ± 22.6	112.0 ± 19.6	123.8 ± 27.2	0.617	0.499		
Change		-8.2 ± 27.8	-1.4 ±31.4		1.2 ± 23.7	13.1 ± 35.4			0.226	0.180
Insulin	7.37 ± 3.46	5.24 ± 2.15	5.67 ± 3.01	5.20 ± 1.06	5.18 ± 1.87	5.41 ± 2.13	0.341	0.412		
Change		-2.13 ± 4.12	-1.69 ± 3.22		-0.01 ± 1.87	0.22 ± 2.23			0.097	0.085
HOMA-IR	1.93 ± 0.97	1.20 ± 0.45	1.47 ± 0.88	1.42 ± 0.40	1.40 ± 0.48	1.67 ± 0.93	0.209	0.241		
Change		-0.73 ± 1.08	-0.46 ± 1.03		-0.02 ± 0.61	0.25 ± 0.74			0.057	0.058
HOMA-βcell	-36.96 ± 14.10	-41.92 ± 10.75	-42.98 ± 10.62	-45.63 ± 4.11	-45.46 ± 8.06	-46.65 ± 6.42	0.655	0.49		
Change		-4.96 ± 16.73	-6.02 ± 13.75		0.17 ± 8.00	-1.03 ± 9.06			0.218	0.192
Total Cholesterol	177.1 ± 25.6	177.7 ± 29.2	178.5 ± 31.4	216.1 ± 35.5	211.6 ± 28.7	216.1 ± 40.8	0.832	0.814		
Change		0.64 ± 16.7	1.4 ± 14.5		-4.5 ± 14.5	0.00 ± 21.0			0.278	0.435
HDL-C	57.4 ± 12.4	8.0 ±11.4	51.09 ± 19.8	53.0 ±11.1	53.8 ± 14.1	51.4 ± 9.6	0.693	0.368		
Change		0.6 ± 6.7	-6.3 ± 14.4		0.75 ± 4.3	-1.7 ± 5.1			0.471	0.198
LDL-C	98.7 ± 28.5	102.1 ± 28.8	103.6 ± 31.7	134.1 ± 29.4	132.5 ± 22.8	135.9 ± 28.8	0.781	0.615		
Change		3.4 ± 13.7	4.9 ± 12.7		-1.6 ± 16.0	1.8 ± 6.2			0.238	0.327
Triglycerides	105.4 ± 46.1	88.5 ± 27.4	95.0 ± 27.4	144.9 ± 36.1	126.4± 33.2	144.6 ± 40.2	0.806	0.288		
Change		-16.9 ± 43.2	-10.4 ± 44.0		-18.5 ± 58.3	-0.3 ± 52.3			0.473	0.327

Units: glucose (mg/dl), insulin (Mu/l), HOMA-βcell function (%), cholesterol and triglycerides (mg/dl)

Statin use

The inclusion of individuals currently on a statin medication regimen for at least one year was to ensure that the medication was well tolerated and any symptoms of muscle and joint pain were absent. However recent reports suggest that statin use may be detrimental to improving aerobic fitness and mitochondrial function when paired with a training program[81]. For this reason we compared the participants randomized into the intervention group who were taking statins vs. those not taking a statin. Four individuals in the intervention group were taking a statin medication ranging from 20mg to 40 mg per day, three on atorvastatin and one on simvastatin, while only one individual in the control group was a statin user. Changes from baseline to 3 weeks and baseline to 12 weeks for statin users and non users in the intervention group for measures of interest are presented in table 9, with no significant differences detected. Further repeated measures ANOVA analysis was performed with statin users excluded from the analysis between intervention and control groups; however any changes failed to reach significance (data not shown).

Table 9- Changes in select measures over three and 12 weeks of RT for statin users and non users in the intervention group; all P>0.05 (mean ± SD)

	Change from baseline to 3 weeks		Change from baseline to 12 weeks	
	Statin users (n=4)	Non- users (n=7)	Statin users (n=4)	Non- users (n=7)
Mitochondrial measures:				
β-HAD activity	3.20 ± 5.34	1.80 ± 5.45	9.90 ± 3.33	5.17 ± 10.88
CS acivity- Homogenate	-8.12 ± 13.61	17.39 ± 26.52	9.69 ± 17.40	-6.26 ± 32.96
CS acivity- Isolated mito	72.58 ± 138.62	269.55 ± 221.27	-6.31 ± 155.76	376.69 ± 456.41
Acid soluble metabolites- Homogenate	-1.20 ± 6.07	0.41 ± 3.41	0.26 ± 6.06	2.01 ± 3.77
Acid soluble metabolites- Isolated mito	-6.39 ± 9.62	1.23 ± 14.94	-4.87 ± 7.87	8.21 ± 14.61
CO_2 produced during FAO	-0.57 ± 0.72	0.90 ± 3.36	-0.36 ± 0.32	-0.23 ± 1.36
PDH acivity- Homogenate	-154.84 ± 220.03	-17.98 ± 178.58	37.06 ± 222.50	214.70 ± 322.07
PDH acivity- Isolated mito	-304.08 ± 566.25	-265.29 ± 877.61	663.88 ± 1010.33	825.66 ± 1655.08
Reverse flow electrons	-106.50 ± 334.73	-226.91 ± 479.50	0.42 ± 279.99	-198.74 ± 274.73
ROS complex I	-67.58 ± 79.02	-64.76 ± 75.60	-27.17 ± 25.54	-12.73 ± 42.37
ROS complex II	-58.17 ± 132.36	-44.62 ± 97.73	27.17 ± 107.33	-9.38 ± 48.26
TFAM expression	0.06 ± 0.16	-0.47 ± 0.92	5.45 ± 10.41	-0.32 ± 0.49
Total FAO- Homogenate	-1.58 ± 6.21	0.27 ± 4.06	0.03 ± 6.42	1.92 ± 3.94
Total FAO- Isolated mito	-6.95 ± 10.25	1.64 ± 15.97	-5.23 ± 8.05	8.56 ± 14.27
Measures from fasting blood draw:				
Blood glucose	-18.0 ± 32.8	-2.6 ± 25.5	-17.3 ± 42.7	7.6 ± 21.6
Insulin	-1.25 ± 2.47	-2.63 ± 4.94	0.18 ± 3.06	-2.76 ± 2.98
HOMA-IR	-0.70 ± 1.06	-0.75 ± 1.18	-0.34 ± 1.31	-0.53 ± 0.95
HOMA-βcell	-0.64 ± 7.98	-7.43 ± 20.37	4.36 ± 12.95	-11.95 ± 10.89
Total Cholesterol	-2.3 ± 13.8	2.3 ± 19.0	-6.3 ± 4.3	5.7 ± 16.8
HDL	3.3 ± 6.6	-1.0 ± 6.8	-13.3 ± 22.5	-2.3 ± 6.3
LDL	-32.5 ± 37.8	-8.0 ± 46.3	-27.3 ± 60.5	-0.7 ± 33.1
Triglycerides	0.8 ± 15.4	4.9 ± 13.7	-0.8 ± 6.7	8.1 ± 14.6
Anthropometrics/strength				
Lean mass	-0.36 ± 0.81	0.68 ± 1.28	1.09 ± 1.06	0.41 ± 1.44
Fat mass	0.11 ± 0.51	-0.63 ± 1.11	-0.92 ± 1.72	-0.21 ± 1.66
Leg press max			92.50 ± 42.72	80.00 ± 33.67
Chest press max			42.50 ± 15.00	35.71 ± 18.13

Correlations

The primary clinical measure significantly changed with RT was strength. Therefore we sought to determine if changes in mitochondrial measures were correlated with changes in strength in the intervention group. Improvements in Leg strength were negatively correlated with changes in β-HAD at both baseline to week 3 (R=-0.553, p=0.039) and baseline to week 12 (R=-0.612, p=0.023). Assessing the primary significant change from the mitochondrial targets (ROS and CS) we determined that changes in complex III ROS at each time point were negatively correlated with baseline to week 12 changes in fat mass (bsl to 3 wk: R=-0.781, p=0.002; bsl to 12 week: R=-0.511, p=0.045). The baseline to week 3 change in complex III ROS (bsl to 3 wk) was positively correlated with changes in PDH activity in homogenate from week 12 (R=0.673, p=0.012). Baseline to week 12 changes in ROS at complex III were correlated with changes in β-HAD from baseline to week 3 (R=0.617, p=0.022). Baseline to week 12 changes in mitochondrial CS activity were negatively correlated with complex I ROS changes from baseline to week 3 (R=-0.586, P=0.029), but positively correlated with change from baseline to 12 weeks (R=0.668, p=0.012). Baseline to week 3 changes in mitochondrial CS activity were negatively correlated with baseline to week 12 changes in β-HAD (R=-0.529, p=0.047).

