THE MITOCHONDRIAL THEORY OF AGING IN HUMAN SKELETAL MUSCLE: EFFECTS OF RESISTANCE EXERCISE

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

AGING, OXIDATIVE STRESS, AND THE EFFECTS OF RESISTANCE EXERCISE

Doctor of Philosophy — McMaster University

(Kinesiology) — Hamilton, Ontario

TITLE: The Mitochondrial Theory of Aging in Human Skeletal Muscle: Effects of Resistance Exercise

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NUMBER OF PAGES: x; 248

Abstract

Introduction. The mitochondrial theory of aging proposes that a vicious cycle of events including an age-related increase in mitochondrial (mt) reactive oxygen species (ROS) cause damage to mtDNA, resulting in electron transport chain dysfunction (ETC). This, in turn, causes a loss in the ability to maintain cellular energy requirements and results in a further increase in ROS production. Based on several reports of normal ETC activity in skeletal muscle of older adults, there is some question as to whether or not the mitochondrial theory is applicable to skeletal muscle. Resistance exercise (RE) is an efficacious therapy for increasing strength and muscle mass. RE training results in a recovery of strength and muscle fiber cross-sectional area in older adults. As such, resistance exercise should slow or reverse the underlying mechanisms associated with skeletal muscle aging. We conducted a series of experiments to: 1) examine the mitochondrial theory of aging in human skeletal muscle; and 2) examine the effects of RE on variables associated with the mitochondrial theory of aging. We hypothesized that aging would be associated with several aspects of the mitochondrial theory of aging such as an increase in oxidative stress, an alteration in antioxidant capacity, an increase in mtDNA deletions, and a reduction in ETC enzyme activity. Furthermore, we hypothesized that regular RE training would result in an increase in antioxidant enzyme activity, a reduction in oxidative damage to proteins and DNA, and reduction in mtDNA deletions. **Methods.** Muscle biopsy specimens and urine samples were collected from young and old men, and subsequently analyzed. An initial study examined the effects of aging on mtDNA deletions, oxidative damage to proteins and DNA, antioxidant enzyme

activity, and oxidative enzyme activity. Two subsequent studies examined the impact of resistance exercise training on the same variables mentioned above. **Results.** 1. Oxidative damage to protein and DNA was higher in older vs. younger men. Antioxidant enzyme activity, in particular MnSOD and catalase were higher in old as compared to young. Oxidative enzyme activity was not lower as a function of age. In fact, complex I+III activity was higher in older as compared to younger men. There was a reduction in full-length mtDNA, and the appearance of mtDNA deletions associated with aging. 2. Whole-body RE training for 14 weeks resulted in a decrease in oxidative damage to DNA in older men. Catalase, CuZnSOD, and MnSOD protein content were unchanged, however, mtCK protein content was higher after training. Oxidative enzyme activity was unchanged with the exception of complex IV, which demonstrated a specific up-regulation in response to RE training. RE had no effect on full-length mtDNA or mtDNA deletions 3. Unilateral resistance training (12 weeks) had no effect on oxidative damage to protein. Antioxidant enzyme activity, in particular CuZnSOD, and catalase was up-regulated following resistance exercise training. There was no change in oxidative enzyme activity or protein content. Full-length mtDNA was unchanged, and there was no effect of resistance exercise on mtDNA deletions. **Conclusion.** Aging was associated with an increase in oxidative stress, mtDNA deletions, and significant changes in antioxidant enzymes, suggesting that aspects of the mitochondrial theory of aging were applicable to skeletal muscle. Regular RE training resulted in a decrease in oxidative damage to DNA, and was sufficient to cause a further up-regulation of antioxidant

enzyme activity, beyond that induced by aging. Finally, complex IV of the ETC may have indirect antioxidant properties by improving ETC efficiency.

ACKNOWLEDGEMENTS

I would like to first thank my advisor, Dr. Mark Tarnopolsky for his support and friendship over the last eight years. To jot a quick thank-you on paper does not give justice to the appreciation and indebtedness that I feel.

To my advisory committee, Dr. Martin Gibala, and Dr. Stuart Phillips who always seem to make time to talk shop or otherwise. It has truly been a pleasure working with you both, and you have been a great example of what I will strive for in an academic career.

I would like to thank the folks in the lab for tolerating my sometimes grumpy demeanor, and to Doug Mahoney, whom I have had the privilege of sharing an office for the last two years. Our conversations and "debates" have surely made me a better scientist.

To all the grad students who have made the most intolerable times tolerable, and to Dave Ditor who is always willing to share my misery.

To John Cullen, who ultimately is responsible for the existence of this thesis. His computer skills saved the data and the write up on more than one occasion.

To my family, who provided me with the structure, support and encouragement to follow my dreams. All of my accomplishments are simply an extension of my parents hard work, and my sisters great example.

Finally, to my best friend and wife, Kristen, who gave me unconditional support over the last year, and has worked so hard to pick me up when I've been down. Without her understanding and encouragement this thesis would never have been possible.

ABBREVIATIONS

8-OHdG	8-oxo-2-deoxyguanisine
ATP	adenosine triphosphate
BER	base excision repair
Ca^{+}	calcium
CAT	catalase
COX	cytochrome oxidase
CS	citrate synthase
CuZnSOD	copper, zinc superoxide dismutase
DNA	deoxyribonucleic acid
ETC	electron transport chain
GPX	glutathione peroxidase
H_2O	water
H_2O_2	hydrogen peroxide
MnSOD	manganese superoxide dismutase
Mt	mitochondria
mtCK	mitochondrial creatine kinase
mtDNA	mitochondrial DNA
O_2	oxygen
O_2^{-}	superoxide
OGG-1	8-oxoguanine glycosylase 1
·OH	hydroxyl radical
RE	resistance exercise
RNA	ribonucleic acids
ROS	reactive oxygen species
rRNA	ribosomal ribonucleic acids
SOD	superoxide dismutase
TBARS	thiobarbituric acid residues
TCA	trichloroacetic acid
tRNA	transfer ribonucleic acids
XL-PCR	extra long polymerase chain reaction

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1.0. Introduction

There are few certainties in life, however, one event we all expect and experience is the process of senescence. Changes related to aging can be attributed to development, genetic defects, the environment, disease, and the inborn process we call 'aging'. From a biological perspective, aging is associated with changes in physiological function that leads to morbidity, a loss of functional autonomy, and ultimately death.

The process of aging remains poorly understood despite a mounting body of scientific evidence. Many theories have been proposed, however one theory in particular has been at the forefront of aging research for the last 50 years. The 'free radical theory of aging' was first proposed in 1954 by Denham Harman (Harman 2001). A fundamental assumption of the theory was that aging was controlled by a single common process, modifiable by genetics and environmental factors. Harman postulated that the aging process is controlled by the initiation of free radical reactions. These reactions were thought to be responsible for the progressive deterioration of biological systems by their ability to induce random change. In 1972, Harman modified the free radical theory of aging based on suggestions that mitochondria were the major producers of free radicals, and that oxidative damage to the mitochondria determined life span (Harman 1972). As such, the theory has now become recognized as the mitochondrial theory of aging. At the heart of the mitochondrial theory of aging is a vicious cycle of events that ultimately results in cellular aging (fig. 1 on page 42). Components of the vicious cycle include mitochondrial DNA (mtDNA) damage related to impaired ETC proteins, which lead to

greater production of ROS, and this in turn causes more damage to mtDNA. Identifying the causal factor in a vicious circle of events is a difficult task, however the main tenet of the mitochondrial theory of aging is that the generation of excessive ROS represents the 'first domino to fall' in this chain of events.

Aging has been associated with an increase in oxidative damage in many tissues including skeletal muscle, and it is generally believed that this is a function of an age-related increase in the production of reactive oxygen species (ROS). It is hypothesized that oxidative stress may be related to an accumulation of mutations in the mitochondrial genome with advancing age, which could theoretically result in electron transport chain (ETC) dysfunction. Interestingly, reports on ETC function with aging are equivocal with as many studies reporting no change (Rasmussen et al., 2003, Chretien et al., 1998, Barrientos et al., 1996, Zucchini et al., 1995) as there are studies demonstrating an age-related decrease (Boffoli et al., 1994, Trounce et al., 1989, Hsieh et al., 1994, Cardellach et al., 1989). Alternatively, oxidative damage may be a result of a decrease in the function of cellular antioxidant systems (Ji, 1993), which may exacerbate oxidative stress associated with age.

Given the growing proportion of older adults in society there is a need for effective therapies to maintain quality of life in advanced age. One non-pharmacological therapy that has proven to be effective is resistance exercise training. Over the last twenty years improvements in strength, increases in muscle mass and muscle fiber cross-sectional area, and improvements in function have been documented following regular resistance exercise training in older adults. Resistance exercise training appears to slow and in some

cases reverse biological aging. However, the effects of resistance training on variables pertinent to the mitochondrial theory of aging remain largely unexplored. Given that the mitochondrial theory of aging represents the leading theory on aging, and that resistance exercise has profound effects on biological age, it stands to reason that resistance exercise may induce changes in the vicious cycle of events associated with the mitochondrial theory.

All biological tissues are affected by the aging process, however this dissertation will focus on skeletal muscle, oxidative stress, mtDNA deletions, mitochondrial adaptations, and the benefits of resistance exercise training. It will attempt to answer the question of whether resistance training alters components of the mitochondrial theory of aging in a favourable manner? The primary purpose of the studies described herein was to: (1) Draw comparisons between younger and older skeletal muscle with respect to: oxidative damage to proteins and DNA, antioxidant enzyme activity, oxidative enzyme activity, and mtDNA: and (2) Examine the effect of regular resistance exercise training on the same variables listed above. It was hypothesized that aging would be associated with a higher level of oxidative damage, higher antioxidant enzyme activity, lower electron transport chain activity, and accumulating mtDNA deletions. In addition, it was hypothesized that regular resistance exercise would decrease oxidative damage by increasing antioxidant enzyme activity, and reduce mtDNA deletions.

2.0. Aging

Skeletal muscle aging is characterized by a number of hallmark events including changes such as a loss of function (ie. strength, and activities of daily living), and changes in muscle fiber morphology. Less well documented events include alterations in electron transport chain enzyme activity, oxidative stress, changes in antioxidant enzyme activity, and alterations in the mitochondrial genome. Cumulatively, these age-associated events will be reviewed and comprise the focus of the first chapter of this dissertation.

2.1. Functional Decline with Age

One of the most common consequences of aging is a decrease in physical function. Falls are a primary cause of injury, morbidity, and mortality in the elderly, commonly related to a weakening of the ankle, knee, and hip muscles (Province et al., 1995). A reduction in maximal voluntary torque produced in the upper and lower body has been documented. For example, maximal voluntary isometric torque produced by the knee extensors and elbow flexors was 35% lower in a group of older (69 years old) as compared to younger men (28 years old) (Klitgaard et al., 1990). Muscle power, or the ability to produce force rapidly, also decreased with age, and this was associated with impairments in physical function (Martin et al., 2000).

Recent work that examined the effect of age on dynamic strength revealed a linear age-related decrease in arm and leg muscle quality (ie. strength per cross-sectional area of muscle mass) of similar magnitude in men and women (Lynch et al., 1999). In support of this finding, single-fibre analysis have suggested that contractile proteins become less effective with age, resulting in loss of functional capacity as discussed below in section 2.2. (Frontera et al., 2000).

The documented loss in strength associated with aging is thought, in large part, to be due to decreases in muscle cross-sectional area, a shift in fibre-type, and a reduction in muscle quality.

2.2. Muscle Fibre Morphology

Skeletal muscle aging is primarily characterized by a decrease in type II fibre cross-section (Klitgaard et al., 1990; Forsberg et al., 1991; Coggan et al., 1992; Grimby et al., 1983; Grimby et al., 1982; Larsson et al., 1978; Lexell et al., 1983; Tomonaga 1977). Some have also suggested that the relative proportion of type II muscle fibres decreases with age (Larsson et al., 1978; Jakobsson et al., 1990) while the proportion of type I fibres increases (Larsson 1983; Larsson et al., 1979). Single-fibre analysis has recently revealed that muscle fibres from older adults have a higher proportion of fibres co-expressing myosin heavy chain type IA and IIA, and type IIA and IIB, as compared to young adults, suggesting that fibre type transitions are an age-related event. The shift in fibre-type has effects on whole muscle performance, with an increased proportion of type I fibres

resulting in fatigue resistance, whereas a reduced proportion of type II fibres results in a decrease in peak torque (Cupido et al., 1992).

In addition, deterioration in the quality of individual muscle fibres has been described (Frontera et al., 2000). Isolated type I and IIA muscle fibres from old men were significantly weaker compared to those of young men, even after correcting for cross-sectional area.

The mechanisms underlying these age-related alterations remain largely unknown, however, the mitochondrial theory of aging represents a potential hypothesis that may explain some of these age-associated events. The following section will focus on the mitochondrial theory of aging as it pertains to skeletal muscle.

3.0. Skeletal Muscle and the Mitochondrial Theory of Aging

Central to the mitochondrial theory of aging are distinct age-associated changes in redox status, which may include enhanced oxidant production or a reduced antioxidant capacity, rearrangements of mtDNA, and declines in ETC enzyme activity. All of these are proposed to be linked in a vicious circle of events that progresses until cell death (Fig 1 on page 42). The following section examines the mitochondrial theory of aging as it pertains to skeletal muscle.

3.1. Reactive Oxygen Species

Reactive oxygen species (ROS), and the damage they induce over the life-time are key elements of the mitochondrial theory. Reactive oxygen species is a generic term given to molecules with the ability to oxidize the cellular environment. An oxygen free radical is a compound that contains at least one unpaired electron in its orbital, and is also considered a ROS. Most biological molecules are non-radicals and contain only paired electrons. Since electrons are more stable when paired together in orbitals, radicals are generally more active than non-radicals and seek to pair with another electron in order to be stabilized. A free radical reacts to accept an electron from another molecule resulting in the production of a stable molecule and another radical. Hence, free radical reactions often proceed as chain reactions where one radical begets another. This type of chain reaction continues until a termination reaction occurs. Thus, there are three stages involved in free radical reactions identified as initiation, propagation, and termination (Matsuo and Kaneko 2000). Termination does not occur until either two radicals react to form a molecule with a paired electron, or an antioxidant donates an electron to stabilize the free radical. In any event, if a free radical chain reaction proceeds without regulation, severe damage to biological tissues can occur.

Although there are many reactive oxygen and nitrogen species that are able to damage the cellular environment, several common ROS are believed to be responsible for the majority of biological damage. Specifically, superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$) will be discussed in further detail.

3.1.1 Superoxide Radical

The O_2^- radical is formed ubiquitously in all aerobic cells (Fridovich 1978). The primary site of O_2^- production has been localized to the inner mitochondrial membrane, where it is thought that inefficiency in the ETC leads to the reduction of oxygen. As atmospheric oxygen concentration increases so to does the production of the O_2^- (Freeman and Crapo 1981). In addition, production of O_2^- is thought to be enhanced during accelerated oxygen uptake, such as the respiratory burst of neutrophils, macrophages, lymphocytes, and presumably during exercise. Although there is evidence to support the role of O_2^- in damaging biological tissues, it is generally thought that O_2^- is dangerous to cells because of its ability to be easily and rapidly converted to other reactive species, such as hydrogen peroxide, which has a much longer half-life and the ability to freely cross through biological membranes.

3.1.2 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a non-radical ROS, as it does not have an unpaired electron in its orbital. In fact, H_2O_2 is a relatively stable compound with a long half-life. Thus in biological systems it is long-lived and is able to travel great distances (Matsuo and Kaneko 2000). The fact that H_2O_2 can readily pass through biological membranes increases the likelihood that it may cause wide-spread cellular damage. Hydrogen peroxide is known to inactivate several enzymes, usually through oxidation of important

thiol groups. For example, Glyceraldehyde phosphate dehydrogenase is inactivated by hydrogen peroxide, suggesting that exposure to high concentrations of H_2O_2 can lead to ATP depletion through inhibition of glycolysis (Brodie and Reed 1987). Despite this potential for damage, H_2O_2 is not a very reactive ROS. In fact, the most dangerous characteristic of H_2O_2 may be its ability to be converted, in the presence of metal transition ions, to the very reactive ·OH.

3.1.3 Hydroxyl Radical

The main source of the hydroxyl radical (·OH) in biological systems is *via* a reductive cleavage of H_2O_2 in the Fenton reaction (Wardman and Candeias 1996). This reaction requires metal transition ions such as iron (II) or copper (I). *In vivo*, the OH· has the unique ability to react with just about any biomolecule that is near its formation site. Although very reactive, the ·OH is also very short-lived, and is not able to react far from the site of formation. This suggests that damage induced by the ·OH should be site-specific. For example, generation of the hydroxyl radical close to DNA could modify its bases or cause a strand break (Mello Filho and Meneghini 1984). As discussed, reaction of the ·OH with a biomolecule will produce other, less reactive, radicals that may have the ability to diffuse far from the site of formation causing further damage.