Discussion

ROS production and the potential antioxidant effects of RT:

      A major and novel finding of the present study is that RT decreases ROS production after three weeks, and that this decrease (at least in complex I ROS) is sustained over 12 weeks. As previously noted, ROS is a major player in the aging process, and has been proposed to play a role in numerous other diseases. Furthermore, this reduction in ROS production occurred absent of changes in substrate oxidation or markers of mitochondrial content and biogenesis (citrate synthase, PGC1-α, TFAM, PPARγ) indicating a very selective mitochondrial adaption to RT. It is generally accepted that aging results in a loss of efficiency in the transfer of electrons, resulting in a greater proportion of electrons transferred to oxygen, leading to the formation of ROS. This elevated level of ROS production in aging often accompanies an increase in antioxidant defense systems such as enzymes MnSOD and catalase; however, the persistence of elevated oxidative damage despite these elevated antioxidant enzymes suggests that these defense mechanisms are simply not able to cope with the enhanced levels of oxidants produced with advancing age [82, 83]. Exercise is another stimulator of ROS formation in skeletal muscle, as with increased mitochondrial respiration oxidative damage within skeletal muscle is also elevated[84, 85]. As such, during exercise skeletal muscle is exposed to increased concentrations of ROS. This induction of ROS presumably serves to elicit a beneficial response with exercise; however, the efficacy of RT in the attenuation of ROS has yet to be eluded. Previous work with RT in older adults investigating oxidative damage has yielded varying results, as one study showed no changes in MnSOD, CuZnSOD, and catalase in after 14 weeks of RT[47], while another study found increases in CuZnSOD and catalase after 12 weeks of RT, but none in MnSOD[86]. Neither of these studies measured ROS production, while our current investigation did not

measure these enzymes, leaving a gap in the literature to be explored. Western blot analysis was performed for UCP3, a mitochondrial uncoupling protein found primarily in skeletal muscle capable of uncoupling oxidative phosphorylation and dissipating the mitochondrial proton gradient, reducing the amount of electrons donated to oxygen and therefore decreasing ROS production[87]. An increase in UCP3 may help attenuate ROS production, although it is possible that our measurements of maximal ROS produced ex vivo would not be affected by UCP3 protein content. In any event, we were unable to detect changes in UCP3 with RT. This is in line with other work indicating that UCP3 does not change with endurance training[88]; however, others speculate that UCP3 is lower in trained subjects to prevent the uncoupling of respiration from ATP synthesis and increasing efficiency of mitochondrial energy production[89]. Research in this area has primarily focused on endurance training, where respiration and substrate oxidation rates are often influenced by the increased demand for oxygen during aerobic exercise. Although absent of mitochondrial respiration measurements, the present study did not observe changes in substrate utilization potentially due to the low demand for oxidative phosphorylation with the resistance training protocol. Another potential reason for the lack of changes in protein expression with the present study and previous work may be due to the possibility of antioxidant defense systems up-regulating through post-translational mechanisms. This has been supported through work in rats that have shown the ability to immediately up-regulate antioxidant enzyme activity following an acute bout of exercise, which would be too soon to change protein content[90]. Therefore it appears that muscle has the capacity of responding to training in such a manner as to reduce the potential harm arising from the accumulation of oxygen free radicals stemming from the enhanced metabolic activity that accompanies exercise, and this response could be apart from any changes in the protein levels of mitochondrial antioxidant enzymes.

To glean further insight into the attenuated ROS production the current study uncovered, 4-Hydroxynonenal (4-HNE) was measured via western blot analysis. 4-HNE is one of the major end products of lipid peroxidation and has been widely accepted as an inducer of oxidative stress at high levles[91]. However, at concentrations slightly above endogenous levels and lower than lethal concentrations, 4-HNE can act as a signaling molecule stimulating the expression of the antioxidative and detoxifying enzymes inducing an adaptive response[92, 93]. Levels of 4-HNE did not significantly increase with training after either 3 or 12 weeks. However, it is unknown if the mild elevations observed were enough to elicit the positive adaptive response 4-HNE may yield, potentially offering an explanation to the observed ROS attenuation in the present study. More research in this area is needed before any certain conclusions can be made.

Further considerations for the decreased ROS phenomena observed include the source of ROS during RT, the localization, and potential indirect antioxidant properties of other RT-induced changes in the mitochondria. Although the overwhelming majority of ROS is produced in the mitochondria as a result of oxidative phosphorylation it is possible that ROS production during RT may be localized to the cytosol. This is in line with the thinking that RT doesn't rely on oxidative phosphorylation as heavily as endurance training, potentially leading to less mitochondrial ROS being produced and promoting the production of ROS apart from the mitochondria to initiate the upregulation of other antioxidant defense systems. This thought is supported by one study that showed increases in cytosolic CuZnSOD and catalase after 12 weeks of RT, but none in mitochondrial MnSOD[86]. Cytosolic ROS could be induced by the inflammatory process that occurs 24-48 hours after RT from phagocytic cells that release hydrogen peroxide and hydroxyl radicals to initiate the repair process[94, 95]. A loss of Ca+ homeostasis during RT may also play a role in ROS production apart from the mitochondria, as

alterations in Ca+ may result in the conversion of xanthine dehydrogenase to xanthine oxidase, which catalyzes the reaction of hypoxanthine to xanthine and produces superoxide and hydrogen peroxide as byproducts[96]. The potential indirect antioxidant properties of other RT-induced changes in the mitochondria may also be a mechanism for the attenuated ROS production after RT. Complex IV activity has been shown to be increased with RT[47], which has the ability to improve the oxidant status of the cell. Complex IV is the terminal electron acceptor in the ETC and increases in its activity may help to draw electrons down the chain and reduce the production of ROS, thereby having indirect antioxidant effect[97].

Enzyme activities

A second intriguing finding of the present study is the fact that Citrate Synthase activity did not change when measured in whole muscle homogenate; however, CS activity did increase with RT when measured in isolated mitochondria. This increase (p=.012) was significant after 12 weeks of RT, where the intervention group increased activity by 237.41 ng/mg protein/min while the intervention saw a decrease in activity.

Citrate synthase is an enzyme that is located solely in the mitochondria[98] and whose activity has been an accepted biomarker of mitochondrial content measured in whole muscle homogenates[99]. It has therefore been subject to measurement in exercise studies, many concluding that CS activity, and thus mitochondrial content, are increased with training[88, 100-103]. However, these studies all used endurance or interval training protocols and measured CS activity in whole muscle homogenates. One RT study concluded that CS activity does not change[47], but this was again measured only in muscle homogenates. The measurement of CS in isolated mitochondria after RT has never been done before, and the result of increased

mitochondrial CS activity in the present study may lead to further work in this area to uncover potential consequences of this novel finding.

Mitochondrial content/biogenesis

The synthesis of new mitochondrial proteins (referred to as mitochondrial biogenesis) functions to increase of the density of mitochondria and can occur in response to several physiological stimuli, including exercise, cold exposure and calorie restriction[104, 105]. PGC-1α is the best-known intracellular mediator of mitochondrial biogenesis, functioning as a transcriptional coactivator that enhances the activity of specific transcription factors coordinating the expression of key nuclear-encoded mitochondrial genes[106, 107]. The present study did not show an increase in PGC-1α expression with RT, which was not surprising taking into account that mitochondrial content (CS activity in muscle homogenates) was not increased. The present study also did not detect a change in PPARγ mRNA levels. PPARγ is responsible for regulating gene expression by binding to specific DNA sequences, peroxisome proliferator response elements (PPRE), in the promoter regions of target genes to regulate fatty acid and glucose metabolism[108]. It again was not surprising that we did not observe a change in PPARγ expression as this coincided with the absence of any adaptations to substrate oxidation with RT. The third gene target assessed in the present study was TFAM. This gene encodes a mitochondrial transcription factor that is a key activator of mitochondrial transcription and mitochondrial genome replication[66]. Interestingly it has been found that ROS enhance the expression of nuclear mitochondrial biogenesis genes NRF-1 and Tfam[109]. Therefore the decreased ROS production in the present study may be a reason these mitochondrial biogenesis-

inducing genes were not expressed. This is just speculative work; however, and requires more

research on the topic.

The "dilution effect"

A potential barrier to using RT as a means of improving mitochondrial function lies in

the possibility of a "dilution effect" that may take place with long-term RT programs. RT

induced increases in myofibrillar volume of both type I and type II muscle fibers may lower the

density of skeletal muscle mitochondria and reduce the activity of muscle oxidative enzymes

when enzyme activity is expressed relative to protein content[110-112]. A recent study observed that

RT resulted in significant increases in muscle CSA, while succinate dehydrogenase (SDH)

activity decreased by 13%. The authors concluded that muscle hypertrophy associated with RT

results in reduced density of regionally distributed mitochondria[51]. To avoid this, we assessed

the degree of mitochondrial adaption with a short-term RT program. It is generally believed that

improvements early in a RT program are neural in nature (better motor unit coordination and

increased ability to recruit high threshold muscle fibers) with muscle hypertrophy occurring later

in a RT program[113-116]. Eight, six, and even four weeks of RT have been shown to improve

strength and lean body mass in both younger and older adults[54, 113, 117-119]. Others demonstrated

that a two-day per week protocol significantly increases strength after 4 weeks of training but not

2 weeks[116]. Assessments of a three-week RT regimen found improvements in strength, but little

change in muscle hypertrophy[114]. This is evidence for the notion that strength may increase after

just three weeks of RT primarily due to neural factors such as the increased voluntary activation

of the trained muscles. Both neural factors and hypertrophy play a role in the further increase in

strength, with hypertrophy becoming the dominant factor after the first three to five weeks[115]. Performing assessments after the initial three weeks of a RT program allowed us to capture the changes in mitochondrion function taking place in present mitochondrion absent of muscle hypertrophy (i.e. the dilution effect). Whereas assessments performed after the 12-week RT intervention gave us the ability to assess mitochondrial function after participants accrued muscle hypertrophy and increases in lean mass. It has been speculated that longer-term RT programs are needed to initiate gene shifting to an adequate degree for improvements in mitochondria function to be detected[120]; however, assessing the short-term effects of RT may preclude any possible confounding gains in muscle hypertrophy (i.e. dilution effect). Based on studies showing that aerobic capacity can improve with as little as seven days of endurance training[121, 122], as well as the absence of a dilution effect, it is reasonable to speculate that changes at the mitochondrial level are taking place during an initial three week period of a RT program. In support of this, we observed some interesting trends with many of the measures presented in tables 2-9. Examining the change scores for many of the substrate oxidation measures we observed trends indicating a decrease from baseline to 3 weeks, and then an increase from baseline to 12 weeks. This was observed in PDH activity, total FAO and acid soluble metabolites in both homogenate and isolated mitochondria. It was also shown that ROS, HDL, blood glucose, insulin, and HOMA-IR decreased more after the first 3 weeks than after 12 weeks of RT. We can rule out the dilution effect as an explanation for this, since lean mass increased at both time points and to a very little degree (0.30 kg after three weeks and 0.66 kg after 12 weeks) representing a change in percent lean mass of only 0.05 and 0.22% respectively. It appears that there must be another underlying adaptive element to RT that has not been eluded, as this is the first study to assess both the short

and longer-term effects of RT on mitochondrial function. Again this finding opens doors for future work to be done in the present field.

Stain use

An interesting finding the present study uncovered upon subsequent data analysis was the potential effects statin medication had on the outcomes of the participants randomized into the training group. Statins (3-hydroxy-3methyl-glutaryl coenzyme A reductase [HMG-Co A reductase]) inhibitors are widely used in the treatment of elevated cholesterol levels[123], and four of the participants randomized to the training group had been taking a stain medication for >1 year upon enrolling in the present study. Adverse effects of skeletal muscle have been reported with statin treatment ranging from severe (rhabdomyolysis) to mild (muscle pain), with prevalence being reported below 10%[124]. Although no member of the present study had reported any side-effects of stain treatment, it is still possible that the molecular mechanisms statins work to reduce cholesterol biosynthesis impact their ability to respond favorably to the RT program. Statins act via the mevalonate pathway to reduce the formation of cholesterol, but also affect the formation of the mitochondrial cofactor coenzyme Q_{10} (ubiquinone) an essential electron transporter of the mitochondrial respiratory chain[123]. Indeed it has been shown that patients treated with statins display an increased lactate/pyruvate ratio, suggesting impaired mitochondrial function[125]. It has been confirmed that statins diffuse into the mitochondria and inhibit complex I of the respiratory chain[126-129]. This depolarizes the inner mitochondrial membrane, triggering a calcium release through the permeability transient pore and sodium calcium exchanger, resulting in the elevation of cytoplasmic calcium that is taken up by the sarcoendoplasmic reticulum calcium pump (SERCA) to the sarcoplasmic reticulum (SR). The

SR becomes overloaded with calcium and spontaneously releases calcium through the ryanodine receptor (Ryr1) to generate a calcium wave that has the ability to further alter mitochondria and induce apoptosis[128, 130, 131]. The physiological effects of this molecular pathway is supported by a recent study examining the effects of simvastatin on changes in cardiorespiratory fitness and skeletal muscle mitochondrial content in response to exercise training, with the finding that Simvastatin attenuated increases in cardiorespiratory fitness and skeletal muscle mitochondrial content[81]. This prompted us to analyze our data to determine if there were any differences in the response to the RT intervention among the four participants in the intervention group who were taking a statin vs. the seven who were not.

Due to the low sample size (n=4 for statin users and n=7 for non-users) definitive conclusions cannot be made, as indicated in table 8, we failed to detect a significant change in any of the mitochondrial measures between stain users and non-users. However we believe our findings should not go unnoticed as there were some interesting trends. Enzyme activities of BHAD and CS in homogenate were both increased after 12 weeks in the statin users compared to non-users, indicating that statin use may not have obstructed in any training-induced effects of these enzymes. However, when looking at CS activity in isolated mitochondria, those on a statin medication appear to be at a disadvantage, as non-users saw an improvement after 12 weeks of 376.69 while users experienced a decrease by 6.31 ng/mg protein/min. Similar trends followed for PDH activity, total FAO, FAO CO2 produced, and ASM at both time points. ROS at complex III between the baseline to 12 week period increased for statin users by over 27 AU while non-users experienced a decrease. Clinical blood parameters may also be affected by statin use, for example, HMOA-βcell function among statin users after 12 weeks increased by 4.36% and non-users decreased by 11.95%. These T-tests between change scores did not

produce a significant effect and there were additionally no significant changes to any of the p-values when only non-statin users were included in the repeated measures ANOVA of all the variables assessed, more than likely due to a lack of power as this was not the primary aim of the current study. The small sample size is certainly a limitation in this post-hoc analysis and requires further research and consideration, especially since this therapeutic is the world's fastest growing class of drugs and many more individuals will figure to be prescribed statin medication in the near future[132].