3.2. Oxidative Damage and Aging

Advancing age is associated with an increase in oxidative damage to biomolecules including DNA, protein, and lipids. The consequences of an accumulation of oxidatively modified products remains unknown, however it is conceivable that such an accumulation may interfere with normal cellular function.

Early studies of the common house fly showed that the amounts of reactive carbonyl derivatives, a marker of oxidative damage to proteins, accumulated with age, and was correlated with senescence (Sohal and Dubey 1994; Sohal et al., 1993). In addition, studies of caloric restriction in rodents and primates have correlated increases in longevity with a lower level of oxidative damage in lipids, protein and DNA (Weindruch 1996). Indeed, human aging has been associated with an accumulation of oxidative damage to protein, lipids and DNA in various tissues including skeletal muscle.

Extensive experiments in rodents have reported an irrefutable age-related increase in 8-hydroxy-2-deoxyguanosine (8-OHdG) in many tissues (Hamilton et al., 2001). 8-OHdG is a measure of modified guanosine bases in DNA by ·OH radical attack. Levels of 8-OHdG varied from tissue to tissue and appeared to be related to metabolic rate, and whether the tissue was post mitotic or actively differentiating. In addition, an important finding lending support to the mitochondrial theory of aging was that 8-OHdG also accumulated with age in mtDNA of several tissues, but more important was the finding that mtDNA contained much higher levels of 8-OHdG as compared to nuclear DNA (Hamilton et al., 2001). In a similar fashion, thiobarbituric acid reactive substance

(TBARS), a measure of lipid peroxidation, was reported to be higher in brain, heart, liver, kidney, and skeletal muscle of aged rodents (Leeuwenburgh et al., 1994; Xia et al., 1995; Ji et al., 1991). Moreover, oxidative damage to proteins was shown to be higher in various tissues of older animals as compared to young animals (Sohal and Dubey 1994; Sohal et al. 1993; Butterfield et al., 1997).

There is limited data available regarding oxidative damage in human skeletal muscle, however results of all studies conducted to date are consistent with the data reported for animals (Fano et al., 2001; Pansarasa et al., 2000; Pansarasa et al., 1999; Miro et al., 2000; Mecocci et al., 1999). Pansarasa and colleagues (2000) reported significantly higher levels of lipid peroxidation and protein carbonyl content in human skeletal muscle of older (66-91 years) and middle aged (41-65 years) individuals when compared to younger individual (17-40 years). Mecocci and colleagues (1999) also demonstrated significant age-associated increases in 8-OHdG, malondialdehyde, and protein carbonyls in human skeletal muscle from individuals aged 25-93 years.

In addition to an increase in oxidant production, antioxidant status is also an important factor in determining redox status. In fact, it has been suggested that in many tissues oxidative stress is caused by a reduction in antioxidant capacity as opposed to enhanced oxidant production (Ji et al., 1990). The following section will focus on the antioxidant systems and how they are affected by age.

3.3. Antioxidant Enzymes

Throughout evolution, as the environment changed from a primarily reducing environment to a primarily oxidizing environment, cells evolved to have the capability to cope with toxic oxygen species. Three primary antioxidant enzymes have been identified in mammalian cells: 1) superoxide dismutase (SOD), 2) glutathione peroxidase (GPX) and, 3) catalase (CAT). All three of these enzymes possess the ability to detoxify ROS to less active ROS, or to neutralize them entirely to H_2O. Superoxide Dismutase promotes the dismutation of O_2^- to H_2O_2 and O_2. GPX utilizes glutathione (GSH) as an electron acceptor to convert H_2O_2 to oxidized glutathione and H_2O. Finally, CAT converts H_2O_2 to H_2O and O_2. The following section provides an overview of each of these antioxidant enzymes.

3.3.1. Superoxide Dismutase

SOD represents the first line of defense against O_2^- radicals. Specifically, SOD dismutates O_2^- to form H_2O_2 and O_2, as illustrated in the following reaction.

$$2O_2^- + 2H+ \rightarrow H_2O_2 + O_2$$

Two isoforms of SOD have been identified in mammalian cells that differ in their molecular structure by the metal ion bound to its active site. CuZnSOD is found

primarily in the cytosol (SOD1), although a nominal amount is also found in the interstitial space (SOD3). Alternatively, MnSOD is found primarily in the mitochondrial matrix (SOD2). Both isoforms catalyze the dismutation of O_2^- with similar efficiency (Ohno et al., 1988). In skeletal muscle, between 15-35% of total SOD activity is found in the mitochondria, 65-85% is found in the cytosol, and a negligible amount (~ 10%) is located in the interstitium (Leeuwenburgh and Ji 1996). Furthermore, it is generally accepted that SOD activity is highest in highly oxidative muscles (Type I), as compared to muscles with low oxidative capacity (Type IIa, IIb) (Criswell et al., 1993; Powers et al., 1994).

3.3.2. Glutahtione Peroxidase

Glutathione peroxidase catalyzes the reaction of H_2O_2 or organic hydroperoxide to H_2O and alcohol in the following two reactions respectively. In both of these reactions GSH is oxidized to form glutathione disulfide (GSSG):

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

$$2GSH + ROOH \rightarrow GSSG + ROH$$

GPX is a selenium dependent enzyme, existing in one isoform, and is found in the cytosol as well as the mitochondria (Ji and Fu 1992). Although GPX is highly specific for its electron donor (GSH), it has a relatively low specificity for hydroperoxides, thus allowing

it to reduce anything from H_2O_2 to various complex organic hydroperoxides (Buettner 1993). This characteristic makes GPX a very effective enzyme in protecting against ROS-mediated damage.

Approximately 45% of GPX activity is found in the cytosol, with the remaining 55% found in the mitochondria (Ji et al., 1998). Similar to SOD, GPX activity is highest in type I fibers and lowest in type IIb fibers, again demonstrating that highly oxidative fibers require a greater amount of antioxidant protection (Powers et al., 1994; Ji et al., 1988).

The glutathione system is unique in that it requires a second enzyme, glutathione reductase (GR), for the GPX system to function efficiently. Glutathione is a requirement for the GPX reaction, thus once it has been oxidized it must be reduced in a similar fashion or the GPX pathway is rendered non-functional. This is accomplished by using NADPH as a reducing agent in the following reaction catalyzed by GR:

$$GSSG + NADPH \rightarrow GSH + NADP^{+}$$

Although, GR is not considered to be a primary antioxidant enzyme, it is essential for the normal function of GPX. Not surprisingly, GR shares a similar cellular distribution pattern as GPX, and is also higher in highly oxidative fibers as compared to less oxidative fibers.

3.3.3. Catalase

The function of CAT is to catalyze the decomposition of H_2O_2 to H_2O and O_2 as outlined in the following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Although CAT appears to have the same function as GPX, the two enzymes differ by their affinity for H_2O_2. It is estimated that the Km for GPX is ~ 1μM, while the Km for CAT is 1 mM. This suggests that at lower concentrations GPX plays a greater role in the decomposition of H_2O_2, whereas at higher concentrations CAT may play a more significant role (Powers et al., 1999).

CAT has a wide cellular distribution, with the highest concentrations found in peroxisomes and the cytosol, and lower concentrations found in the mitochondria (Halliwell and Gutteridge 1989). Consistent with other antioxidants, the highest activities of CAT are found in highly oxidative fibers, with lower activities found in the less oxidative fibers (Powers et al., 1994)

3.4. Aging and Antioxidant Enzymes

Recent literature demonstrates that when *C. elegans* were exposed to antioxidants their life span increased dramatically (Melov et al., 2000), suggesting that loss of antioxidant

enzymes over time may, at least in part, be responsible for the aging process. In addition, aging has been shown to be associated with a reduction in the capacity of enzymatic and non-enzymatic antioxidant systems in several organs including liver, brain, heart, and kidney (Ji 1993). Interestingly, the vast majority of data from skeletal muscle suggests an age-related increase in antioxidant capacity. Barja and colleagues (1994) examined the biological differences of species of similar size but of varying life-spans. They reported that rat tissue had a much higher antioxidant capacity than pigeon tissue, despite the fact that pigeons live approximately 35 years longer than rats, and have the capacity for approximately double the O_2 consumption of rats (Perez-Campo et al., 1998). These results suggest that maximum life-span is associated with a lower antioxidant enzyme capacity. To clarify these paradoxical results, it was shown that ROS production was significantly greater in rats than in pigeons, and the authors concluded that long-lived animals have a more efficient ETC, which produces less ROS, and as a consequence elicits a smaller antioxidant response (Barja et al., 1994).

It is now generally accepted that senescent skeletal muscle is associated with an increase in antioxidant enzyme capacity (Leeuwenburgh et al., 1994; Lammi-Keefe et al., 1984; Vertechy et al., 1989; Ji et al., 1990; Lawler et al., 1993; Luhtala et al., 1994; Oh-Ishi et al., 1995). Lammi-Keefe and colleagues (1984) were the first to report that total SOD activity increased with ageing in rat skeletal muscle between 2 to 23 months of age. Further analysis revealed that both CuZnSOD and MnSOD accounted for the increase in total SOD. Interestingly, Vertechy and colleagues (1989) reported increases in GPX, GR, CAT, and a tendency towards an increase in MnSOD activity in rat gastrocnemius

between the ages of 3, 15, and 27 months, however, they reported no age-related changes in CuZnSOD. Perhaps the most comprehensive examination of age-related changes in rat skeletal muscle was performed by Ji and colleagues (1990). They reported a progressive increase in CuZn and MnSOD activity from rats aged 4, 26 and 31 months of age, such that by 31 months there was a 135% and 218% higher activity respectively. Furthermore, CAT activity was twice as high at 31 months as compared to 4 months of age, and GPX was 2-fold higher in cytosolic and 50% higher in mitochondrial fractions of *vastus lateralis* muscle. In accordance, Leeuwenburgh and colleagues (1994) reported similar increases in antioxidant enzymes in rat deep vastus lateralis and soleus muscles. In addition, they reported that γ-glutamyl transpeptidase , an enzyme important for GSH uptake into muscle, was significantly higher in DVL of older as compared to younger rats, suggestive of an increased demand for antioxidant capacity with age. In humans, Pansarasa and colleagues (1999) reported no age associated changes between the ages of 17-91 years for GPX and CAT, whereas MnSOD increased in *vastus lateralis, rectus abdominus,* and *gluteus maximus* muscles. The same group (Pansarasa et al., 2000) reported contradictory results in a subsequent study demonstrating higher activities of CAT, and MnSOD in men and women aged 66-91 years old, as compared to 17-41 year old men and women. This is a particularly interesting finding since MnSOD is localized to the mitochondria indirectly implicating the mitochondria as a primary source of ROS. Although the exact mechanism for an age-related increase in antioxidant enzymes remains unclear, it is generally thought to reflect a shift in cellular redox status towards oxidation (Ji 1993).

It is long established that aging mitochondria have a tendency to produce more O_2^- and H_2O_2 (Nohl and Hegner 1978). It has also been suggested that senescent muscle may be more susceptible to injury during muscular contraction (Radak et al., 2002). A higher level of injury may be associated with an inflammatory response that includes a lysosomal oxidative burst, producing a second source of ROS. Together, these events may induce a chronic oxidative stress in senescent muscle. There is little direct evidence to support a relationship between oxidative stress and antioxidant enzymes, however, reducing the production of ROS in rats, *via* dietary restriction, resulted in an attenuated increase in GPX and CAT activities in *quadriceps femoris* from 34 month old rats (Luhtala et al., 1994). Not surprisingly, antioxidant enzymes in skeletal muscle are closely related to oxidative capacity of the muscle fiber. Lawler and colleagues (1993) reported the lowest levels of GPX was in white gastrocnemius (fast twitch), as compared to red gastrocnemius and soleus muscle (slow twitch), and this did not change as a function of age. In addition, Oh-Ishi and colleagues (1995) reported that the activity and protein content of CuZnSOD was significantly higher in both soleus (slow twitch) and extensor digitorum longus (EDL; fast twitch) in 24 month old rats, as compared to 4 month old rats. MnSOD was higher in soleus, however, protein content remained unchanged with age, and GPX and CAT activities were only significantly higher in soleus muscle. Interestingly, mRNA for CuZnSOD was unchanged with aging, whereas mRNA for MnSOD decreased with age. Lack of an increase in gene expression of these antioxidant enzymes as a function of age suggests that the observed age-related increase in antioxidant enzyme capacity is either due to translational or post-translational

phenomena. This was confirmed in a recent study that found that CuZnSOD protein content was higher with aging despite no change in mRNA, and that mRNA for MnSOD was lower in all three muscles despite significant increases in enzyme activity, and no differences in protein content (Hollander et al., 2000). Moreover, both nuclear factor κβ (NF-κβ) and activator protein-1 (AP-1) were lower as a function of aging in all three muscles. It has been established that NF-κβ, and AP-1, are important in the signaling process for gene expression of MnSOD (Meyer et al., 1993, 1994). Both NF-κβ and AP-1 binding sites are found in the promoter region of MnSOD, and it has been shown that oxidative stress activates their binding (Warner et al., 1996).

To date, only one study has examined the effect of aging on antioxidant enzyme systems in different fibre types of humans (Pansarasa et al., 2002). Pansrasa and colleagues (2002), examined MnSOD, GPX, CAT, and lipid peroxide levels in subjects aged 65-90 years. The subjects were pre-screened using myosin ATPase staining, and then grouped into either >40% type II fibre distribution or <40% type II fibre distribution. Results showed that CAT and GPX were not different between the groups, however, total SOD was significantly lower in the <40% type II fiber distribution group, and lipid peroxides were higher. These results suggest that total SOD activity is lower in human muscle exhibiting greater type I fibre content, which is inconsistent with the animal literature. Furthermore, these results make little teleological sense, since one would assume that fibres with greater oxidative potential would also have a greater antioxidant capacity. It is difficult to reconcile these results with the animal literature, however it should be noted that muscle samples from this study were isolated from three different muscles including

the *vastus lateralis*, *rectus abdominus*, and the *gluteus maximus*. It is possible that antioxidant enzymes in these muscles are basally active to varying levels, and thus the adapted state of each muscle may be significantly different making results difficult to interpret.

Due to the paucity of data in humans it is difficult to make any concrete conclusions regarding antioxidant capacity in skeletal muscle, although preliminary evidence provides some support to those findings reported in rodents. It is also difficult to explain some of the variability between studies, however the differences may be related to muscle fibre-type, assay methods, gender, and species (Oh-ishi et al., 1995). See summary table 1 on page 44.

3.5. Mitochondrial DNA

Mitochondria, present in virtually all mammalian cells, are the site of essential functions including the citric acid cycle, amino acid biosynthesis, fatty acid oxidation and oxidative phosphorylation. Unlike any other organelle, the mitochondria contains its own genetic material that codes for essential ETC subunits. The human mitochondrial genome contains 16,569 base pairs (bp), and is a double stranded circular molecule (Fig 2 on page 43) (Anderson et al., 1981). One strand of the mtDNA is rich in purines G and A, and is referred to as the heavy strand, while the complimentary strand is rich in pyrimidines C and T, and is referred to as the light strand. It is hypothesized that the proto-mitochondrion contained over 4000 genes encoding for polypeptides involved in protein

import, beta oxidation, the urea cycle, and oxidative metabolism, among others, most of which have relocated to the nuclear genome. Today, mtDNA contains only 37 genes, 13 of which are polypeptides involved in the ETC, 2 of which are rRNA's, and the remaining 22 are tRNA's. In short, mtDNA contain 13 genes essential for oxidative phosphorylation, and all of the material necessary for translation inside the organelle. In addition, unlike nuclear DNA, mtDNA contains no introns, and thus represents a molecule dense in genetic information, an important point when considering the relative vulnerability of mtDNA to oxidative damage as previously discussed above. Mitochondrial DNA is located in the matrix of the mitochondria in relatively high abundance. Each mitochondrion contains 2-10 copies of DNA, and each cell contains 10-100's of mitochondria, depending on the energy requirement of the cell. Since mtDNA only encodes for 13 polypeptides, nuclear DNA encodes for the remainder of the polypeptides involved in the ETC. As such, an elegant relationship has developed between the mitochondria and the nucleus, and several proteins have now been identified as mediators in communication between the nucleus and the mitochondria (Schmidt et al., 2001). Five distinct complex enzymes comprise the ETC. Complex I is composed of seven mitochondrial encoded subunits, and 35 nuclear encoded subunits. Complex II is composed of no mitochondrial encoded subunits and 4 nuclear encoded subunits. Complex III is composed of one mitochondrial encoded subunit, and 10 nuclear encoded subunits. Complex IV is composed of three mitochondrial encoded subunits and 10 nuclear encoded subunits, and Complex V is composed of two mitochondrial encoded subunits and 12 nuclear encoded subunits. Thus, a mutation in either nuclear or mtDNA

can result in an impairment of energy production, and/or an increase in the production of ROS (Robinson 1994). Mitochondrial diseases, characterized by loss of ETC function due to mutations in the mitochondrial genome, provide a model to highlight the relevance of the role of damage to mtDNA in aging.