Strengths and limitations:

To date, no study has comprehensively investigated mitochondrial function in older adults who RT. This investigation included assessment of isolated mitochondria as well as whole muscle homogenates, mRNA and protein samples obtained from muscle biopsies to offer a very comprehensive look into what is taking place at the mitochondrial level with RT. A second strength of the present study is the novel design, as the assessment of both a short-term (3 week) and longer-term (12 week) RT effects on mitochondrial function has yet to be done, with many RT research studies only performed measurements after 12 or 14 weeks, and even these are scarce. A further strength is the use of a randomized controlled design to allow us to directly attribute any changes assessed during the three and 12-week periods to the RT program. Limitations of the present study include the small sample size, as our total n=19 may have limited statistical power. With the high number of variables included in the RM ANOVA analysis, the possibility of alpha inflation may have presented itself. Alpha inflation refers to the phenomenon that the more statistical tests, the more likely one could find a significant result when it is actually not. However, with only a select few variables in our analysis with a Type I

error below 5%, we felt it was not necessary to correct for or lower our significance level from 0.05. The use of only non-obese, white male subjects reduces the generalizability of the study; however this preliminary data is important for proposing future larger-scale trials in this area.

Future Directions

A number of important questions remain in regards to aging and mitochondria function. The present investigation has offered insight into the role RT may have on mitochondrial function, specifically ROS production and CS activity in isolated mitochondria. This added insight we have offered will ideally lead to future research investigating the potential explanations into the mechanisms affecting the aging phenotype, expanding our knowledge in the relatively unexplored area of mitochondrial function and aging. This should shift more clinical attention to the problems affecting aged skeletal muscle, bringing about new treatment recommendations and strategies with the goal of improving the health of our aging population and lessening the monetary burden on our health care system.

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Chapter 3- CONCLUSIONS AND FUTURE DIRECTIONS

Maintaining skeletal muscle mitochondrial content and function is important for sustained health throughout the lifespan. Perturbations in mitochondrial content and function can directly or indirectly impact skeletal muscle function and consequently whole-body health and wellbeing[1, 2]. The molecular mechanisms positively regulating mitochondrial metabolism continue to be researched with a great deal left unsolved. Many major myopathies, neuromuscular diseases and conditions such as diabetes and aging all experience dysregulated mitochondrial function[3]. Impaired mitochondrial metabolism can directly influence skeletal muscle atrophy and contribute to the risk and severity of the age-related loss in skeletal muscle and strength. The potential use of physical exercise holds great promise as an approach to improve health in these conditions as well as maintain cellular/ mitochondrial health through the lifespan. Exercise activates key stress signals that positively impact major transcriptional pathways that transcribe genes involved in skeletal muscle mitochondrial biogenesis, fusion and metabolism. The positive impact of exercise is not limited to younger healthy adults but also benefits skeletal muscle from diseased populations and the elderly[4]. Future research in this area will not only improve skeletal muscle mitochondrial health but also enables us to identify molecular mechanisms that may be attractive targets for therapeutic manipulation.

Different types of exercise have different outcomes on health parameters due to the different molecular stresses and mechanisms of adaption[5]. Resistance training has been shown to primarily lead to improved muscle strength and improved physical function among older individuals, being a useful intervention to counteract the age-related losses in muscle mass, increase strength, hypertrophy and functionally ability [6-8]. Much less is known about the

potential use this type of training may have in alleviating the declines in mitochondrial metabolism seen in the aged population.

The present study has served to shed light on several yet to be explored intricacies regarding RT and mitochondrial metabolism. The vast array of measurements the present study employed allowed us to uncover two novel findings that may provide a focus for future interventions. The conclusion that ROS are reduced with RT at Complex I after both three and 12 weeks of RT, and ROS at complex III are reduced after 3 weeks warrants further investigation. Since mitochondria are the primary players in ROS production, one can speculate that RT acts on the mitochondria to lead to either less ROS being produced or an enhanced antioxidant defense system similar to aerobic training. Alternatively, RT may act to lower ROS independent of classic mitochondrial antioxidants through various pathways such as upregulation of complex IV, Ca+ homeostasis disturbance, and cytosolic antioxidants that have not been extensively alluded[9]. In addition to the ROS discovery, the present study has found that citrate synthase activity is upregulated in isolated mitochondria while remaining unchanged in whole muscle homogenates. This tells us that mitochondrial content may not be increasing, rather, the functional ability of individual mitochondria are improving. This novel finding will again hopefully bring more attention to this area of research and foster future studies to uncover the molecular mechanisms and further our understanding of this topic.

Another important contribution to this study was the analysis of those taking statin medications and their ability to respond to the RT program. Although we failed to detect any significant differences between intervention participants taking a statin (n=4) to those who were not on a statin (n=7), this was an underpowered post-hoc analysis and not the primary aim of the study. The trends presented should again set the stage for future research to focus on the issue of

RT and statin use, an area that has yet to be investigated. The topic has extreme clinical relevance, as statins represent the fastest growing class of medications[10]. With more adults, especially older adults, beginning statin regimens it would be of great interest to uncover any potential negative aspects to a stain medication regimen.

The translatability component of the current study must also be considered. The great majority of older adults are sedentary, overweight or obese, and experiencing age-related declines in muscle strength and functional capacity[11, 12]. Alongside these harmful physical characteristics, metabolic and mitochondrial health are also a major problems, leading to conditions such as diabetes, cancer, heart disease and other diseases that are affecting our nation's health and wellbeing. RT offers a promising intervention that may be more easily accepted and adopted in this population than recommendations for dieting and weight loss to address these issues.

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<u>Appendix A: Institutional Review Board Full Approval</u>

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MEMORANDUM

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DATE: September 18, 2012

Office of Research Compliance
Institutational Review Board
2000 Kraft Drive, Suite 2000 (0497) Blacksburg, VA 24060
540/231-4606 Fax 540/231-0959 email irb@vt.edu
website http://www.irb.vt.edu

TO: Brenda Davy, Kevin Davy, Kyle Flack, Matthew Wade Hulver, Richard A Winett, Madlyn Irene Frisard

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires May 31, 2014)

PROTOCOL TITLE: The Effects of Resistance Training on Aged Skeletal Muscle and Mitochondrial Function

IRB NUMBER: **12-652**

Effective September 10, 2012, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the New Application request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Full Review**
Protocol Approval Date: **September 10, 2012**
Protocol Expiration Date: **September 9, 2013**
Continuing Review Due Date*: **August 26, 2013**
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included

VirginiaTech

Appendix B: Institutional Review Board Amendment Approval

MEMORANDUM

DATE: March 20, 2013

Office of Research Compliance
Institutational Review Board
2000 Kraft Drive, Suite 2000 (0497) Blacksburg, VA 24060
540/231-4606 Fax 540/231-0959 email irb@vt.edu
website http://www.irb.vt.edu

TO: Brenda Davy, Kevin Davy, Kyle Flack, Matthew Wade Hulver, Richard A Winett, Madlyn Irene Frisard

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires May 31, 2014)

PROTOCOL TITLE: The Effects of Resistance Training on Aged Skeletal Muscle and Mitochondrial Function

IRB NUMBER: 12-652

Effective March 19, 2013, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Full Review**
Protocol Approval Date: **September 10, 2012**
Protocol Expiration Date: **September 9, 2013**
Continuing Review Due Date*: **August 26, 2013**
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

Invent the Future

Appendix C: Institutional Review Board Continuing Review Approval

MEMORANDUM

DATE: September 13, 2013

Office of Research Compliance
Institutational Review Board
North End Center, Suite 4120, Virginia Tech
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TO: Brenda Davy, Kevin Davy, Kyle Flack, Matthew Wade Hulver, Richard A Winett, Madlyn Irene Frisard

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires April 25, 2018)

PROTOCOL TITLE: The Effects of Resistance Training on Aged Skeletal Muscle and Mitochondrial

Function

IRB NUMBER: 12-652

Effective September 13, 2013, the Virginia Tech Institution Review Board (IRB) Chair, David M Moore, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

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(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: **Full Review**
Protocol Approval Date: **September 13, 2013**
Protocol Expiration Date: **September 12, 2014**
Continuing Review Due Date*: **August 25, 2014**

Appendix D: Flack KD, Davy KP, Hulver MW, Winett RA, Frisard MI, Davy BM. Aging, resistance training, and diabetes prevention. J Aging Res. 2010;2011:12731

Review Article

Aging, Resistance Training, and Diabetes Prevention

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With the aging of the baby-boom generation and increases in life expectancy, the American population is growing older. Aging is associated with adverse changes in glucose tolerance and increased risk of diabetes; the increasing prevalence of diabetes among older adults suggests a clear need for effective diabetes prevention approaches for this population. The purpose of paper is to review what is known about changes in glucose tolerance with advancing age and the potential utility of resistance training (RT) as an intervention to prevent diabetes among middle-aged and older adults. Age-related factors contributing to glucose intolerance, which may be improved with RT, include improvements in insulin signaling defects, reductions in tumor necrosis factor-α, increases in adiponectin and insulin-like growth factor-1 concentrations, and reductions in total and abdominal visceral fat. Current RT recommendations and future areas for investigation are presented.

1. Introduction

With the aging of the baby boom population and an increased life expectancy, individuals aged 65 years or older are the fastest growing segment of our population [1]. Increases in the number of individuals aged 65+ years will increase demands on health care and health care costs, which could lead to inadequate public resources and less care for the aged [1]. Chronic conditions such as diabetes exert a profound economic impact on our nation; this disease and its associated comorbidities are a major cause of disability and death [2]. In 2007, the total estimated cost of diabetes was $174 billion, which included $116 billion in medical costs and $58 billion in reduced productivity [3].

Total diabetes prevalence (undiagnosed and diagnosed) is currently estimated to be 14% of the U.S adult population [4] and is highest in those aged ≥65 years [2]. Prediabetes, that is, impaired fasting glucose (IFG; 100 mg/dl (5.6 mmol/l)–125 mg/dl (6.9 mmol/l)), or impaired glucose tolerance (IGT; 2-h plasma glucose 140 mg/dl (7.8 mmol/l)–199 mg/dl (11.0 mmol/l)) [5] is also becoming more prevalent in the United States [6]. Individuals with prediabetes

are at increased risk for developing diabetes, with the progression of diabetes within 6 years of those with IFG and IGT being 65%, as compared to a 5% progression rate for those with normal blood glucose levels [7]. Recent estimates indicate that by the year 2050, diabetes prevalence could be as high as 33% [4]. The increased prevalence of diabetes among older adults, coupled with the aging of our population, suggests a clear need for effective diabetes prevention strategies. The purpose of the present paper is to review what is currently known about changes in glucose tolerance with advancing age, and the potential utility of resistance training (RT) as an intervention to prevent diabetes among middle-aged and older adults. Current RT recommendations and areas for future investigation are also presented.

2. Aging: Changes in Body Composition and Glucose Tolerance

2.1. Aging and Sarcopenia. Aging brings about a decline in skeletal muscle mass termed sarcopenia [8–10]. Muscle mass declines at a rate of 3–8% each decade after the age of 30 [11]. This loss of muscle mass increases the risk of developing

glucose intolerance and diabetes due to the fact that muscle tissue is the primary site of glucose disposal [12–14]. There are many potential causes of sarcopenia including a reduction in muscle cell number through apoptosis, loss of motorneurons, and a reduction in calcium pumping activity. In addition, a decrease in the muscle twitch time and force is experienced, which can be considered a cause or an effect of sarcopenia [9, 15–17]. Increases in inflammatory cytokines and oxidative stress may also contribute to sarcopenia [18]. Other consequences of this decline in muscle mass include reduced muscle strength, reduced resting metabolic rate, reduced lipid oxidative capacity, and increased adiposity (reviewed in [8]). Many clinical studies have shown that increasing lean body mass (primarily muscle mass) parallels the improvements in glucose tolerance seen with resistance training among older adults [19–31]. However, others have suggested that the prevalence of glucose intolerance in older individuals is not a direct reflection of one's lean body mass, but a result of age-associated increases in abdominal fat [32–34]. Although lean mass may not be the most robust predictor of glucose tolerance, the results of numerous clinical trials suggest that increases in lean body mass with RT are associated with improvements in glucose tolerance [19–31]. Therefore, increasing lean mass regardless of baseline levels should improve glucose tolerance and insulin-resistance, which may be an important strategy to combat the age-related increases in insulin-resistance and glucose intolerance.

2.2. Aging and Body Fat Distribution.

2.2. Aging and Body Fat Distribution. Along with reductions in lean mass, older individuals often experience increases in adipose tissue [35–37]. Aging is strongly associated with increases in body weight and body fatness [38, 39]. Based on the 2007-2008 National Health and Nutrition Examination Survey (NHANES), 78.4% of men and 68.6% of woman $\geq$60 years were considered overweight or obese (BMI $\geq$ 25 kg/m^2). This represents the highest prevalence of overweight or obesity across all age groups [40]. This could partially be due to reductions in physical activity; for example, older adults average 37% fewer steps per day when compared to younger adults, and perform significantly less moderate to vigorous physical activity [41]. Older adults often do not achieve the recommended amount of physical activity (i.e., $\geq$30 minutes of moderate physical activity on five or more days/week) proposed by organizations including the World Health Organization, Center for Disease Control and Prevention, Health Canada, and the Department of Health and Ageing [41].

Body fat accumulation is associated with an increased risk of premature mortality and morbidity [39], as well as hyperinsulinemia and glucose intolerance [38, 42]. Older individuals also demonstrate changes in body fat distribution, with increasing levels of upper body fat [35–37]. This increase in upper body fat (specifically abdominal visceral fat) has been linked to glucose intolerance and diabetes [43–45], and abdominal visceral fat is an independent predictor of glucose intolerance [34, 45]. This adipose tissue depot is sensitive to lipolytic stimuli, and

in obese states may lead to increased circulating free fatty acid (FFA) concentrations [46, 47]. Visceral fat lipolysis may be responsible for 5–10% of circulating FFAs in lean individuals, while this value may increase to 20–25% in obese individuals [47, 48]. However, upper body, nonvisceral fat is the primary contributor to FFA concentrations [48]. Increased FFA concentrations have been implicated in the development of insulin-resistance and metabolic inflexibility [47–50].

3. Aging and Glucose Intolerance: Potential Contributing Factors

Although factors such as a reduction in lean body mass, physical inactivity, obesity, and changes in fat distribution may contribute to glucose intolerance, age appears to be an independent determinant of impaired glucose tolerance [42, 51, 52].

3.1. Insulin Signaling within Skeletal Muscle. Insulin's effects on peripheral tissues (i.e., skeletal muscle, adipose tissue) involve a complex framework of signaling pathways that result in the translocation of GLUT4 transporters to the cell surface, which are responsible for the transport of glucose across the plasma membrane into the target cell [53]. An alteration in any of the related pathways reduces insulin's effectiveness and leads to the insulin-resistance and glucose intolerance associated with advancing age. The insulin signaling process is complex and not fully understood (reviewed in [53, 54]). Both diabetes and age-associated declines in glucose tolerance are hallmarked by a decreased uptake of glucose by peripheral tissues, primarily skeletal muscle. The age-associated reduction in glucose uptake is not due to impaired insulin binding, but instead to a defect in the postreceptor intracellular insulin signaling pathway [53, 55–57]. This defect has not been fully elucidated; however, a reduction in the number of insulin-stimulated glucose transport units occurs with aging [56]. Thus, fewer GLUT-4 transporters and/or postreceptor defects in the insulin signaling cascade results in insulin-resistance. Exercise-induced, contraction-mediated GLUT4 translocation to the muscle membrane is independent of insulin and occurs via an alternative mechanism (reviewed in [58]). Importantly, older adults with diminished glucose tolerance do not demonstrate a decline in exercise-induced contractile-mediated GLUT4 translocation [59].

3.2. Aging and Pancreatic Beta Cell Function. Insulin secretion decreases at a rate of 0.7% per year with advancing age, and is accelerated twofold in individuals with glucose intolerance [60]; yet it is uncertain the extent to which reduced insulin secretion is due to β-cell dysfunction or reduced β-cell mass [60]. Individuals with glucose intolerance demonstrate a 50% reduction in β-cell mass [61], which may be attributed to increased β-cell apoptosis. The aging of β-cells appears to decrease proliferation and increase sensitivity to hyperglycemia-induced apoptosis [62].