3.6. Mitochondrial DNA damage and Aging

Oxidative damage to mtDNA is a central component to the mitochondrial theory of aging. Unlike the nuclear genome, mtDNA does not contain introns, is not protected by a histone coat, and is located in close proximity to the primary free radical producer (DiMauro et al., 2002). Three main types of damage to mtDNA have been identified. As discussed previously, an age-related increase in oxidative nucleotide modification has been demonstrated in several species including humans (ie. 8-OHdG). Nucleotide point mutations have been shown to increase with age, and the main observation most commonly reported is an age-related increase in mtDNA deletions. The following section will be the focus of these observations.

3.6.1. Nucleotide Point Mutations and Aging

Nucleotide point mutations occur when a nucleotide on a DNA strand is replaced with a different nucleotide. This can cause deleterious effects by the insertion of an incorrect amino acid during translation, which can result in diminished, or complete loss of protein

function. This event occurring in the mitochondrial genome can have deleterious consequences, since all proteins encoded in the mtDNA code for ETC subunits and vital translational machinery.

An A to G point mutation of $tRNA^{lysine}$ has been described in mtDNA of older adults (Munscher et al., 1993). This mutation is associated with defects of oxidative phosphorylation resulting in an impairment of energy production. The mutation was described in extra-ocular muscle of "healthy" individuals aged 74-89 years, accounting for approximately 2% of total mtDNA. The mutation was also present in six of nine subjects aged 20-70 years (Munscher et al., 1993). A similar A to G point mutation of $tRNA^{leucine}$ at position 3243 was reported to be present in various tissues from older individuals (Zhang et al., 1993). Seven out of 38 adult tissues tested positive for this mutation, as compared to none of 16 infant tissues tested. In addition, point mutations at position 3243 and 10006 in mtDNA of extraocular muscle were demonstrated to increase in an age-related fashion (Munscher et al., 1993).

Lack of described phenotypes to point mutations during aging has led to the conclusion that although mutations may be an age-associated phenomenon, they are not a causal link to aging (Pak et al., 2003). Moreover, analyses in tissue homogenates have estimated that mutations accumulate to less than 2%, suggesting that mutations may not be a critical link to cellular aging. However, recent single fiber analysis has demonstrated that point mutations in fact do accumulate to high levels and are both age and tissue specific (Nekhaeva et al., 2002). For example, a mutation at position 414 located in the control region of mtDNA was detected in skeletal muscle of individuals 30 years and older and

found to accumulate with age, however, the mutation was not present in brain tissue mitochondria harvested from individuals aged 23 to 93 years of age (Murdock et al., 2000).

In addition, mtDNA point mutations were shown to colocalize with cytochrome oxidase-negative (COX-) fibres, suggesting that mutations in mtDNA may be physiologically relevant (Lin et al., 2002; Fayet et al., 2002). Lin and colleagues (2002) demonstrated that advanced aging of brain tissue was associated with an increased aggregate mutational burden as compared to young, and correlated negatively with COX activity, suggesting that multiple mtDNA point mutations have physiological consequences. In addition, single muscle fibers from deltoid, quadriceps femoris, and tibialis anterior muscle taken from 14 individuals aged 69-82 years of age, corresponded to high levels of point mutations in tRNA genes of mtDNA. However, these mutations were only detected in fibers identified as COX negative. The point mutations occurred at position 3243, 8344, 4460, 4421, and a novel 3-bp deletion in the $tRNA^{leucine}$ gene. Together, these results suggest that mtDNA point mutations are associated with reduced COX activity with aging, and that focal accumulation results in significant impairment of mitochondrial ETC function in individual cells (Fayet et al., 2002).

The role of nucleotide point mutations in aging is a growing field, however, the most often reported age-associated mtDNA alterations are mtDNA deletions, which will be the focus of the following section. See summary table 2 on page 45.

3.6.2. Mitochondrial DNA Deletions and Aging

Mitochondrial DNA deletions represent sections of the mitochondrial genome that have been "excised" resulting in a genetic code lacking large regions of genetic material. Thus, essential genes encoding ETC proteins, mitochondrial tRNA, and mitochondrial rRNA are not available for transcription, and can lead to ETC dysfunction.

In patients with mitochondrial myopathies and encepaphalopathies, deleted regions of mtDNA was reported in 20-80% of all mitochondrial genomes (Holt et al., 1988; Schon et al., 1994), and was associated with a specific phenotype largely characterized by muscle fatigue and weakness. A 5 kb deletion was identified as common in 30-50% of all diagnosed patients (Wallace 1992). Appropriately, this deletion became known as the "common" deletion ($mtDNA^{4977}$), and was found to extend between nucleotides 8483 and 13459 in humans, and is flanked by a 13 bp direct repeat.

The first reports of an age-associated increase in mtDNA deletions suggested that the $mtDNA^{4977}$ deletion increased in various tissues of older adults, however, it was undetectable in infant and fetal tissue (Linnane et al., 1990; Cortopassi and Arnheim 1990). Interestingly, post-mitotic tissue, such as muscle and nerve, appear to have the highest levels of deletion products (Cortopassi et al., 1992). Since these early reports, many investigations have confirmed the presence of mtDNA deletions in aging tissue (Yarovaya et al., 2002; Cormio et al., 2000; Zhang et al., 1998; Liu et al., 1998; Kovalenko et al., 1997; Eimon et al., 1996; Melov et al., 1995; Schwarze et al., 1995;

Simonetti et al., 1992; Katayama et al., 1991). There are three commonly reported findings associated with aging. First, a decrease in the amount of full-length mtDNA. Second, total mtDNA does not decrease, rather there is an increase in mtDNA deletions. Finally, a significant amount of oversized mtDNA is detected in old adults, indicative of mtDNA insertions (Kovalenko et al., 1997). Furthermore, histochemical analysis of COX activity revealed that COX - fibres correlated strongly with age and the extent of mtDNA deletions (Linnane et al., 1997).

The observation that mtDNA deletions in tissue homogenates occurred in relatively low abundance with aging (<0.1%) led some to conclude that mtDNA deletions had little physiological significance, and probably did not contribute to muscle dysfunction, at least in aging. However, studies of single human fibres have presented a different scenario (Brierley et al., 1998; Kopsidas et al., 2002). Brierley and colleagues (1998) were the first to report that single fibres identified as COX - also contained high levels of mtDNA deletions demonstrating that clonal expansion is present during normal aging. In addition, although only a small percentage of fibres in cross-section are COX -, individual fibres are deficient only over part of their length, suggesting that deficiency is segmental. Thus, it is possible that one or more segments of a high proportion of fibres are COX -, and therefore it is possible that overall function of a muscle fibre deficient in COX activity at one or more segments in its length may be impaired. This mosaic may play a significant role in the decline of overall muscle function with age. Accordingly, Kopsidas and colleagues (2002) also identified type I muscle fibres that were ETC deficient. Amplification of mtDNA from these fibres demonstrated a reduction in full-length

mtDNA, and an accumulation of mtDNA deletion products. In fact, many fibres that were COX negative and SDH hyper-positive (indicative of mitochondrial proliferation) contained no detectable full-length mtDNA. Moreover, Kopsidas and colleagues (2002) suggest that the reduction in full-length mtDNA is the critical event leading to the loss of ETC function as opposed to the accumulation of mtDNA deletions and mutations. Together, these observations suggest that mtDNA deletions and mutations occur in a stochastic manner throughout the muscle. Thus in tissue homogenates, mtDNA deletions appear to be present in inconsequential amounts, however at the level of the ETC deficient single fibre, mtDNA deletions appear to play an integral role in the loss of mitochondrial bioenergetics. Recent reports have since confirmed these findings in humans as well as rats (Wanagat et al., 2001; Kovalenko et al., 1998; Kopsidas et al., 1998).

The mechanism underlying the acquisition of mtDNA deletions remains unknown, however, it has been suggested that deletions may occur spontaneously during replication of DNA. The 5 kb common deletion is flanked by a 13 bp perfect direct repeat. Since the majority of mtDNA deletions detected in aged individuals contained direct repeats at their breakpoints, a "slip replication" hypothesis of deletion formation was proposed (Shoffner et al., 1989). This hypothesis suggested that during replication a displaced heavy strand direct repeat pairs with a downstream light strand direct repeat that is exposed because of the replication fork. Completion of mtDNA replication results in one normal mtDNA molecule from the original light strand and one deleted mtDNA molecule from the slip-replicated heavy strand (Shoffner et al., 1989). It has also been suggested

that there may be other mechanisms underlying deletion formation, since deletions have been identified without direct repeats at their breakpoints(Lee et al., 1994; Chung et al., 1994). These mechanisms are less well understood and have been hypothesized to involve illegitimate or homologous recombination, secondary structure formation, and/or topoisomerase cleavage.

The results discussed in this section draw a link between mtDNA deletions and ETC dysfunction, as assessed via histochemistry. Much of the work examining whole muscle homogenates however, report equivocal results with respect to ETC enzyme activity in aging. The following section will focus on the effects of aging on ETC function. See summary table 3 on page 46.

3.7. Muscle Enzymes

Recent evidence has implicated peripheral factors as potentially limiting in the rate of O_2 consumption in advanced age (Hepple et al., 2003). Oxygen consumption was measured in rat hind-limb and was shown to be reduced in old vs. young rats, despite the same convective O_2 delivery. Moreover, complex I+III activity was 45% lower in old vs. young animals. However, recent evidence based on human vastus lateralis, demonstrated no difference in ETC enzyme activity as a function of age (Rasmussen et al., 2003). The authors concluded that this is evidence that the mitochondrial theory of aging was not supported in human skeletal muscle. Several reports are in accordance with these findings (Chretien et al., 1998; Barrientos et al., 1996; Zucchini et al., 1995), however

numerous reports have demonstrated an age-related reduction in respiratory chain activity (Boffoli et al., 1994; Trounce et al., 1989; Cooper et al., 1992; Cardellach et al., 1989). In isolated mitochondria from human vastus lateralis, state III (activated) mitochondrial respiration was significantly lower in old as compared to young adults (Boffoli et al., 1994; Trounce et al., 1989). More specifically, complex IV and complex I activity were reported to be lower, whereas complex II activity appeared to be unaffected by age (Hsieh et al., 1994). These results are particularly interesting since complex II is the only enzyme in the respiratory chain that is exclusively encoded by nuclear genes (DiMauro and Schon 2003), while all other enzyme complexes are encoded by a combination of both mitochondrial and nuclear genes. In contrast, at least two investigations in humans have reported no apparent relationship between aging and complex I, II, III, or IV activity in mitochondria isolated from human deltoid muscle (Chretien et al., 1998; Barrientos et al., 1996). In another study, mitochondrial oxygen uptake was found to decrease, however when confounding variables, including physical activity and tobacco consumption were included in a multiple regression analysis, no age – related differences were apparent (Barrientos et al., 1996).

ETC activity in different fibres are also differentially affected by aging. This is not a surprise since type I fibres depend primarily on oxidative metabolism, whereas type IIX fibres possess a relatively low oxidative potential. Studies of young and old rat gastrocnemius muscle, containing primarily type II fibers, demonstrated normal complex IV activity. However, rat soleus muscle, containing primarily type I fibers, was shown to have a decreased complex IV activity with advanced age (Pastoris et al., 1995). In

contrast, Desai and colleagues (1996), reported a 54-74% reduction in the activity of complex I, III, and IV activity in mouse gastrocnemius muscle. In addition Vmax of complex IV decreased 90% from 10 months to 26 months of age. Lack of an appropriate definition for "aged" animals may contribute to the varying results reported in the literature. Rodent studies have examined animals ranging anywhere from 20-36 months of age, which is a considerable span in age, given that rodents have a life-span of approximately 36 months. In addition, there is reason to believe that considerable changes occur quickly, as it has been reported that at age 20 months high affinity binding sites on complex IV accounted for 46% of total binding sites, and decreased significantly to 33% by age 26 months (Desai et al., 1996). Thus, examination of a group aged 20 months, may in fact be a different population than a group aged 30 months, even though both age groups have traditionally been considered "old adults".

Inconsistencies in the literature regarding ETC enzyme activity are attributable to biochemical inconsistencies between mitochondria from different tissues, the restriction of studies to a particular tissue (Kwong and Sohal 1998), and methodological differences. In addition, variable results concerning the effect of aging on ETC enzyme activities may be attributable to the fact that comparisons are often made between activities of specific enzymes between young and old. There is ample evidence to suggest that it is more accurate to compare activity ratios of different complexes (Kwong and Sohal 2000). Changes in activity ratios have been shown to be indicators of potential imbalance between the complexes and may be a marker of ETC inefficiency (Chretien et al., 1998; Geromel et al., 1997). A case study of a patient presenting with the severe phenotype

associated with mild ETC deficiency demonstrated that although activities of specific enzyme complexes fell into the normal range, an imbalance in electron flow was suggested by the comparison of activity ratios (Geromel et al., 1997). Furthermore, a linear inverse relationship between the level of protein carbonyls, and the activity ratio of complex IV and complex I in rat brains of varying age were reported, suggesting that a decrease in the ratio of complex IV to complex I led to greater production of ROS (Martinez et al., 1996). Finally, an age-related shift in activity ratios among different complexes have been reported in skeletal muscle of aging mice. It was hypothesized that a shift from the steady state condition of electron flow led to a loss of potential for generating the proton gradient, resulting in respiratory inefficiency (Kwong and Sohal 2000).

The effect of aging on ETC activity in whole muscle is not clear, despite evidence of age-related mtDNA anomalies, and age-associated oxidative stress. Given the growing number of older adults in society it is of critical importance to identify effective therapies to maintain quality of life through advancing age. One therapy that has been proven to be effective is regular resistance exercise training. The following section will examine the potential role for resistance exercise in the mitochondrial theory of aging. See summary table 4 on page 47.

4.0. Resistance Training in Older Adults

Resistance training is known to induce hypertrophy and strength gains in young men and women (Deschenes and Kraemer 2002). In the quest to discover effective therapies to reduce age-associated muscle atrophy, and subsequent losses in strength, resistance training has become a popular form of therapy. Indeed, many studies have examined the effects of resistance training as a means of developing muscle strength and muscle mass in older individuals (Spirduso and Cronin 2001). Perhaps more importantly, several investigations have examined the potential for resistance training to improve activities of daily living such as walking, and stair climbing (Fiatarone et al., 1990; Fiatarone et al., 1994; Schlicht et al., 2001; Hunter et al., 2000).

4.1. Effects on Strength

High intensity resistance training in older adults has been demonstrated to increase muscle strength in men and women (Frontera et al., 1988; Charette et al., 1991). These increases have been attributed to both improved neuromuscular recruitment (Moritani and deVries 1979), as well as muscle fiber hypertrophy (Frontera et al., 1988; Charette et al., 1991; Brown et al., 1990).

Since the early 1980's a variety of resistance exercise protocols have been utilized to examine the effects of resistance training on strength gains in older adults. It appears that irrespective of the protocol, high intensity resistance exercise training conclusively

increases muscle strength. It is well documented that regular strength training increases 1RM strength in a variety of upper and lower limb exercises in men and women aged 60-78 years (Frontera et al., 2000; Charette et al., 1991; Brown et al., 1990; Pyka et al., 1994; Roth et al., 1999; Hikida et al., 2000; Ivey et al., 2000; Roth et al., 2000). More importantly, the adaptive potential to resistance training does not appear to become reduced even in advanced age (Fiatarone et al., 1990). Fiatarone and colleagues (1990) demonstrated that 8 weeks of resistance training in frail elderly (86-96 years) also increased knee extension 1RM by 174%. These results suggest that resistance exercise is an efficacious and potent therapy for increasing muscle strength in advanced age.

4.2. Effects on Body composition

Early reports suggested that strength gains during resistance exercise training in older adults were primarily related to adaptations in neural factors (Moritani and deVries 1980). For example, Fiatarone and colleagues (1990) reported that 8 weeks of resistance exercise training in physically frail older adults (86-96 years) increased thigh muscle strength, however the increase in muscle cross-sectional area was much smaller than the increase in strength, suggesting that the majority of the increase in strength was accounted for by neural adaptations. Moreover, several studies have suggested that resistance training does not result in increases in muscle mass in the elderly (Ades et al., 1996; Bermon et al., 1998). However, these studies utilized less sensitive techniques to estimate fat-free mass (FFM) such as anthropometry (Bermon et al., 1998) or hydrodensitometry (Ades et al.,

1996), which have been shown to lack the sensitivity to detect subtle changes following resistance training (Lohman 1981).

Despite these findings, there is a significant body of literature attesting to whole-body as well as site-specific increases in muscle mass/fat-free mass (FFM) following resistance training in older adults (Fiatarone et al., 1994; Frontera et al., 1988; Brown et al., 1990; Nichols et al., 1993; Taaffe et al., 1999; Treuth et al., 1994; Hurley et al., 1991). Reported increases in FFM are usually accompanied by a reduction in fat mass. Treuth and colleagues (1994) reported a 2 kg increase in FFM, while fat mass decreased by the same magnitude following 16 weeks of resistance training in subjects aged 51 and 71 years. Hunter and colleagues (2000) reported a 3.4% decrease in body fat, a 3.1 kg decrease in fat mass, and a 2 kg increase in FFM in subjects aged 61-77 years after 26 weeks of strength training.

4.3. Effects on Muscle Morphology

One of the hallmark signs of muscle aging is a decrease in muscle fibre cross-sectional area, and an increase in the proportion of type I muscle fibres. There is now conclusive evidence that muscle fibre cross-sectional area of type I, IIa, and IIx fibres all increase significantly following short-term high intensity resistance training in older adults (Frontera et al., 1988; Charette et al., 1991; Brown et al., 1990; Pyka et al., 1994; Hikida et al., 2000; Hagerman et al., 2000; Brose et al., 2003). In studies ranging from 10-16 weeks, there was an increase in type I muscle-fibre cross-sectional area ranging from 14-

46%, and an increase in type II muscle-fibre cross-sectional area ranging from 19-52% (Frontera et al., 1988; Charette et al., 1991; Brown et al., 1990; Pyka et al., 1994; Hikida et al., 2000; Hagerman et al., 2000; Brose et al., 2003). It should be noted that the greatest increase in cross-sectional area was observed in the study of longest duration (16 weeks), suggesting that there is a dose-response relationship between hypertrophy and duration of training in older adults. Only two studies examined the effects of resistance training on type IIx fibres, and these reported increases of 19% and 52% increase in muscle fibre cross-sectional area (Hikida et al., 2000; Hakkinen et al., 1998). Regular resistance exercise training thus favourably affects strength, body mass, and muscle fibre morphology in elderly individuals. The goal of exercise prescription in aging includes improving the ability of individuals to perform tasks of daily living. The following section will focus on the effects of resistance training on functional capacity.

4.4. Effect on Functional Capacity

With an increasing proportion of older adults in society it is paramount that therapies designed to attenuate age-related declines in muscle mass also convey improvements in functional capacity. Surprisingly, very few studies have specifically examined the effects of resistance exercise on functional capacity in older adults. This may be due in part to the fact that these variables are difficult to control, thus measuring practical applications in the lab does not always result in significant outcomes, despite improvements in strength. Nonetheless, eight weeks of resistance exercise training has been shown to

result in significant improvements (48%) in gait speed in frail elderly individuals (86-96 years) (Fiatarone et al., 1990). In a similar study, 10 weeks of regular resistance exercise resulted in a significant increase in strength, which was correlated to an increase in gait velocity (12%), and an increase in stair-climbing power (28%), in older adults aged 72-98 years (Fiatarone et al., 1994). Finally, a recent study demonstrated that 8 weeks of resistance exercise training resulted in a 17% increase in walking speed, and a 15% decrease in a 5- repetition sit-to-stand timed test in older adults aged 61-87 years (Schlicht et al., 2001).

There is growing evidence that there may be a role for strength training in improving mitochondrial function in patients with mitochondrial disease *via* a process referred to as "gene shifting" (see below). Strikingly, advanced age presents a very similar phenotype to patients with mitochondrial disease, thus it stands to reason that the positive effects of resistance exercise on mitochondrial function in disease, may also be realized in older adults. The use of resistance exercise to induce adaptations in mitochondrial function is an unorthodox concept, and the following section will describe the cellular events resulting in these adaptations.

4.5. Resistance Training and "Gene Shifting"

Traditional concepts in exercise physiology suggest that in order to induce oxidative adaptations one must stress the oxidative systems, through continuous exercises such as running or cycling. It appears, however, that in patients with compromised mitochondrial

function, oxidative adaptation may be induced following resistance exercise. These adaptations occur through a concept termed "gene shifting". This concept is based on the notion that muscle satellite cells have a low mutational burden (Fu et al., 1996). In other words, because satellite cells are known to remain quiescent unless induced, it is theorized that these cells do not accumulate mtDNA mutations and deletions (*via* ETC flux and subsequent oxidative stress) at the same rate as mtDNA from mature myofibres. It is well known that satellite cells are activated in response to muscle injury (Bischoff 1997). Upon activation, satellite cells proliferate, giving rise to myogenic precursor cells that fuse with mature myofibers. During the fusion process the organelles of the satellite cell, such as nuclei, mitochondria, and molecular machinery are donated to the existing myofiber. Hence, the process of muscle damage and regeneration may aid in maintaining the ratio of wild-type to mutant mtDNA above the threshold at which the metabolic demands can no longer be met. The theory of "gene shifting" was tested in patients using experimental muscle damage induced by a biopsy-rebiopsy technique, in which a biopsy was taken from the vastus lateralis and one week later the same region was re-biopsied (Shoubridge et al., 1997). One week following the injury, all regenerating fibres were stained positively for COX suggesting that mtDNA donated from satellite cells were intact and functional. Similar results were reported in a study that used injection of bupivicaine hydrochloride to induce muscle injury (Clark et al., 1997). Given the capacity for acute exercise to induce muscle damage, it was proposed that resistance exercise may be a viable therapy for patients with mitochondrial disease, as the adaptation to repeated cycles of degeneration and regeneration would lead to a more favourable ratio

of wild-type to mutant mtDNA (Taivassalo et al., 1999). Taivassalo and colleagues (1999) examined the effect of concentric resistance training in one arm vs. eccentric training in the other. The results demonstrated a striking 19.5% and 43.5% decrease in the number of COX^- fibres in the eccentric and concentric arms, respectively. These results corresponded to an 8% and 33% increase in the amount of wild-type mtDNA in the eccentric and concentric arms, respectively. Given the propensity for eccentric exercise to induce significantly greater muscle damage than concentric exercise, the results were somewhat confusing, as one would have expected a greater response in the eccentrically trained arm. Nonetheless, these results provide encouraging evidence for the use of resistance training to improve mitochondrial function in patients with mitochondrial disease. Since aging is associated with an accumulation of mtDNA deletions and mutations, it follows that "gene shifting", induced through resistance exercise, may result in improved oxidative function in older adults. It should be noted however, that endurance exercise training in patients with mitochondrial disease appears to result in a decrease in the proportion of wild-type mtDNA, suggesting a trend towards preferential proliferation of mutant genomes (Taivassalo et al., 2001). These authors concluded that the long-term implication of a downward shift in wild-type mtDNA needs to be assessed before aerobic exercise can be proposed as a treatment option, however, resistance exercise appears to be safe and effective.

4.6. Resistance Exercise Induced Oxidative Stress

Limited information is available regarding the effects of resistance exercise on the induction of oxidative stress. In fact, the effect of resistance exercise on antioxidant systems, and oxidative function in older adults remains unknown. A limited number of studies, have examined the effects of an acute bout of resistance exercise and the effect of resistance training on markers of oxidative damage. Malondialdehyde, a marker of lipid peroxidation, was shown to be elevated above base-line following an acute bout of resistance exercise (McBride et al., 1998). More importantly, resistance exercise training for 6 months in older adults resulted in a decrease in malondialdehyde and hydroperoxides following an acute aerobic challenge as compared to before training (Vincent et al., 2002). However, a recent study conducted on older adults demonstrated no statistically significant effect of resistance training on urinary excretion of 8-OHdG, despite an ~ 40% decrease (Rall et al., 2000).

Despite minimal evidence to support a role for resistance exercise training in inducing adaptations in oxidative and antioxidant systems, there is a strong theoretical argument to suggest that resistance training may be a source of oxidant production, and hence may elicit an appropriate and beneficial response. Studies examining the inflammatory response following an acute damaging bout of eccentric exercise report that neutrophil infiltration into cells peaks approximately 24 hr following exercise in humans (Beaton et al., 2002; Stupka et al., 2000)and rats (Takekura et al., 2001). It is well known that neutrophil function is associated with a respiratory "burst". This burst is accomplished

via the enzyme NADPH oxidase, which catalyzes the production of a series of ROS including O_2^-, H_2O_2, and ·OH (Babior 1978). The respiratory burst is thought to be necessary to induce further damage before the clean up process is initiated by macrophage infiltration. As a result, a cytosolic oxidant stress is induced.

In addition, a consequence of damaging exercise is an increase in cytosolic calcium concentration (Armstrong et al., 1991). Xanthine oxidase is a cytosolic enzyme that produces O_2^- and H_2O_2 during the conversion of hypoxanthine to xanthine. Under normal conditions, this enzyme is present as an NAD+ dependent dehydrogenase, generating NADH and xanthine as reaction products. Under conditions of increased calcium load, a calcium dependent protease converts the dehydrogenase to an oxidase that uses molecular oxygen instead of NAD^+ as the electron acceptor, with xanthine, O_2^- and H_2O_2 as reaction products (Kondo et al., 1993; McCord 1985). Together, these events represent potential cytosolic sources of oxidative stress following a damaging bout of resistance exercise. Indeed, following stretch injury in rabbits, non-mitochondrial oxidant production was elevated at 12h and 48h following the injury. Maximum oxidant production by neutrophils and macrophages was also elevated in the damaged leg 48h following the injury, and myeloperoxidase activity, a measure of activated neutrophils was increased at 4h and 48h after injury. Moreover, xanthine oxidase, and SOD activity were elevated at 24h and 4 h respectively (Brickson et al., 2001). These results suggest that acute muscle injury can result in non-mitochondrial mediated oxidative stress and elicit an antioxidant response. Thus, regular resistance exercise that induces cycles of damage and regeneration may lead to an oxidant stress, and result in a greater protection against

oxidants via antioxidant up-regulation. Given the increased vulnerability to oxidants in advanced age, resistance exercise training may be an effective means of managing age-associated oxidative stress.

5.0. Statement of Purpose

The purpose of the experiments detailed in this dissertation were two fold. First, to describe a series of experiments conducted in order to assess the mitochondrial theory of aging in human skeletal muscle, with particular attention to oxidative damage to proteins and DNA, antioxidant enzyme activity, oxidative enzyme activity, and mtDNA. Second, to examine the effects of resistance exercise on the underlying mechanism(s) of aging as they pertain to the mitochondrial theory.

Figure 1. The Vicious Cycle

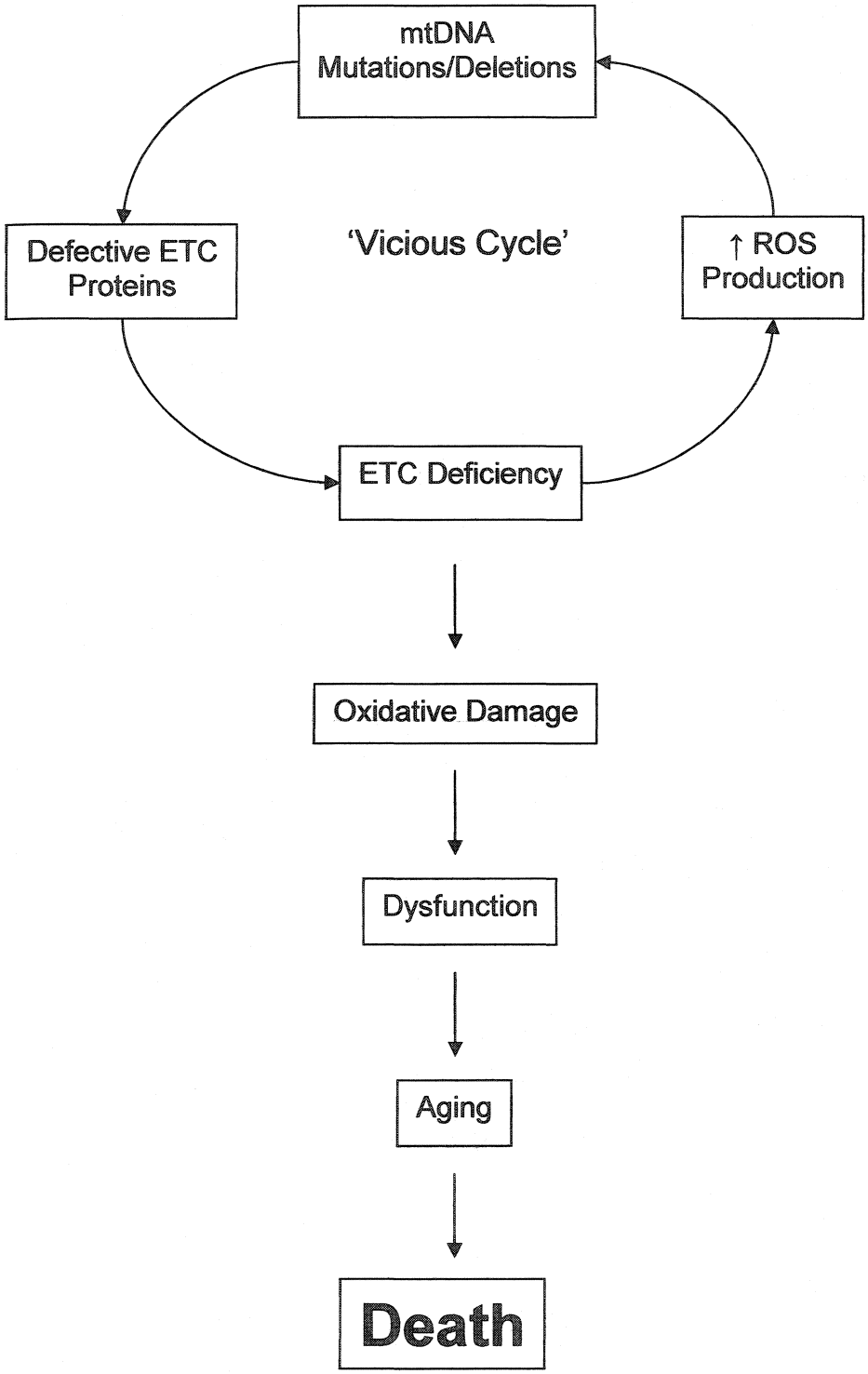

Figure 1. The 'vicious cycle' as proposed in the mitochondrial theory of aging.

Figure 2. The Mitochondrial Genome. (Adapted from A. Gemin 2003)

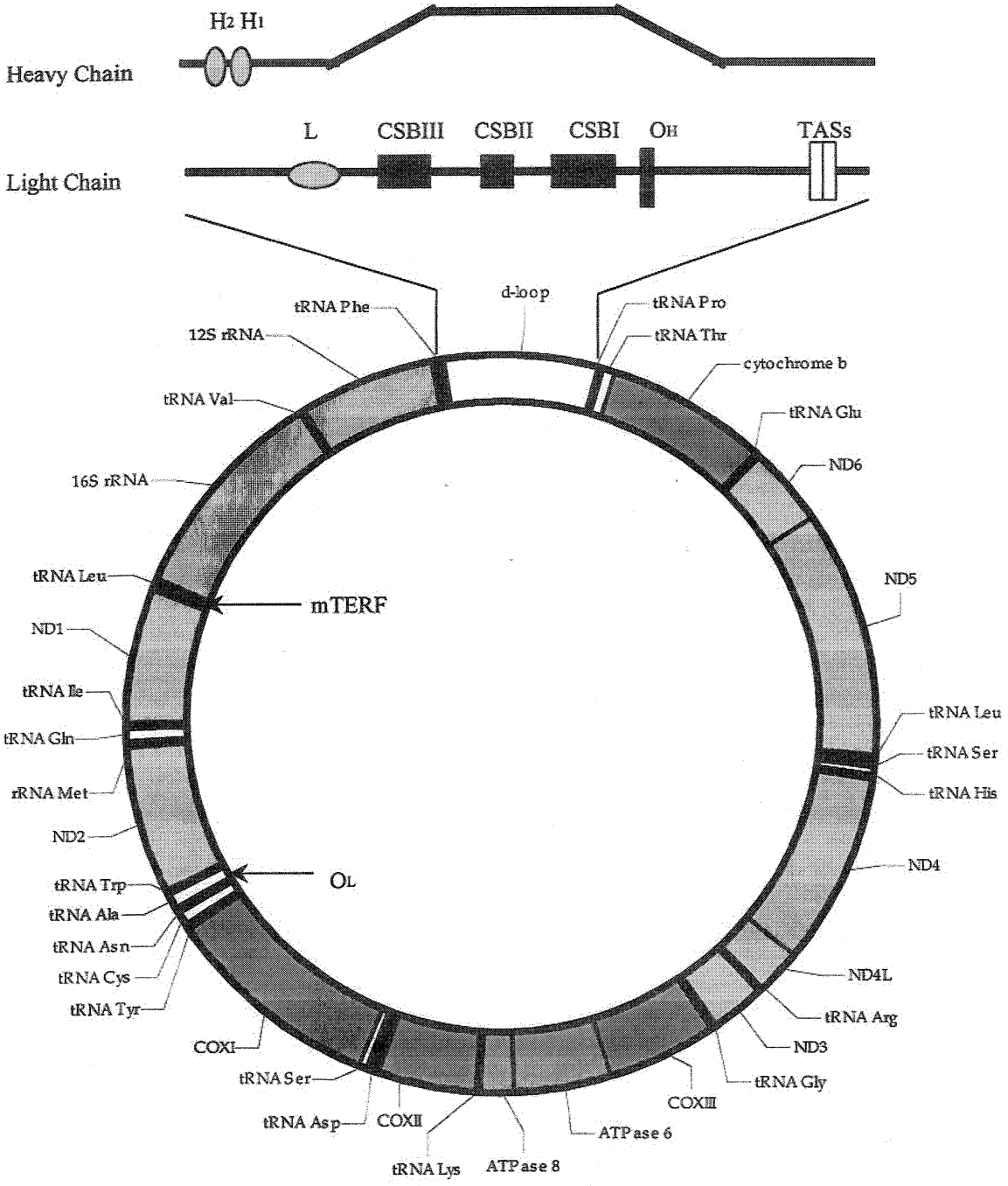

Figure 2. : Map of the human mitochondrial genome. Protein, tRNA and rRNA genes are represented by boxes. H1 and H2: Heavy strand promoters; L: Light strand promoter; CSB: Conserved Sequence blocks; TASs: Termination Associated Sequences; mTERF: mitochondrial transcription termination factor; OL: Orign of light strand replication; OH: Origin of heavy strand replication