Diminished β-cell function has been reported among individuals with glucose intolerance, which decreases as fasting plasma glucose concentrations increase [63]. Therefore, a combination of β-cell dysfunction and β-cell apoptosis may contribute to age-related declines in glucose tolerance.

3.3. Aging and Mitochondrial Function. A reduction in mitochondrial function may also contribute to age-related declines in glucose uptake [64–67], possibly arising from increases in mitochondrial DNA deletions and mutations [67, 68]. This may lead to a 40% decrease in mitochondrial oxidative metabolism in older adults compared to younger individuals [66]. Specifically, cytochrome c oxidase gene expression and enzyme activity are reduced in aged skeletal muscle [67]. This mitochondrial dysfunction contributes to the decline in physical fitness and oxidative capacity older adults may experience [67, 69]. Insulin resistance is related to increased plasma FFA concentrations and enhanced FFA influx into skeletal muscle [66, 70–72]; decreased mitochondrial oxidative capacity may cause intramyocellular accumulation of fatty acid metabolites such as fatty acyl coenzyme-A, diacylglycerol, and ceramide to accumulate and produce insulin-resistance through serine kinase activation [65, 66, 72]. Serine kinases impede insulin signaling by reducing IRS phosphorylation [64, 65, 72] which leads to a decline in insulin-stimulated GLUT4 translocation and impaired skeletal muscle glucose uptake [64, 65].

3.4. Aging: Adiponectin, Tumor Necrosis Factor Alpha, and Insulin-Like Growth Factor-1. Two strong correlates of aging and insulin-resistance include adiponectin and tumor necrosis factor alpha (TNF-α), with low concentrations of adiponectin and high concentrations of TNF-α being linked to insulin-resistance [43, 73–75]. Both may also play a role in body fat distribution [37, 43, 44, 76, 77] and sarcopenia [18]. Adiponectin is secreted by adipose tissue (i.e., an adipokine) and is a key modulator of insulin sensitivity [43, 75, 78]). Low plasma adiponectin concentrations are associated with insulin-resistance, diabetes, obesity, body fat percentage, body fat distribution, and BMI [37, 43, 76, 79–82]. Adiponectin is believed to activate $5'$-AMP-activated protein kinase (AMPK), which activates insulin-independent glucose uptake by the muscle, downregulates gluconeogenic enzymes and increases muscle fatty acid oxidation [83].

Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine secreted by adipose tissue, macrophages, and other cells, which appears to influence insulin-resistance. Elevated TNF-α concentrations are linked to obesity and insulin-resistance, while obese mice lacking TNF-α are protected from insulin-resistance [84]. Inflammatory pathways that impair insulin signaling at the level of IRS proteins are activated in the presence of TNF-α [73, 84]. TNF-α is correlated with body fat distribution [77] and sarcopenia [18] which may also lead to insulin-resistance among individuals with elevated TNF-α concentrations.

Unlike adiponectin and TNF-α, insulin-like growth factor-I (ILGF-I) is not secreted by adipose tissue, but instead a peptide hormone which possesses insulin-like properties such as the promotion of glucose uptake by peripheral tissues [85, 86]. Insulin-like growth factor-I concentrations decline with age, and is associated with the age-related changes in body composition by both increasing fat mass and decreasing muscle mass [87–89], thus potentially being a modulator of insulin-resistance. Administration of recombinant ILGF-I improves glucose uptake in those with insulin-resistance and type 2 diabetes. Other factors may be involved in the role of ILGF-I and glucose metabolism including binding proteins, hybrid receptors, and growth hormone secretion [90].

4. Resistance Training: Influence on Insulin Resistance

The diabetes prevention program (DPP) demonstrated that lifestyle modification reduces the development of diabetes by focusing on weight loss, increased physical activity, and dietary modification. Lifestyle modification decreased the incidence of type 2 diabetes by 58%, as compared to the 31% among individuals taking metformin [91]. The physical activity component of the DPP recommended that individuals accumulate 150 minutes/week of moderate physical activity. The DPP stressed brisk walking as the physical activity of choice, but also lists aerobic dance, skating, bicycle riding, and swimming as options [91]. In support of the DPP's recommendations for aerobic training (AT), regular AT improves glucose control and insulin sensitivity [92, 93]. The American Diabetes Association (ADA) recommends that individuals with diabetes perform at least 150 minutes of moderate-intensity AT per week [94]. However, factors such as obesity, arthritis, low back pain, and physical disabilities affecting many older adults may preclude this population from regularly performing AT [95–97]. Environmental factors such as unsafe neighborhoods or streets also may discourage engagement in many types of aerobic activity [97]. Therefore, alternative approaches for increasing physical activity among older adults should be considered.

Resistance training is one such alternative that can be safe and effective for older adults, including the elderly [95, 98–102]. The ADA encourages individuals with type 2 diabetes to perform resistance exercise three times a week targeting all major muscle groups, progressing to three sets of 8–10 repetitions at high intensity [103]. By using machines that provide external resistance with controlled movements, even those confined to a wheel chair or a walker can perform some types of RT. Though older adults demonstrate reduced overall muscle protein synthesis (MPS) relative to younger adults after a bout of resistance training [104], clinical trials investigating RT interventions among older adults have shown improvements in insulin-resistance and sarcopenia, by increasing lean body mass [19–31].

To identify published research relevant to the focus of this paper, a literature search was conducted using the PubMed search engine, developed by the US National Library of Medicine of the National Institutes of Health, without restrictions on publication date. Additional inclusion criteria

were as follows: randomized controlled trial study design, studies conducted in middle-aged and older adults, study duration greater than one month. Intervention studies which met inclusion criteria are described in Table 1. Of the RT intervention studies reviewed, most reported improvements in glucose uptake, and reduced diabetes risk (i.e., 4 of 5 interventions report beneficial effects of RT on diabetes-related outcomes). Intensity appears to influence the magnitude of improvement in these outcomes; high intensity RT (defined as training loads above 75% one-repetition maximum (RM) [105]) produces greater improvements than RT performed at a moderate or low intensity (training loads between 50%–74% of one RM and below 50% one RM, respectively [105]) [102, 106]. Although AT has been an accepted (see DPP [91]) and recommended (ADA [94, 103]) exercise intervention to improve glucose metabolism, some investigations of the combined effects of RT and AT conclude that RT + AT exercise programs enhance diabetes related outcomes [23, 30], while others have suggested that RT-alone programs have benefits comparable to that of AT-alone programs [22, 107–109]. Evidence to support one mode of training (RT versus AT) over the other is limited and should be further investigated before conclusions can be made as to the superiority of one form of exercise over the other.

Two RT modes were used in the five investigations included in Table 1. Four interventions utilized weight-training machines [27, 31, 79, 109] while one used therabands [110]. Interestingly, all four studies using a weight-training machine protocol reported improvements in diabetes-related measures, whereas the RT intervention utilizing therabands did not lead to differences between exercise and control groups. The number of studies in this area is limited, yet these findings suggest that RT mode may be an important issue with regard to improvements in glucose metabolism.

Although Table 1 only includes studies investigating chronic RT effects, others have investigated glucose metabolism with acute bouts of RT, and reported conflicting results. Black et al. found that a single RT session performed at either low or high intensity, using either a multiple set or single set protocol, improved 24-hour postexercise insulin sensitivity measured via fasting plasma glucose [106]. Conversely, Jimenez et al. assessed insulin sensitivity using the euglycemic-hyperinsulinemic-clamp technique preexercise, and 12 and 36 hours postexercise, and reported no differences between control and exercise groups [111]. Methodological difference may have contributed to the conflicting findings (i.e., RT protocol, outcome measures, study population). Thus it remains uncertain the extent to which improvements in glucose/insulin metabolism with RT could be attributed to an acute exercise bout versus a result of chronic training.