Table 1. Antioxidant Enzymes and Aging

Author	Species	Age	Tissue	Results
Lammi-Keefe et al., 1984	Rat	3, 23 mths	cardiac and skeletal muscle	Cardiac M - ↑ MnSOD, Skeletal M - ↑ MnSOD, ↑ CuZnSOD
Vertechy et al., 1989	Rat	4, 15, 27 mths	cardiac and skeletal muscle	Cardiac M - ↑ GPX, Skeletal M - ↑ GPX, ↑ GR, ↑ CAT
Ji et al., 1990	Rat	4, 26, 31 mths	skeletal muscle	↑ CuZNSOD, ↑ MnSOD, ↑ CAT, ↑ GPX, ↑ GR, ↑ GST
Leeuwenburgh et al., 1994	Rat	4, 26, 31 mths	skeletal muscle	↑ GPX, ↑ GR, ↑ SOD, ↑ CAT
Luhtala et al., 1994	Rat	11, 26, 34 mths	skeletal muscle	↑ CAT, ↑ GPX
Oh-Ishi et al., 1995	Rat	various ages	skeletal muscle	↑ CuZNSOD, ↑ CAT, ↑ GPX
Lawler et al., 1993	Rat	4, 24 mths	skeletal muscle	↑ GPX
Pansarasa et al., 1999	Human	17-91 years	skeletal muscle	↑ MnSOD
Pansarasa et al., 2000	Human	17-91 years	skeletal muscle	↑ MnSOD

Table 1. Summary table of studies examining the effect of age on antioxidant enzyme activity.

Table 2. Mitochondrial DNA Point Mutations with Aging

Author	Species	Age	Tissue	Results
Muscle Homogenates				
Munscher et al., 1993	Human	various ages	extraocular muscle	↑ 3243, 10006 mutation
Munscher et al., 1993	Human	74-89 years	extraocular muscle	↑ 8344 mutation
Lin et al., 2002	Human	various ages	Brain	↑ aggregate mutational burden
Murdock et al., 2000	Human	23-93 years	skeletal muscle	↑ 414 mutation (control region)
Liu et al., 1998	Human	Jan-90	skeletal muscle	↑ 3243 mutation
Zhang et al., 1998	Human	0-90 years	skeletal muscle	↑ 3243 mutation
Single Fibers				
Fayet et al., 2002	human	69-82 years	skeletal muscle	↑ 3242, 8344, 4460, 4421, in ETC deficient fibers

Table 2. Summary table of studies examining the accumulation of mtDNA point mutations with aging.

Table 3. Mitochondrial DNA Deletions with Aging

Author	Species	Age	Tissue	Results
Muscle Homogenates				
Yarovaya et al., 2002	Rat	various ages	skeletal muscle	↑ mtDNA deletions, ↓ full-length mtDNA
Cormio et al., 2000	Human	<40 - >70	skeletal muscle	↑ mtDNA deletions
Zhang et al., 1998	Human	0-90 years	skeletal muscle	↑ mtDNA deletions
Liu et al., 1998	Human	0-90 years	skeletal muscle	↑ mtDNA deletions
Kovalenko et al., 1997	Human	90 years	skeletal muscle	↑ mtDNA deletions, ↓ full-length mtDNA, ↑ oversized mtDNA
Eimon et al., 1996	Mouse	various ages	skeletal muscle	Proportionately more mtDNA deletions in major arc of mtDNA
Melov et al., 1995	Human	various ages	skeletal muscle	↑ mtDNA deletions
Schwarze et al., 1995	Monkey	various ages	skeletal muscle	↑ mtDNA deletions
Simonetti et al., 1992	Human	0-84 years	skeletal muscle	↑ mtDNA deletions
Katayama et al., 1991	Human	60-90 years	skeletal muscle	↑ mtDNA deletions in the minor arc
Cortopassi et al., 1990	Human	44-104 years	heart, brain	↑ mtDNA deletions
Linnane et al., 1990	Human	0-87 years	skeletal muscle	↑ "common" deletion
Single Fibers				
Kopsidas et al., 2002	Human	various ages	Skeletal muscle	ø full length mtDNA and ↑ mtDNA deletions in ETC deficient fibers
Wanagat et al., 2001	Rat	5, 18, 38 mths	skeletal muscle	↑ mtDNA deletions in ETC deficient fibers
Kovalennko et al., 1998	Human	various ages	skeletal muscle	↑ "common" deletion
Brierley et al., 1998	Human	62-85 years	skeletal muscle	↑ mtDNA deletions in ETC deficient fibers
Kopsidas et al., 1998	Human	5, 90 years	skeletal muscle	ø full length mtDNA and ↑ mtDNA deletions in ETC deficient fibers

Table 3. Summary of studies examining the accumulation of mtDNA deletions with aging

Table 4. ETC Enzymes and Aging

Author	Species	Age	Tissue	Results
Hepple et al., 2003	Rat	8, 28-30mths	skeletal muscle	↓ VO2max, ↓ complex I+III activity
Rasmussen et al., 2003	Human	20+, 70+ years	skeletal muscle	↔ Respiratory Chain
Chretien et al., 1998	Human	0-65 years	skeletal muscle	↔ Respiratory Chain
Barrientos et al., 1996	human	15-95 years	skeletal muscle	↔ Respiratory Chain
Boffoli et al., 1994	human	17-91 years	skeletal muscle	↓ complex I, II, IV
Trounce et al., 1989	human	16-92 years	skeletal muscle	↓ Respiratory Chain
Cooper et al., 1992	Human	20-30, 60-90 years	skeletal muscle	↓ complex I, IV
Hseih et al., 1994	Human	various ages	skeletal muscle	↓ complex IV
Pastoris et al., 1995	Rats	4, 12, 24, mths	skeletal muscle	↔ complex IV
Desai et al., 1996	Mice	10, 20 mths	skeletal muscle	↓ complex I, III, IV
Kwong and Sohal, 2000	Mice	3.5, 12-14, 28-30 mths	skeletal muscle	↓ complex II, III, IV

Table 4. Summary of studies examining ETC enzyme activity with aging

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7.0 MANUSCRIPT 1

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Oxidative Stress and the Mitochondrial Theory of Aging in Human Skeletal Muscle

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Acknowledgements: This work was supported by the Natural Science and Engineering Research Council.

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The Mitochondrial Theory in Aging Skeletal Muscle

Abstract- The mitochondrial theory of aging proposes that an age-related increase in oxidative stress is responsible for cellular damage and ultimately cell death. Despite compelling evidence that supports the mitochondrial theory of aging in some tissues, data regarding aging skeletal muscle are inconsistent. We collected resting muscle biopsies from the *vastus lateralis* of young (N=12, ~22 years), and old (N=12, ~72 years) men. Urinary 8-OHdG was significantly higher in older men as compared to young (Old: 7714 ± 1402, Young: 5333 ± 1191 $ng \cdot g^{-1}$ creatinine: *p=0.005*), as were levels of protein carbonyls (Old: 0.72 ± 0.42, Young: 0.26 ± 0.14 $nmol \cdot mg^{-1}$ protein: *p=0.007*). MnSOD activity (Old: 7.1 ± 0.8, Young: 5.2 ± 1.8 $U \cdot mg^{-1}$protein:*p=0.04*) and catalase activity (Old: 8.5 ± 2.0, Young: 6.2 ± 2.4 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein: *p=0.02*) were significantly higher in old as compared to young men respectively, with no differences observed for total or CuZnSOD. Full-length mtDNA was 37% lower (*p=0.02*) in old as compared to young men, and mtDNA deletions were present in 6/8 old and 0/6 young men. The maximal activities of Citrate synthase, and complex II+III, and IV was not different between young and old men, however, complex I+III activity was higher in older as compared to younger men (Old: 2.5 ± 0.5, Young: 1.9 ± 0.5 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww: *p=0.03*) respectively. In conclusion, healthy aging is associated with oxidative damage to proteins and DNA, a compensatory up-regulation of antioxidant enzymes, and a decrease in full-length mtDNA.

Keywords- Aging, oxidative damage, 8-OHdG, protein carbonyls, mitochondrial DNA damage, antioxidant enzymes, electron transport chain enzymes

INTRODUCTION

The mitochondrial theory of aging represents one of the leading theories on skeletal muscle aging [17]. This theory postulates that the aging process is mediated by a vicious cycle of events ultimately leading to cellular senescence. Central to the theory is an increase in oxidative stress, mediated by an increased production of reactive oxygen species (ROS), and/or a reduced antioxidant capacity. The electron transport chain (ETC) is thought to be the main producer of ROS in skeletal muscle, and it has been demonstrated that ROS, produced by the mitochondria, are maintained at a relatively high level inside the mitochondrial matrix [26]. Given the proximity of mitochondrial DNA (mtDNA) and several functional mitochondrial proteins to the primary ROS generator, it is a possibility that these molecules are at a greater risk of incurring oxidative insults, potentially leading to mitochondrial dysfunction. Congruent with this hypothesis, markers of oxidative damage to DNA are much higher in the mitochondria as compared to the cytosol [16]. Accordingly, age-related oxidative stress is thought to lead to mitochondrial DNA (mtDNA) damage, which results in defective electron transport chain (ETC) proteins, defective ETC activity, and enhanced production of ROS. Ultimately, these events may lead to cellular aging and cell death. Although this theory describes an elegant relationship between oxidants and aging, many of the studies designed to examine the mitochondrial theory have yielded equivocal or conflicting results.

The literature on human skeletal muscle strongly supports an age-associated increase in oxidative damage to proteins, lipid, and DNA [14;34;40;41]. Higher levels of

lipid peroxidation, protein carbonyl content and 8-OHdG (oxidative DNA damage) have been demonstrated in human skeletal muscle of older individuals when compared to younger individuals [14;34;41].

It has been suggested that age-related oxidative stress may be a function of a reduction in antioxidant capacity [20], but this has not been demonstrated in human skeletal muscle. In fact, all investigations of aging human skeletal muscle have reported significantly higher levels of at least one antioxidant enzyme [40;41]. Of the few reports in aging skeletal muscle, an increase [41] or no change [40] in catalase activity has been observed, along with increases in MnSOD, the mitochondrial isoform of superoxide dismutase [40;41]. Interestingly, CuZnSOD, the cytosolic isoform of superoxide dismutase, decreases with age [40;41], suggesting that there may be a compartmentalization of the origin of oxidant stress in aging. Together, these results suggest that antioxidant capacity is not implicated as a mechanism in age-related oxidative stress, but rather is responsive to age-related oxidative stress.

In support of the mitochondrial theory of aging, a progressive relationship has been established between age and the accumulation of mtDNA deletions and mutations in human skeletal muscle [10;11;13;15;21;22;24;25;29;31;35;44;45;48;49;51]. Investigations in skeletal muscle homogenates have reported an accumulation of mtDNA point mutations [38;39;50] as well as deletion products [11;25;29] as a function of age, albeit to low levels of total mtDNA. However, isolated single muscle fibers presenting with ETC dysfunction accumulate mtDNA point mutations [15;28] and deletion products [6;48] to significant proportion of total mtDNA. Together, this data suggests that age-

associated mtDNA anomalies have physiological significance, but may be undetectable in whole muscle homogenates. Conversely, it remains unclear whether or not anomalies in single fibers can account for the global effects observed in aging skeletal muscle.

Despite a strong relationship between aging and oxidative damage, and the recent correlation between ETC dysfunction and mtDNA deletion and mutations in single muscle fibers, the literature on the effect of aging on ETC function remains equivocal in humans. Many studies demonstrate a significant age-related reduction in ETC complex enzymes [4;7;18;47], while others demonstrate no age-associated decrease in human skeletal muscle [3;9;43;52]. It has been suggested that the reported age-related reductions in ETC function are not related to the aging process *per se*, but rather due to other confounding variables such as tobacco consumption and physical inactivity [3].

The purpose of the present study was to conduct a comprehensive examination of the mitochondrial theory of aging in human skeletal muscle, with particular attention to oxidative damage, antioxidant enzyme activity, ETC function, and mtDNA.

MATERIALS AND METHODS

Subject Characteristics

Healthy older men (N=12, 72 ± 2 years), and healthy young men (N=12, 22 ± 3 years) were recruited for the present investigation (Table 1). Subjects were recruited using a strict exclusion criteria including: coronary heart disease, congestive heart failure, uncontrolled hypertension, chronic obstructive pulmonary disease, diabetes mellitus,

renal failure, major orthopedic disability, and smoking. Subjects were recreationally active, and had no known pathology or family history of mitochondrial disease. All subjects gave informed consent and the study was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board.

Tissue and Urine Collection

A muscle biopsy was taken from the *vastus lateralis* muscle of the dominant leg, 20 cm proximal to the knee joint using a modified Bergström needle (5 mm diameter) with suction modification. The biopsy specimen was dissected of fat and connective tissue and immediately frozen in liquid nitrogen. All samples were stored at -80 °C for subsequent analysis.

Spot urine samples were collected at the time of the muscle biopsy. Urine was aliquoted into 2 ml eppendorf tubes and stored at -80 °C for subsequent analysis of 8-OHdG and creatinine.

All subjects were required to abstain from strenuous physical activity for 72 hours prior to the testing session.

Markers of Oxidative Damage

Urinary 8-OHdG was determined using an enzyme-linked immunoassay. The assay was carried out in triplicate utilizing the manufacturers instructions (Genox Corp., Baltimore, MD, USA). Urinary creatinine levels were determined using a standard colorimetric UV spectrophotometric assay (Sigma Chemical Co., St. Louis, MO, USA).

Intra-assay CV for 8-OHdG was 6.4%, and 8.2% for creatinine, determined by comparing the variance between five replicates. 8-OHdG was expressed relative to creatinine to account for between subject differences in muscle mass.

Protein carbonyls were determined by measuring the reactivity of carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) as previously described [27]. Briefly, the tissue was homogenized in ice cold Hepes buffer (pH 7.2 – 7.4), containing leupeptin (0.5 $\mu g \cdot ml^{-1}$), pepstatin (0.7 $\mu g \cdot ml^{-1}$), aprotinin (0.5 $\mu g \cdot ml^{-1}$), and phenylmethylsulfonyl fluoride (40$\mu g \cdot ml^{-1}$). The soluble protein fraction was separated with centrifugation and then treated with strepatmycin sulfate to remove nucleic acids. The protein sample was pipetted into two glass borosilicate tubes and precipitated with trichloroacetic acid (TCA). DNPH (10 mM in 2 M HCl) was added to one tube, and HCl (2 M) to the other. The tubes were incubated at room temperature for 1 hour, with vortexing every 10 min. TCA (10% final concentration) was added to both tubes and pelletes were recovered via centrifugation. Pellets were washed 3 x with ethanol-ethyl acetate to remove free DNPH. The precipitated protein was redissolved in 6 M guanidine solution, and the absorbance of both solutions was measured at 370 nm . All samples were analyzed in duplicate on an HP spectrophotometer (Delaware City, DE. UV Visible Chemstation 8453).

Electron Transport Chain Enzymes and Citrate Synthase

ETC Enzymes and Citrate Synthase. Tissue preparation and analysis were performed as previously described [8]. Briefly, approximately 30 mg of wet muscle

tissue was homogenized using a glass homogenizer. Assays were preformed on fresh homogenates.

Citrate synthase (CS) activity was determined by adding 10 µl of muscle homogenate to a solution of 1 ml of Tris buffer plus 2 µl of acetyl CoA (30 mM in H_2O), and 10 µl of oxaloacetate in a cuvette warmed to 37°C. Absorbance was recorded at 412 nm every 30 s for 3 min. CS activity was calculated and reported as $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Three replicates for each sample were compared and intra-assay CV was 3.1%.

Complex I+III activity was measured by the reduction of cytochrome c in the presence of NADH, thus measuring the flux of electrons from Complex I through complex III to cytochrome c. This was achieved by adding 20 µl of muscle homogenate to 940 µl of potassium phosphate (KPi) , 30 µl of oxidized cytochrome c, and 10 µl of NADH in a cuvette warmed to 37°C. Absorbance was measured at 550 nm every 30 s for 3 min. In a separate cuvette, the same sample was analyzed under identical conditions, with the addition of rotenone (0.1 mM). The difference between the two cuvettes represents complex I activity. Enzyme activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 6.6%.

Complex II+III activity was measured by the reduction of cytochrome c in the presence of succinate, thus measuring the flux of electrons from complex II through complex III to cytochrome c. 15 µl of muscle homogenate was added to 930 µl of KPi buffer 30 µl of oxidized cytochrome c, and 25 µl of succinate (0.4 M) in a cuvette warmed to 37°C. Absorbance at 550 nm was recorded every 30 s for 3 min. Enzyme activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 5%.

Complex IV was determined by measuring the oxidation of cytochrome c, by the flow of electrons through cytochrome oxidase. Stock cytochrome c (oxidized), was reduced by sodium ascorbate and KPi (10 mM). 15 µl of muscle homogenate was added to 950 µl of KPi, and 30 µl of reduced cytochrome c to a cuvette warmed to 37°C. Absorbance was recorded at 550 nm every 30 s for 3 min. Complex IV activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 4.5%.
All samples were analyzed in duplicate on a Hewlett Packard spectrophotometer (Delaware City, DE. UV Visible Chemstation 8453).

Western Blots

Muscle samples were homogenized and prepared for electrophoresis using methods previously described [46]. The muscle homogenates from young and old men were probed using mouse monoclonal antibodies specific to human subunit II of cytochrome oxidase (Molecular Probes –A6404). This antibody recognizes a polypeptide with a molecular weight of ~ 25.6 kDa. Post-transfer blots were stained with coomassie blue to ensure equal protein loading between lanes. No difference in protein load were observed between lanes.

Antioxidant Enzymes

Muscle catalase activity was determined by measuring the kinetic decomposition of H_2O_2. Muscle catalase activity was measured by combining 970 ul of K_2HPO_4 buffer (50 mM with 50mM EDTA, and 0.01% Triton X-100, pH 7.2-7.4), with 30 ul of muscle

homogenate. 10 ul of H_2O_2 (1 M) was added to the cuvette and mixed by inversion to initiate the reaction. Absorbance was measured at 240 nm every 15 s for 2 min. Catalase activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$. Intra-assay CV was 8.2%.

Muscle total SOD activity was determined by measuring the kinetic consumption of O_2^- by superoxide dismutase in a competitive reaction with cytochrome c. Muscle total SOD activity was measured by combining 955 ul of K_2HPO_4 buffer (50 mM with 0.1 mM EDTA, pH 7.8) containing cytochrome c (25 mg/100 ml) and Xanthine (5 μmol). 30 ul of muscle homogenate was added to the cuvette and mixed by inversion. 15 ul of xanthine oxidase (0.2 U/ml) was added to initiate the reaction, and absorption at 550 nm was observed every 15 s for 2 min. One unit of SOD activity was defined as the amount of enzyme which caused a 50% inhibition of the reduction of cytochrome c. Activity was calculated and expressed in $U \cdot mg\ protein^{-1}$.

In a separate cuvette, the same sample was analyzed under identical conditions with the addition of 10 ul of 0.2 M KCN (pH 8.5-9.5) for determination of Mn SOD activity. KCN is known to inhibit CuZn SOD activity as described by Higuchi and colleagues (1985). CuZn SOD was approximated by subtracting Mn SOD activity from total SOD activity, and was expressed in $U \cdot mg\ protein^{-1}$.

All samples were analyzed in duplicate in a Hewlett Packard spectrophotometer (Delaware City, DE. UV Visible Chemstation 8453).

Mitochondrial DNA Deletions

Mitochondrial DNA deletions were determined using XL-PCR as previously described [25]. Briefly, DNA was extracted from approximately 25 mg of wet muscle using a PUREGENE® DNA isolation kit, as per the instruction of the manufacturer (Gentra Systems, Minneapolis, MN). Two PCR primer sets were designed approximately 2000 bp apart, and all samples were run using both sets of primers. One set of primers were designed to begin amplification in the 16s rRNA region (3310L (30nt) – CCC ATG GCC AAC CTC CTA CTC CTC ATT GTA, 2690H (28nt) – TCT TGC TGT GTT ATG CCC GCC TCT TCA C) and a second pair was designed to begin amplification in the cytochrome b region (15148L (27nt) – GTG AGG CCA AAT ATC ATT CTG AGG GGC, 14841H (26nt) – TCT TGC TGT GTT ATG CCC GCC TCT TCA C). Methodological experiments determined that the optimal template concentrations for the 16s primers was 125 ng of total DNA, whereas the optimal template concentration for the cytb primers was 100 ng of total DNA. Amplification was achieved using the Expand Long Template PCR System (1 681 842, Roche Biochemicals, Mannheim, Germany), utilizing a final concentration of 500 μM of each dNTP, 300 nM of each primer, 2.25 mM of $MgCl_2$ in 10X Buffer 3, and 3.3 units of the Long Template Enzyme mixture. Mineral oil (30ul) was used to prevent evaporation. Optimal conditions for the reactions consisted of 2min hot-start at 92°, 10 cycles of 92° for 10 s to denature, 71° for 30 s for primer annealing, and 68° for 12 m and 30s for elongation. An additional 16 cycles were performed at these same temperatures however for each subsequent cycle after the initial 10 cycles the elongation time was increased by 20 s. At the end of a total of 26 cycles

there was a final 7 m elongation step at 68°. 10 ul of the final product was mixed was run on an agarose gel containing ethidium bromide for 1 hour at 100V. Gels were visualized on a UV light gel documentation system, and analyzed using a commercially available software package.

Statistical Analysis

Statistical analysis was performed using the Statistica software package (Version 5.0, Tulsa, OK). All variables were statistically analyzed using an independent t-test. Alpha was established at $p<0.05$, and all values were reported as mean ± standard deviation (SD).

RESULTS

Subject Characteristics

Older men were significantly heavier ($p=0.03$), had a greater fat-free mass ($p=0.0006$), and had a lower % fat mass ($p=0.017$). These results are evidence that we recruited healthy older individuals. Furthermore, these results suggest that any age-related changes we observed were due to a true effect of the aging process *per se* as opposed to other confounding variables such as inactivity.

Oxidative Damage

Oxidative DNA damage in the form of urinary 8-OHdG was found to be 44% higher in old (N=11, 7714 ± 1402 ng·g creatinine^{-1}) versus young men (N=11, 5333 ± 1191 ng·g creatinine^{-1}) (*p=0.0003*) (figure 1A). In addition oxidative damage in the form of protein carbonyls was 170% higher in old (N=9, 0.72 ± 0.42 nmol·mg protein^{-1}) versus young men (N=9, 0.26 ± 0.14 nmol·mg protein^{-1}) men (*p=0.007*) (figure 1B).

Antioxidant Enzymes

Muscle catalase activity was 37% higher in the old (N=12, 8.5 ± 2.0 μmol·min^{-1}·mg protein^{-1}) compared to young (N=12, 6.2 ± 2.4 μmol·min^{-1}·mg protein^{-1}) men (p=0.02) (figure 2A). Mn SOD was also found to be 37% higher in old (N=6, 7.1 ± 0.8 U·mg protein^{-1}) versus young men (N=6, 5.2 ± 1.8 U·mg protein^{-1}) (p=0.04) (figure 2B). Conversely, CuZn SOD was not different between groups (old:N=6, 11.2 ± 2.8, young:N=6, 10.8 ± 6.1 U·mg protein^{-1}) (figure 2C).

Electron Transport Chain Enzymes

Complex I+III activity was significantly higher in old (N=9, 2.5 ± 0.5 μmol·min^{-1}·mg^{-1} ww) as compared to young (N=9, 1.9 ± 0.5 μmol·min^{-1}·mg^{-1} ww)(*p=0.03*) men respectively. There were no other significant differences for any of the other oxidative enzymes (Table 3).

Mitochondrial DNA Deletions

There were significantly more mtDNA deletion products in the old vs. the young. Six of the eight older individuals examined presented with mtDNA deletions, whereas none of the eight young individuals had any visible deletion products. Older adults also demonstrated 37% less normal length mtDNA than young adults ($p=0.02$) (figure 3). Results were similar using both primer pairs, however figure 3 depicts only results using the 16S rRNA primers.

Western Blots

Western blots for a mitochondrial encoded subunit of COX revealed no age related differences in protein content (figure 4).

DISCUSSION

Although many investigations have examined components of the mitochondrial theory in isolation, the present study represents the most comprehensive examination of the mitochondrial theory in human skeletal muscle. Our data suggest that there is an age-related increase in oxidative damage, associated with an increase in mitochondrial DNA deletions and a reduction in full-length mtDNA. Furthermore, aging was associated with a significant increase in mitochondrial SOD, catalase but not cytosolic SOD. Interestingly, electron transport chain maximal enzyme activity was not reduced by age

suggesting that low level mitochondrial DNA deletions are not associated with electron transport chain defects at the whole muscle level.

Oxidative damage to the cellular environment is a major tenet of the mitochondrial theory of aging. It is thought that accumulation of damaged products ultimately leads to cellular dysfunction, and eventual cell death via a vicious cycle of events. In the present study we demonstrated a 44% higher level of oxidative DNA damage, measured as urinary 8-OHdG, and more impressive 170% higher level of total protein carbonyls in skeletal muscle, in older versus younger men. These results are in accordance with others who have demonstrated significant age-associated increases in oxidative damage to human muscle DNA, proteins and lipids [14;34;36;40;41]. Pansarasa and colleagues (2000), also reported significantly higher levels of lipid peroxidation, and protein carbonyl content in human skeletal muscle of older (66-91 years) and middle aged (41-65 years) individuals as compared to younger individual (17-40 years). Similarly, Mecocci and colleagues (1999) also found a significant age-associated increase in 8-OHdG, malondialdehyde, and protein carbonyls in human skeletal muscle from individuals aged 25-93 years. Although we know that the observed increase in urinary 8-OHdGH is a marker of DNA damage, we cannot distinguish whether the damage is incurred in mtDNA or nuclear DNA. However, it has been demonstrated that higher levels of 8-OHdG in old liver, heart and brain of rats and mice is found to be 6-23 fold higher in mitochondrial DNA as compared to nuclear DNA (Hamilton et al., 2001). Thus, the literature suggests that a greater relative proportion of our measured 8-OHdG is attributable to mitochondrial DNA damage.

Miro and colleagues (2000) demonstrated that despite significant age-associated increases in lipid peroxidation of human heart, there was no measured correlation with oxidative damage and ETC enzyme activity. The authors concluded that ETC dysfunction cannot be considered the main cause of increased oxidative damage associated with aging. In this investigation we also report age-associated increases in oxidative damage in the face of normal ETC function. Recent work has suggested that protein in skeletal muscle is more sensitive to oxidants in old as compared to young rats [42]. This suggests that oxidative damage may not be dependent on ETC dysfunction, and that old proteins may simply be more susceptible to damage.

Age-related oxidative stress can be a result of either an age-associated increase in the production of ROS or a reduction in the antioxidant capacity. Some studies have suggested that a reduction in antioxidant capacity contributes to age-related oxidative stress [19], however, recent studies have demonstrated that antioxidant capacity adaptively increases with age in human skeletal muscle [40;41]. Consistent with these findings, we found a 37% higher level of catalase activity in older men. In a recent study, Pansarasa and colleagues (2000) reported an age-related increase in catalase activity in men and women aged 66-91 years as compared to young men and women. However, in a previous investigation the same group reported no age-related change in catalase activity in human skeletal muscle between the ages of 17 and 91 years [40]. Despite the variable results, neither of these studies suggest an age-related decrease in catalase activity, which might contribute to age-related oxidative stress. In the present study we also demonstrated an age-associated higher level of MnSOD activity (37%) in older versus

younger men, whereas we observed no difference in CuZnSOD between age groups. MnSOD (SOD2) has been found to be localized to the inner membrane of the mitochondria, while CuZnSOD (SOD1) is found primarily in the cytosol. Compartmentalization of these isoforms suggests that they may be differentially affected, depending on the source of the oxidant stress. Pansarasa and colleagues (2000, 1999) demonstrated significantly higher levels of MnSOD in old men and women (66-91 years), as compared to young men and women (17-40 years) in human skeletal muscle. Furthermore, they reported that total SOD activity was lower in the old than in the young, suggesting that CuZn SOD decreased with age since total SOD is comprised primarily of cytosolic CuZn SOD and mitochondrial Mn SOD. Together, these results suggest that aging is associated with a superoxide associated mitochondrial stress, with no apparent cytosolic stress. Given the short half-life of superoxide, and its inability to freely cross bio-membranes [32], any stress from the mitochondrial ETC will be realized only in the mitochondria. Conversely, H_2O_2 has a relatively long half-life and possesses the ability to freely cross bio-membranes [33]. Thus, the cytosol may be associated with an age-related oxidative stress, potentially derived from the mitochondria, however not likely from superoxide. This idea is supported by our observation of an age-associated higher activity of catalase but not CuZnSOD. Together, results in the present study and those reported in the literature demonstrate a rather robust increase in MnSOD activity, no change or a decrease in CuZnSOD activity, and an increase in catalase activity. Interestingly, the consistent age-associated change is an up-regulation of MnSOD activity. In addition, this finding suggests that antioxidant enzymes respond to age-

related oxidative stress by up-regulation, and are not an underlying mechanism of age-related oxidative stress.

The vicious cycle proposed by the mitochondrial theory of aging hypothesizes that an age-related increase in the production of ROS will ultimately lead to damage of mtDNA in the form of point mutations and deletions. These mutations and deletions lead to ETC dysfunction and enhanced production of ROS. In the present study we demonstrate that older men had 37% less full-length mtDNA, and that aging was associated with a significantly greater number of mtDNA deletion products. It has previously been shown that aging is associated with a reduction in full-length mtDNA [25]. Moreover, evidence demonstrates an age-related accumulation of mtDNA deletion products [10;11;13;15;21;22;24;25;29;30;35;44;45;48;49;51]. Kovalenko and colleagues (1997) demonstrated a progressive decrease of deltoid muscle full-length mtDNA across a 5, 37, 69, 75, and 84 year old. It appeared that by 84 years only a small amount of full-length mtDNA was present. In addition to the decline in full-length mtDNA, there was an increase in the total amount and copy number of multiple mtDNA rearrangements. In summary, we demonstrate an age-related change in mtDNA, characterized by a reduction in full-length mtDNA, and an accumulation of mtDNA deletion products. The mechanism(s) responsible for such changes in the mitochondrial genome remain unclear. It is speculated that ROS are central to this process, however a causal link remains elusive. We recently demonstrated that exercise, associated with an increase in 8-OHdG, and total protein carbonyls, resulted in the accumulation of mtDNA deletion products in

young individuals, suggesting that oxidative stress may in fact be responsible for the induction of anomalies in mtDNA (Parise et al., - unpublished observations).

ETC function in human skeletal muscle with aging remains a controversial field of research. To date, results in human skeletal muscle remain equivocal, with studies demonstrating a significant age-related reduction in ETC complex enzymes [4;47], whereas others demonstrate no age-associated decrease [3;9;43;52]. Isolated mitochondria from human *vastus lateralis* demonstrated significant reductions in state III respiration in older as compared to younger adults [4;47]. In addition, individual complex enzymes in human skeletal muscle were shown to decrease in an age dependent manner, with complex IV activity most adversely affected by age [18]. In contrast, Chretien and colleagues (1998), reported that there was no apparent relationship between aging and complex I, II, III, or IV activity in mitochondria isolated from human deltoid muscle. In addition, individual respiratory chain enzyme activities in human muscle were shown to be lower with age, however after including tobacco consumption and physical activity as confounding variables there was no apparent age-related decrease, suggesting that ETC function does not change as a function of "normal" aging [3]. A recent report by Rassmussen and colleagues (2003) demonstrated that there was no age-associated reduction in ETC enzyme activity, leading the authors to conclude that the mitochondrial theory of aging is not supported in human skeletal muscle. Indeed, we also report no decrease in ETC activity between young and old muscle using biochemical measurements of tissue homogenates. In support of this finding we observed no differences in protein expression for a mitochondrial-encoded sub-unit of cytochrome oxidase, despite a

significant decrease in amplifiable full-length mtDNA. This may be due to an increase in mitochondrial mRNA stability with age [2], and/or to the strict exclusion criteria we utilized in recruiting our subjects, accounting a priori for the variables that Barrientos and colleagues (1997) have identified as confounding for ETC function. Interestingly, we measured a 27% higher complex I+III activity in old muscle tissue as compared to young. The assays we used in these experiments followed NADH through the reduction of cytochrome c for complex I+III, and succinate through the reduction of cytochrome c for complex II+III. Given there was no difference between young and old in complex II+III activity, and complex III activity was constant in both of these assays, it is fair to conclude that the significantly higher activity of complex I+III can be attributed to complex I activity alone. This is the first time such an observation has been reported. It is possible that there are protein specific responses in the cell attempting to adapt to age-related changes to allow normal function. In the current study we used whole muscle homogenates in the analysis of ETC function, and thus may have missed subtle age-related changes in single fibers, as recently described [5;6;23;48]. Alternatively, our healthy older population may be demonstrating a true maintenance, or enhanced function in the ETC.