4.1. Resistance Training: Changes in Insulin Signaling, Adiponectin, TNF-α, and ILGF-1.
Resistance-trained muscle has shown increased rates of insulin-stimulated glucose uptake and transport [112, 113]. This has been attributed to the fact that RT increases aspects of the insulin signaling cascade that result in the upregulation of this pathway. Increases in the protein content of the insulin receptor and kinase activity (PIP-3, Akt/PKB, aPKC) are evident in resistance-trained muscle, even without increases in lean mass, and may enhance glucose uptake [96, 112–114]. Akt/PKB, insulin receptor protein, and glycogen synthase activity are increased with RT, all of which are downstream targets in the insulin signaling cascade that may be important in the translocation of GLUT-4 receptors and skeletal muscle glucose uptake [96]. These changes in the insulin signaling cascade are observed even without increases in lean mass [96]. In addition to (or possibly as a result of) the increased activity of the insulin signaling cascade, an increase in GLUT-4 protein concentration has also been observed with RT in humans [96] and rodents [112–114]. Thus, the two possible insulin signaling defects that result in insulin-resistance (decreased number of GLUT-4 transporters and/or post receptor default in the insulin signaling cascade resulting in less GLUT-4 translocation) appear to be improved with RT. Increased insulin signaling activity, along with increases in GLUT-4 protein expression, may lead to increased GLUT-4 translocation thereby increasing glucose transport and reducing insulin-resistance.

Improvements in adiponectin concentrations have been reported with weight loss [115–117], aerobic exercise [78, 80–82, 118] and RT [119, 120]. Since low adiponectin concentrations are associated with obesity, interventions often include weight loss to promote increases in adiponectin. However, some exercise interventions report increases in serum adiponectin concentrations without changes in body weight [20], although others do not [121]. There is also conflicting data on the influence of RT on adiponectin concentrations; some have reported no change [79, 116, 122] while others have reported increases [119, 120]. Methodological differences (i.e., RT intensity, measurement of total versus low/high molecular weight adiponectin) may explain conflicting findings. With regard to TNF-α, high intensity RT appears to reduce TNF-α concentrations and improve insulin sensitivity [20, 123, 124], even when fat mass is unchanged [20].

There is conflicting data on the influence of RT on ILGF-I concentrations. Borst et al. reported that 25 weeks of 1 or 3-set resistance training increased ILGF-I in healthy adults aged 25 to 50 [125]; however, this was not observed in a subsequent study by the same group using adults aged 60–85 years and high and low-intensity resistance training [126]. Conversely, others have reported significant increases in ILGF-I with resistance training in the elderly [99, 127]. These studies concluded that despite atrophy and ultrastructural damage, elders respond to RT with significant increases in musculoskeletal remodeling, cross-sectional area and elevated IGF-I levels [99, 127]. Increases in ILGF-I concentration are also associated with increases in lean mass, indicating that ILGF-I may be important in addressing age-related sarcopenia and insulin-resistance. Although more research is needed in this area, it appears that ILGF-I concentrations can be increased in older adults to augment glucose uptake and improve insulin-resistance.

Table 1: Randomized controlled trials >1 month in duration investigating the effect of resistance training on diabetes-related outcomes among nondiabetic middle-aged and older adults[†].

Source	Study design	Study duration	RT protocol	Study population	Primary findings
Traditional Weight Training only*					
Iglay et al. [27]	RCT ($n = 36$): RT + 0.9/g/kg/d protein intake, $n = 16$ RT + 1.2 g/kg/d, $n = 16$	3 months	3x week, 8 machine exercises, 2 sets 8 reps + one set to voluntary fatigue at high intensity	Healthy individuals, aged 60–62 yrs.	↓ glucose OGTT AUC 25–28% with RT, no differences between diet groups.
Onambélé-Pearson et al. [110]	RCT ($n = 30$): LI (~40% 1RM), $n = 18$ HI (~80% 1RM), $n = 12$	3 months	3x week, 6 exercises using therabands, progressing from 8–11 reps and 2–4 sets, different intensity groups: HI versus LI	Sedentary individuals, aged 55–80 yrs.	↑ fasting plasma glucose (4.8 ± 0.19 to 5.51 ± 0.08 mmol/L) in HI group, no change in plasma glucose for LI, no change in plasma insulin for either group.
Zachwieja et al. [31]	RCT ($n = 15$): RT + GH injections, $n = 6$ RT only, $n = 9$	4 months	4x week, 9 machine exercises, 4 sets, 4–10 reps at high intensity.	Healthy men, aged 64–75 yrs.	↑ in glucose disappearance rate (3.0 ± 0.3 to 4.0 ± 0.4 mg/100 mL/min minimal model of glucose kinetics, IVGTT) with RT only
RT + AT (either alone or combined)					
Ahmadizad et al. [79]	RCT ($n = 24$): AT, $n = 8$ RT, $n = 8$ Control, $n = 8$	3 months	3x week, circuit weight training, 11 machine exercises, 4 sets, 12 reps, at moderate intensity with 30 sec. rest between exercises.	Healthy men, aged 35–48 yrs.	↓ HOMA-IR 35.7 and 38.5% after AT and RT respectfully; no differences between groups.
Smutok et al. [109]	RCT ($n = 37$): RT, $n = 14$ AT, $n = 13$ Control, $n = 10$	4.5 months	3x week, 11 machine exercises, 2 sets, 12–15 reps at moderate intensity.	Men at risk for CHD with either abnormal glucose tolerance, dyslipidemia, or hypertension, aged 41–59 yrs.	↓ plasma glucose at 60, 90, and 120 minutes after glucose ingestion with RT; ↓ plasma glucose at 90 and 120 min after glucose ingestion with AT. ↓ fasting glucose with RT, no changes with AT. Insulin OGTT AUC ↓ 24% for AT and 21% for RT, no changes in control.

*Traditional Weight Training= any muscle strengthening exercises using resistance training machines/equipment, free weights (e.g., dumbbell, barbell) or therabands.
[†]Abbreviations used: AT: Aerobic training, AUC: Area under curve, GH: Growth Hormone, HI: High intensity, HOMA: Homeostasis model assessment, IVGTT: Intravenous glucose tolerance test, IR: Insulin Resistance, LI: Low intensity, OGTT: Oral glucose tolerance test, Reps: Repetitions, RCT: Randomized controlled trial, RT: Resistance training.

4.2. Resistance Training and Body Fat Distribution. Body fat distribution may play a major role in the development of insulin-resistance, particularly abdominal fat [33, 42]. Resistance training reduces abdominal fat, including visceral fat, among individuals with diabetes [116, 128, 129]. Both low intensity RT three times per week [128], and high intensity RT twice per week [116] improve insulin-resistance and reduce body fat mass. Strength training-induced changes in abdominal visceral fat were also reported without significant weight loss [129]. Thus, RT alone may reduce abdominal and visceral fat, which is known to increase with advancing age and influence insulin-resistance.

An overview of how RT may influence age-related physiological changes impacting diabetes risk is presented graphically in Figure 1.

5. Current Recommendations: Aging, Resistance Training, and Diabetes Prevention

Major health organizations such as the American College of Sports Medicine (ACSM), American Heart Association (AHA), and the American Geriatrics Society (AGS) have issued recommendations regarding RT for older or diabetic individuals. As stated previously, the ADA encourages individuals with type 2 diabetes to perform resistance exercise three times per week targeting all major muscle groups, and progressing to three sets of 8–10 repetitions at high intensity [103]. According to the ACSM, older adults should engage in RT at least twice per week. These sessions should include 8–10 exercises of 8–12 repetitions, involving the major muscle groups, done at a moderate
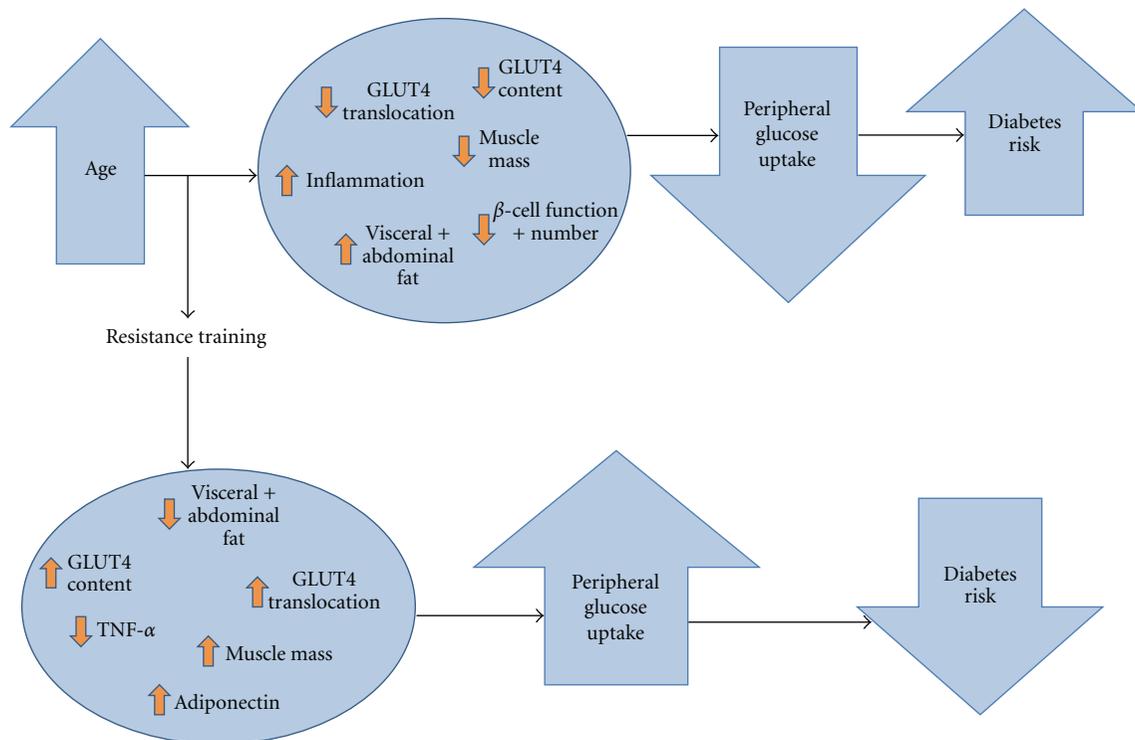
FIGURE 1: Age-related physiological changes and diabetes risk: Potential influence of RT.

to vigorous intensity [130]. Similarly, ACSM's position stand on exercise prescription for diabetes care recommends that individuals engage in RT at least twice per week, with 8–10 exercise involving the major muscle groups to be performed with at least one set of 10–15 repetitions. This position stand recognizes that increased intensity of exercise or adding additional sets may produce greater benefits, but may not be appropriate for some individuals [131]. Both ACSM position stands advocate progressive RT, with increases in resistance as the individual progresses through the program [130, 131]. The AGS recommends 2-3 days per week of RT with 10–15 repetitions at low intensity, 8–10 at moderate intensity, or 6–8 at high intensity [132]. The AHA recommends that older adults engage in resistance training 2-3 nonconsecutive days per week doing one set of 10–15 repetitions at low intensity, and also recognizes that multiple set regimens performed at higher intensities and frequencies (>2 days a week) may provide greater benefits [133]. These recommendations are similar to protocols used in many clinical trials investigating the effect of RT on diabetes-related outcomes among older adults (see Table 1). Two of the four trials included in Table 1 used a high intensity protocol, while 2 used moderate intensities and one had high intensity and low intensity groups. All of the studies used multiple set protocols. Frequency of training was most commonly three days per week ($n = 4$), while one study used a 4 day per week protocol.

Some studies have addressed the issue of RT intensity and volume on insulin sensitivity. High-intensity protocols

show significant increases in insulin sensitivity as compared with moderate intensity protocols [106], and single set protocols may be less effective than multiple set protocols in lowering fasting blood glucose concentrations [106]. A meta analysis concluded that high intensity protocols were more effective than low intensity protocols at increasing strength in older adults [102]. Higher volume interventions are also associated with greater increases in lean body mass in older individuals [134] as well as young men [135]. This suggests the possibility of a dose-response relationship, such that improvements in strength and insulin-resistance are increased as RT intensity and volume increase. Additionally, others have reported that twice weekly RT at low intensity but high volume (three sets of ten repetitions) improved insulin-resistance [136]. Recently, RT interventions stressing volitional fatigue (i.e., the point at which the exercise could not be completed with proper technique) have been conducted [137, 138]; more work is needed to determine if this RT approach is beneficial with respect to blood glucose control and insulin-resistance.

Taken together, existing recommendations and these research studies suggest that high volume and high intensity RT may produce greater improvements in muscle mass gains, insulin-resistance and glucose tolerance; however, it would be prudent for sedentary older diabetic or prediabetic individuals to begin an RT program at low intensity (rate of perceived exertion of ~5-6) and low volume (1 set per exercise, 10–12 reps) twice weekly, and if time and fitness are sufficient, progressively increasing intensity, volume, and frequency [130, 131, 133].

5.1. Future Directions. Research suggests that RT may play a role in improving the age-related increases in insulin-resistance, and prevent the onset of diabetes. Major health organizations have recognized the benefits of RT. However, according to the CDC, only 13% of men and less than 10% of woman aged ≥65 yrs reported engaging in strength training at least two days per week [139]. Possible reasons for low rates of adoption and minimal adherence may include barriers such as the perceived complexity and knowledge needed to perform RT, misinformation of expected RT outcomes (e.g., excessive or undesirable hypertrophy), and the emphasis many public health programs and clinicians place on AT rather than RT. Once effective RT interventions are identified, the translational capabilities of intervention approaches should be investigated. Adherence, simplicity, and cost effectiveness are important for RT interventions to be successful in real-world settings.

Differences in traditional RT versus circuit weight training have not been addressed, as well as differences in protocols using free weights and those using machine weights. It is possible that certain RT approaches lead to greater rates of adoption, adherence and greater cost effectiveness among older, insulin resistant individuals.

Dietary and weight loss interventions in conjunction with RT should be investigated to determine the optimal approach for diabetes prevention with advancing age. For example, the role dietary protein intake may play in reversing insulin-resistance and improving glucose control should be studied more in depth, as high protein diets improve glucose control in individuals with type 2 diabetes when compared to those on a low protein diet [140, 141]. Additionally, a positive relationship between protein intake and change in whole body fat-free mass has been observed after pooling RT studies investigating protein intake in adults aged 50–80 [142]. Based upon these findings, it has been suggested that the RDA for protein intake (0.8 g/kg) is inadequate for older adults who engage in RT [142]. With the possibility that high protein diets can be beneficial to those with impairments in glucose metabolism as well as older adults engaging in RT, the synergistic effect of RT and high protein diets on glucose tolerance warrants further investigation.

Finally, additional work should be done to address mechanisms for RT-induced improvements in insulin-resistance and glucose tolerance. The specific effects of RT on insulin signaling are uncertain, and the effect of RT on pancreatic β-cell function/mass and mitochondrial dysfunction are unknown. It is also possible that other inflammatory markers not yet identified may influence sarcopenia and the response to RT among older adults. Although some work has been done addressing the effect of RT on visceral adipose tissue [76, 77], direct effects on FFA concentrations and gluconeogenesis are uncertain. By continuing to identify the mechanisms by which RT improves insulin-resistance, and by determining optimal combinations of RT with other lifestyle factors to prevent diabetes, interventions can be developed which optimize reduction in diabetes risk with advancing age.

In conclusion, it appears RT may be an effective intervention approach for middle-aged and older adults to counteract age-associated declines in insulin sensitivity and to prevent the onset of type 2 diabetes. Older adults who engage in RT may see benefits with respect to improvements in body composition, body fat distribution, inflammatory markers, and blood glucose homeostasis. Future research investigating mechanisms, optimal RT protocol, and intervention approaches with high translation potential are needed to enhance knowledge in this area, and to increase public awareness and adoption of RT.

Acknowledgments

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