Taken together, unlike Rasmussen and colleagues (2003) we suggest that the mitochondrial theory of aging may be apparent in human skeletal muscle. Based on results in the current study, and those in the literature we propose a model of aging in which there is a degree of redundancy and adaptation throughout the aging process that allows the cell to function normally in the face of accumulating abnormalities. As others

have reported [14;34;36;40;41], we also report accumulating cellular abnormalities in the form of oxidatively modified DNA and proteins. In addition, as others have suggested [12;13;21;25;29;35;44;45], we also report decreases in full-length mtDNA, and an accumulation of deletions. A 37% decrease in full-length mtDNA, with a significant number of visible deletion products, without a concomitant loss of ETC function suggests that there may be a level of redundancy to protect the cell against mitochondrial energy depletion. Congruent with this finding is data from experiments using cybrids that suggests that ETC function is maintained until approximately 90% of mtDNA is deleted [1;37]. As cellular abnormalities accumulate, it is entirely possible that cellular adaptations occur to counteract these events. In the present investigation we report increases in antioxidant capacity, in particular mitochondrial SOD and catalase, in old as compared to young muscle. A somewhat unexpected finding was a specific up-regulation of complex I activity. Although this has never before been described in skeletal muscle, this apparent adaptation may represent an attempt to maintain ETC function. Perhaps in frail adulthood, the combination of accumulating abnormalities and a reduction in adaptive potential cross a threshold by which cellular function cannot be maintained in the "normal" range. Furthermore, we hypothesize that crossing this threshold is associated with frailty and may be the cause of sharp declines in muscle function in advanced age (Fig. 7).

An unresolved issue is the role of low-level mtDNA deletions in skeletal muscle. Traditionally, mtDNA deletions have been reported to accumulate to low levels in whole muscle homogenates [12;13;21;25;29;35;44;45]. Recent evidence, however, has

demonstrated that mtDNA deletions and mutations accumulate to high levels in single fibers, and are associated with ETC dysfunction, as well as muscle fiber atrophy [6;48]. In addition, Brierley and colleagues (1998) have demonstrated that these deletions preferentially amplify in a stochastic manner. It is possible that low-level deletions in whole muscle homogenates simply reflect a relatively small number of fibers with high levels of deletions products. Through middle-age and into healthy old-age whole muscle function may not be compromised, however, crossing our proposed critical threshold, where redundancy and adaptation can no longer cope, may result in accelerated accumulation of mtDNA deletion products, resulting in cell death, and leading to pronounced muscle atrophy.

In summary, our results lend support to the mitochondrial theory of aging in skeletal muscle. Increases in oxidative damage, mtDNA rearrangements, and antioxidant enzyme capacity all implicate oxidative stress as an important factor in the aging process of human skeletal muscle. Lack of a decrease in ETC function associated with age, measured in whole muscle homogenates, continues to cast doubt on the importance of low-level mtDNA deletions to whole muscle function. This phenomenon, however, may be indicative of cellular redundancy and adaptation and does not rule out the mitochondrial theory of aging as a potential mechanism underlying skeletal muscle aging.

Figure Legend

Figure 1. A. The effect of aging on levels of urinary 8-OHdG. B. Demonstrates the effect of aging on levels of muscle protein carbonyls. * denotes a significant difference between young and old. Alpha was set at $p<0.05$.

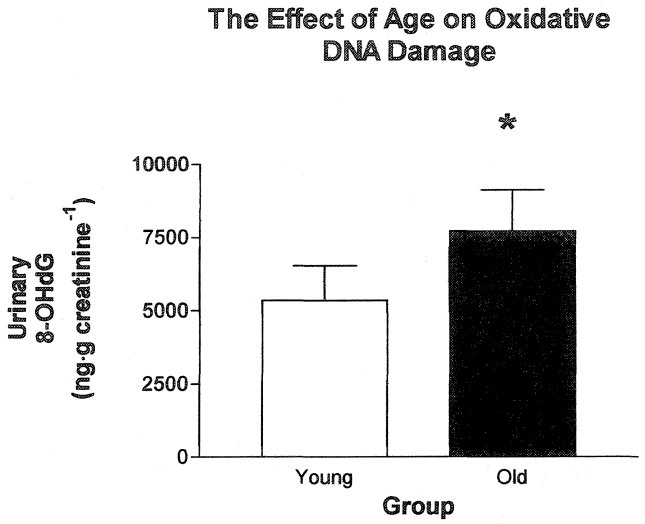
Figure 2. A. The effect of age on muscle catalase activity. B. Demonstrates the effect of aging on muscle MnSOD activity. C. Demonstrates the effect of aging on muscle CuZnSOD activity. * denotes a significant difference between young and old. Alpha was set at $p<0.05$.

Figure 3. The effect of age on mtDNA. Note a significant reduction (37%) in normal length mtDNA ($p<0.05$). We also observed that 6/8 old subjects demonstrated an accumulation of deletion products, whereas none of the young subjects demonstrated any deletion products. This figure is representative of results using the 16S rRNA.

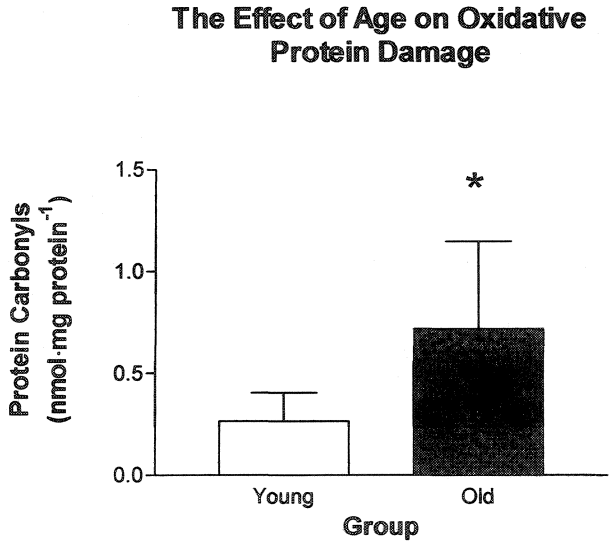
Figure 4. Protein content of subunit II of COX was not different between young and old ($p>0.05$).

Fig 5. A model of aging. Through redundancy and adaptation normal cell function is maintained in the presence of cellular abnormalities. Once a critical threshold is crossed, with accumulating abnormalities, and reduced adaptability, there is a loss of cellular function. This theory is compatible with observations made in human skeletal muscle.

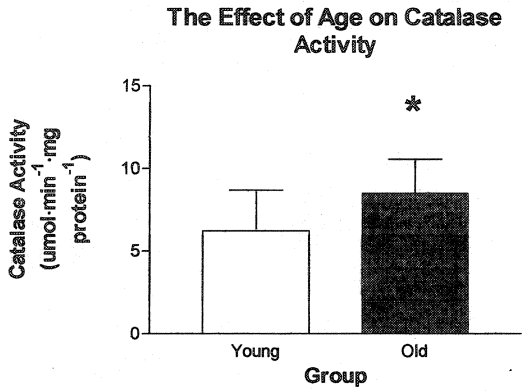
A.


The Effect of Age on Oxidative DNA Damage
Urinary 8-OHdG (ng·g creatinine^{-1})
10000
7500
5000
2500
0
*
Young
Old
Group

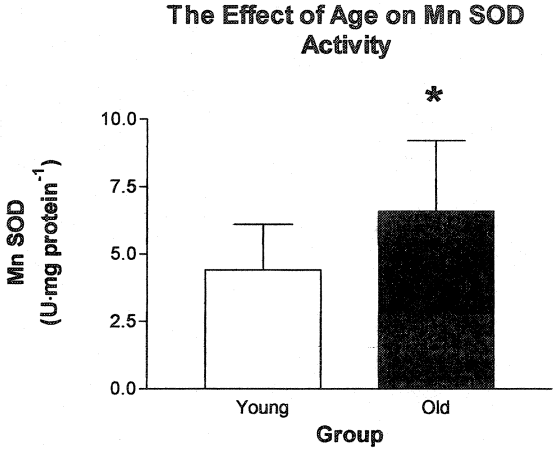
B.


The Effect of Age on Oxidative Protein Damage
Protein Carbonyls (nmol·mg protein^{-1})
1.5
1.0
0.5
0.0
*
Young
Old
Group

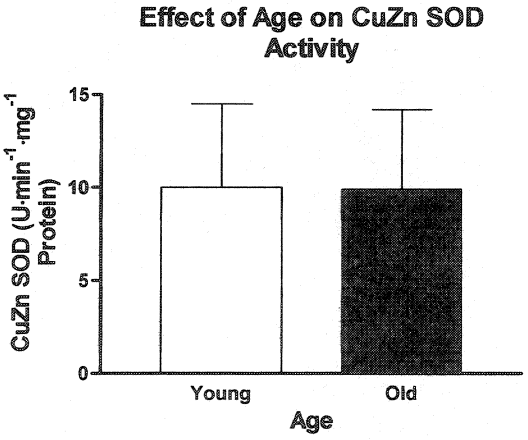
A.

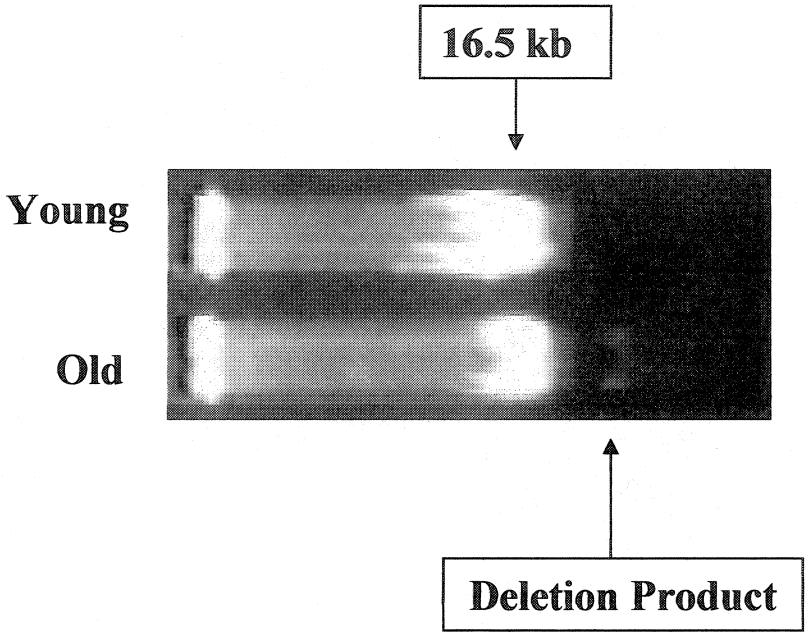

The Effect of Age on Catalase Activity
Catalase Activity (umol·min-1·mg protein-1)
15
10
5
0
*
Young
Old
Group

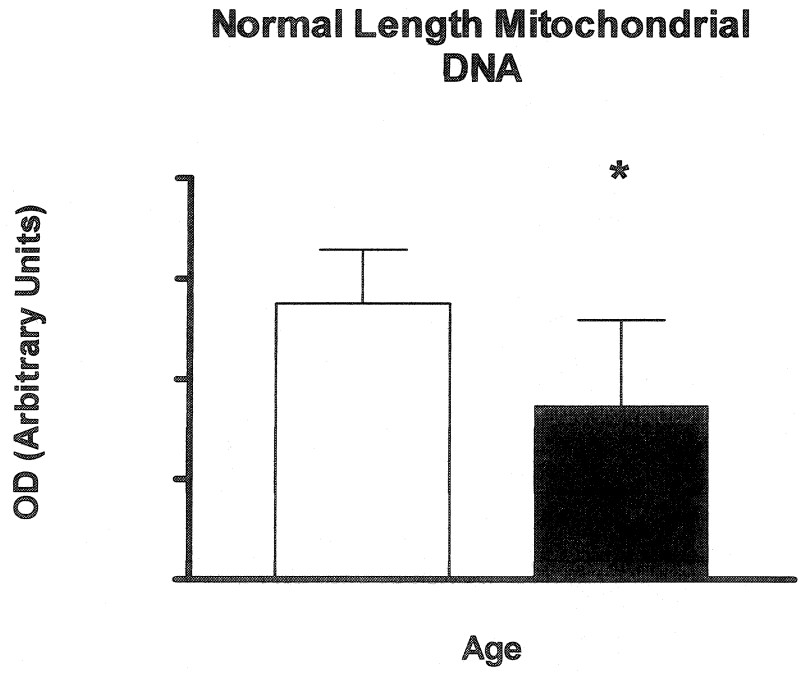
B.


The Effect of Age on Mn SOD Activity
Mn SOD (U·mg protein-1)
10.0
7.5
5.0
2.5
0.0
*
Young
Old
Group

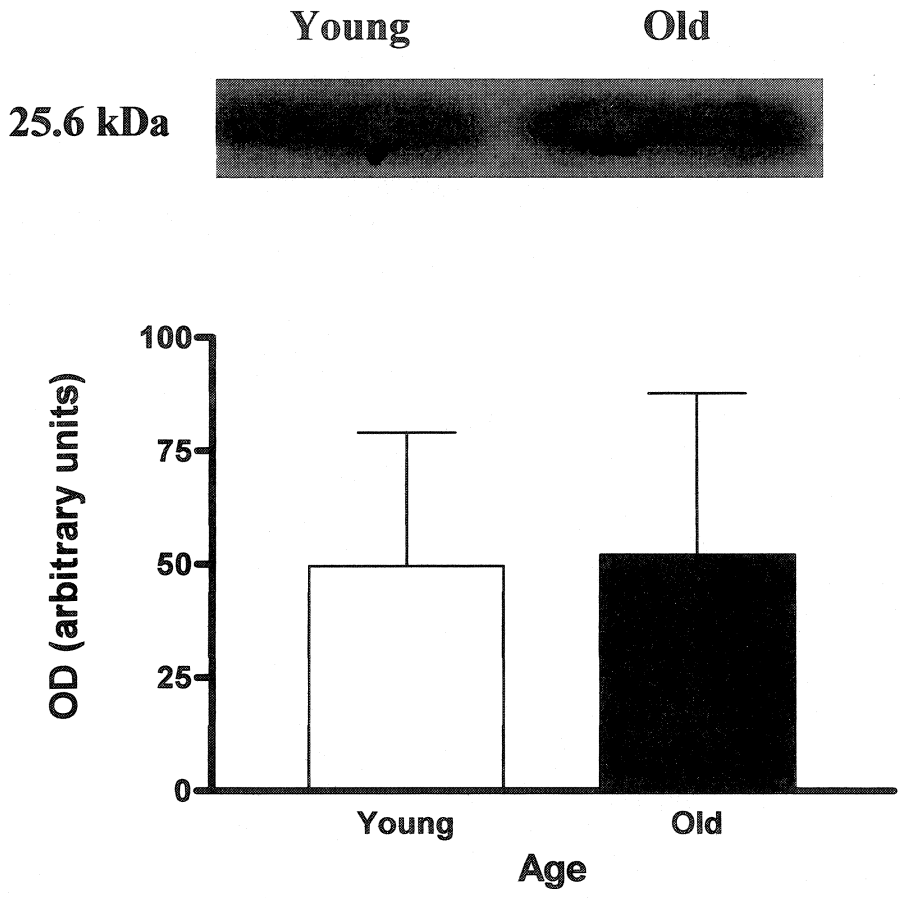
C.


Effect of Age on CuZn SOD Activity
CuZn SOD (U·min-1·mg-1 Protein)
15
10
5
0
Young
Old
Age


16.5 kb
Young
Old
Deletion Product


Normal Length Mitochondrial DNA
*
OD (Arbitrary Units)
Age

The Effect of Aging on Subunit II of Cytochrome Oxidase



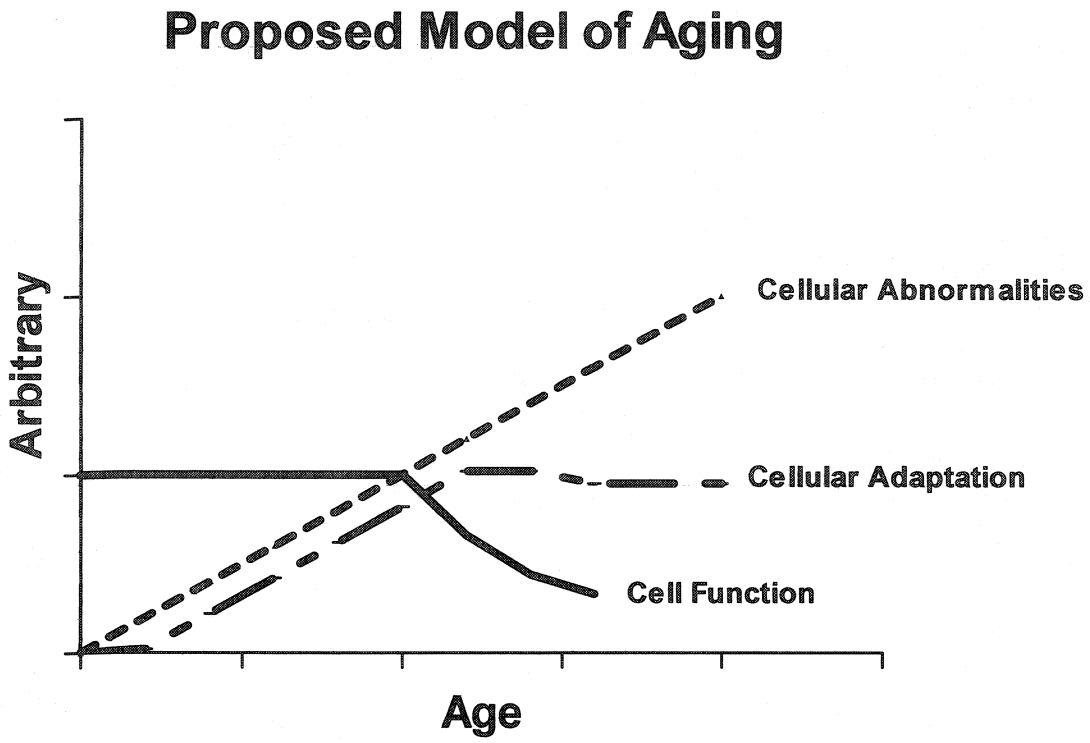

Proposed Model of Aging
Arbitrary
Cellular Abnormalities
Cellular Adaptation
Cell Function
Age

Table 1. Subject Characteristics

Dependent Variable	Young (n = 12)	Old (n = 12)
Age (years)	22.8 ± 3.4	71.8 ± 6.2 *
Height (cm)	175.4 ± 8.2	175.0 ± 5.0
Body Mass (kg)	76.6 ± 13.1	88.1 ± 11.6 *
Body Fat (%)	25.9 ± 4.9	21.3 ± 3.8 *
Fat-free Mass (kg)	56.4 ± 8.1	68.3 ± 5.9 *

Table 1. Subject characteristics. Values are mean ± SD. Older individuals were heavier, had a greater fat-free mass, and had a lower % fat mass. Alpha was set at $p<0.05$.

Table 2. ETC and CS Activity in Young and Old Men

Enzyme	Young (n = 12)	Old (n = 12)
Citrate Synthase	11.5 ± 2.6	13.2 ± 4.5
Complex I+III	1.9 ± 0.5	2.5 ± 0.5*
Complex II+III	1.9 ± 0.6	1.8 ± 0.6
Complex IV	5.7 ± 2.7	6.7 ± 1.0

Table 2. Demonstrates that ETC activity and CS activity was not lower in old vs. young men. Values are mean ± SD, and are expressed as $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$. * denotes a significantly higher complex I+III activity in older adults. Alpha was set at $p<0.05$.

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8.0 MANUSCRIPT 2

PREPARED FOR:

THE JOURNAL OF APPLIED PHYSIOLOGY

Resistance Exercise Training Decreases Oxidative Damage to DNA and Increases Cytochrome Oxidase Activity in Older Adults.

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Running Head: Resistance Exercise and Oxidative Stress in Aging

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Keywords: antioxidants, electron transport chain, mitochondrial theory of aging

Abstract- Resistance exercise is known to increase muscle strength and induce muscle fibre hypertrophy in older adults. Aging and acute exercise are associated with oxidative stress. Oxidative stress and mitochondrial dysfunction are components of the mitochondrial theory of aging. The effect of regular resistance exercise upon mitochondrial function and oxidative stress in older adults is unclear. Twenty-eight older men and women (~ 68.5 ± 5.1 years) performed a whole-body resistance exercise protocol for 14 weeks. Muscle biopsies were taken before and 72 h following the last exercise bout from the *vastus lateralis*. Urine samples were also taken at the time of tissue collection. Resistance exercise training resulted in a decrease in 8-OHdG (Pre: 10783 ± 5856, Post: 8897 ± 4030 $ng \cdot g^{-1}$ creatinine; $P<0.05$). Protein content for CuZnSOD, MnSOD, and catalase, and enzyme activity for citrate synthase, mitochondrial ETC complex I+III, and complex II+III were not significantly different from baseline. However, complex IV activity was significantly higher after training as compared to before training (Pre: 2.2 ± 0.5, Post: 2.9 ± 0.9 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww; $P<0.05$), and the ratio of complex IV to complex I was also higher (Pre: 11.1 ± 9.3, Post: 14.5 ± 10.3; $P<0.05$). There were no changes in normal mtDNA content or visible mtDNA deletion products as a function of training. These data suggest that regular resistance exercise decreases oxidative stress, but does not affect mtDNA. Moreover, increases in complex IV of the electron transport chain may have an indirect antioxidant effect in older adults.

Regular resistance exercise in older adults increases strength (11;21), and induces muscle fiber hypertrophy (7;11;21). More importantly, regular exercise in the elderly improves function (18;19;26;67), and decreases morbidity (20) and mortality (52;65). In essence, progressive resistance exercise is a potent therapy for ameliorating age-related muscle dysfunction.

Despite mounting scientific evidence, the causal factors for cellular aging remain elusive. The mitochondrial theory, as proposed by Harman and colleagues (1972), has been the leading theory on aging for the last 30 years. This theory postulates that aging is a function of mitochondrial mediated oxidative stress (23). At the heart of this theory is an accumulation of mitochondrial DNA (mtDNA) mutations, related to electron transport chain (ETC) dysfunction, and an enhanced production of reactive oxygen species (ROS). Indeed, aging is associated with oxidative stress and accumulating mtDNA deletions and mutations (14-17;33;35-37;44;45;50;68;72;80;81;83). Recent evidence in rats (64), and unpublished observations from our laboratory suggest that acute exercise is associated with oxidative stress and mtDNA deletions in younger persons. Together, these results present a paradox since it is known that regular exercise can slow, and in some cases reverse age-associated dysfunction of skeletal muscle (18;19;26;67). The effects of regular resistance exercise training on antioxidant status, and on mtDNA remain unknown, however, it is possible that regular resistance exercise upregulates antioxidant enzyme activity in response to exercise induced oxidative stress. In addition, studies of patients with mitochondrial disease have suggested that regular resistance exercise may shift the mtDNA profile towards normal (75).

There is a paucity of data regarding the effect of resistance exercise on oxidative stress, despite evidence to suggest a shift in cellular redox status following resistance exercise (47). Malondialdehyde, a marker of lipid peroxidation has been shown to be elevated from base-line following an acute bout of resistance exercise (47). More importantly, resistance exercise training for 6 months in older adults resulted in a decrease in malondialdehyde and hydroperoxides following an aerobic challenge as compared to before training (79). In addition, a recent study demonstrated a non-significant 40% decrease in basal oxidative damage to DNA following resistance training in older adults (62).

In addition to the production of ROS, antioxidant enzymes are also a factor in determining cellular redox status, and have previously been shown to increase in activity in response to acute (27-30;40;43;56;57;59;70), and chronic aerobic exercise (24;25;27;31;32;39-42;51;55;56;69). Few studies have examined the effects of aerobic exercise training in old rats, however, results suggest that there may be an inability to up-regulate antioxidant enzymes in advanced age (41). To date, there have been no investigations examining the effect of resistance exercise on antioxidant enzyme status. Despite the lack of scientific evidence, there is a strong theoretical foundation to suggest that resistance exercise may be a potent stimulus for the up-regulation of antioxidant enzymes. Acute, damaging, eccentric exercise has been associated with a significant infiltration of neutrophils and macrophages in the post-exercise period (4;74). An important function of neutrophils includes what has been termed the respiratory burst. This so-called burst involves the production of many potent ROS, such as the superoxide

radical, hydrogen peroxide, and the hydroxyl radical, *via* the enzyme NADPH oxidase (2). In addition, loss of Ca^{+} homeostasis within the muscle results in the conversion of xanthine dehydrogenase to xanthine oxidase. Both of these enzymes catalyze the reaction of hypoxanthine to xanthine, however xanthine oxidase produces O_2^{-} and H_2O_2 as reaction byproducts (34;48). Presumably, these processes may induce a cytosolic oxidative stress and illicit a beneficial antioxidant response. This possibility however, has not yet been investigated.

Central to the mitochondrial theory of aging is a loss of ETC function due to age-associated deletions and mutations in mtDNA. It is thought that these deletions and mutations not only lead to a loss of ETC function but also enhances the production of ROS. Recently, a phenomenon referred to as "gene shifting" has been described following resistance exercise training in patients with mitochondrial disease (75). It is theorized that due to their quiescent nature, muscle satellite cells do not accumulate mtDNA deletions and mutations at the same rate as mature myofibers. Therefore it is possible that satellite cell-derived myoblasts may donate their mitochondria during the fusion process, and thus increase the amount of normal mtDNA relative to mutant mtDNA. In fact, using resistance exercise as a means to activate satellite cells resulted in a significant increase in the proportion of wild-type mtDNA (75). In addition, there was a significant decrease in muscle fibers that stained negatively for cytochrome oxidase, suggesting a restoration of mitochondrial ETC function (75).

Mutant mtDNA is associated with pronounced ETC dysfunction (8;80), and several reports suggest a reduction in ETC capacity with aging (5;13;78). A loss of ETC function

with age may result in the inability to meet cellular energy requirements. However, it has also been shown that ETC dysfunction may lead to a greater production of ROS. In fact, investigations in rat brain have demonstrated a linear relationship between the complex IV/I and superoxide production (46). More than ten years ago, Harding (1992) proposed that aging may be the most common mitochondrial disease of all. If so, then resistance exercise has the potential to induce "gene shifting" in older adults, in a manner similar to that described in patients with mitochondrial disease (75).

In the present investigation we examined the effects of resistance exercise training on oxidative stress, antioxidant enzyme protein content, oxidative enzymes, and mtDNA in older adults.

METHODS

Subjects. Fifteen men and 15 women (68.5 ± 5.1 years) volunteered to participate in a 14 week resistance training program. See table 1 for subject characteristics. All subjects underwent a thorough screening process before being admitted into the study. Subjects were first screened by telephone, and were then subject to a medical evaluation. Consent from their family physician was required, and then all potential subjects underwent a resting electrocardiogram, and a sub-maximal graded exercise test on a bicycle ergometer. Exclusion criteria included: evidence of coronary hear disease; congestive heart failure; uncontrolled hypertension; chronic obstructive pulmonary disease; diabetes mellitus; renal failure; major orthopaedic disability; and smoking. None of the subjects had previously participated in a structured exercise program, however all subjects were

moderately active and participated regularly in activities such as walking, tennis, and golf. After subjects were advised of the benefits and risks of participation, subjects gave their written informed consent. The study was approved by the McMaster University Medical Ethics Board and conferred to the principles of the declaration of Helsinki. Twenty eight subjects completed the study protocol, as 2 dropped out for personal reasons. Aspects of this study have been previously published (6).

Exercise Training. Resistance training was performed three times weekly on non-consecutive days (Monday, Wednesday, and Friday) for 14 weeks, under strict supervision. Prior to each training session subjects warmed up for 5 minutes on a cycle ergometer, and stretched all major muscle groups. In addition each session was followed by a cool-down period involving stretching of all major muscle groups. Resistance training was performed in a circuit set system with 2 minutes rest between sets. Twelve exercises were used to train all major muscle groups including: seated chest press, latissimus pull-down, leg press, military press, calf raise, arm extension, back extension, abdominal crunch, upright row, knee extension, and knee flexion. All arm exercises included 10 repetitions, whereas 12 repetitions were performed for the remaining exercises. Training progressed from one set of each exercise at 50% of the initial 1 repetition maximum (1RM) to 3 sets at 80% of 1RM over the training period. Training logs were kept to record the volume and intensity of each session. The 1RM was re-evaluated every 2 weeks, and the training load was adjusted accordingly. All exercises were performed on universal strength training equipment (Universal Gym Equipment, Inc., Cedar Rapids, Iowa).

Muscle Biopsy and Urine Collection. A muscle biopsy was taken from the *vastus lateralis* muscle of the dominant leg, 20 cm proximal to the knee joint using a modified Bergström needle (5 mm diameter) with suction modification. The biopsy specimen was dissected of fat and connective tissue and immediately frozen in liquid nitrogen. All samples were stored at -80 °C for subsequent analysis.

Spot urine samples were collected at the time of the muscle biopsy. Urine was aliquoted into 2 ml eppendorf tubes and stored at -80 °C for subsequent analysis of 8-OHdG and creatinine.

All subjects were required to abstain from strenuous physical activity for 48 hours prior to the testing session.

DNA Oxidation. Urinary 8-OHdG was determined using an enzyme-linked immunoassay. The assay was carried out in triplicate utilizing the manufacturers instructions (Genox Corp., Blatlimore, MD, USA). Urinary creatinine levels were determined using a standard colorimetric UV spectrophotometric assay (Sigma Chemical Co., St. Louis, MO, USA). Intra-assay CV for 8-OHdG was 6.4%, and 8.2% for creatinine, determined by examining the variance in 5 replicates. 8-OHdG was expressed relative to creatinine to account for between subject differences in muscle mass.

ETC Enzymes and Citrate Synthase. Tissue preparation and analysis were performed as previously described (10). Briefly, approximately 30 mg of wet muscle tissue was homogenized using a glass homogenizer. Assays were preformed on fresh homogenates. Citrate synthase (CS) activity was determined by adding 10 µl of muscle homogenate to a solution of 1 ml of Tris buffer plus 2 µl of acetyl CoA (30 mM in H_2O), and 10 µl of

oxaloacetate in a cuvette warmed to 37°C. Absorbance was recorded at 412 nm every 30 s for 3 min. CS activity was calculated and reported as $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 3.1%, determined by examining the variance of three replicates for each sample.

Complex I+III activity was measured by the reduction of cytochrome c in the presence of NADH, thus measuring the flux of electrons from Complex I through complex III to cytochrome c. This was achieved by adding 20 µl of muscle homogenate to 940 µl of potassium phosphate (KPi) , 30 µl of oxidized cytochrome c, and 10 µl of NADH in a cuvette warmed to 37°C. Absorbance was measured at 550 nm every 30 s for 3 min. In a separate cuvette, the same sample was analyzed under identical conditions, with the addition of rotenone (0.1 mM). The difference between the two cuvettes represents complex I activity. Enzyme activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 6.6%.

Complex II+III activity was measured by the reduction of cytochrome c in the presence of succinate, thus measuring the flux of electrons from complex II through complex III to cytochrome c. 15 µl of muscle homogenate was added to 930 µl of KPi buffer 30 µl of oxidized cytochrome c, and 25 µl of succinate (0.4 M) in a cuvette warmed to 37°C. Absorbance at 550 nm was recorded every 30 s for 3 min. Enzyme activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 5%.

Complex IV was determined by measuring the oxidation of cytochrome c, by the flow of electrons through cytochrome oxidase. Stock cytochrome c (oxidized), was reduced by sodium ascorbate and KPi (10 mM). 15 µl of muscle homogenate was added to 950 µl of KPi, and 30 µl of reduced cytochrome c to a cuvette warmed to 37°C. Absorbance was

recorded at 550 nm every 30 s for 3 min. Complex IV activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 4.5%.

All samples were analyzed in duplicate on a Hewlett Packard spectrophotometer (Delaware City, DE. UV Visible Chemstation 8453).

Antioxidant Enzyme and Mitochondrial CK Protein Content. Muscle samples were homogenized and prepared for electrophoresis using modifications of methods previously described (77). Muscle homogenates were probed using four different antibodies. The MnSOD specific antibody was a rabbit polyclonal antibody specific to human MnSOD. This antibody recognizes a polypeptide with a molecular weight of ~ 28 kDa (kind gift from Dr. B. Robinson). The CuZnSOD antibody was a mouse monoclonal antibody specific to human CuZnSOD. This antibody recognizes a polypeptide with a molecular weight of 18 kDa (Sigma – S2147). The catalase antibody was a mouse monoclonal antibody specific to human catalase. This antibody recognizes a polypeptide with a molecular weight of 56 kDa (Sigma – C0979). Finally, mitochondrial CK primary antibody used was specific to human sarcomeric mtCK (kind gift from Dr. T Wallimann) (66). This antiobody recognizes a polypeptide with a molecular weight of 45 kDa, and does not recognize cytosolic muscle CK. Post-transfer blots were stained with coomassie brilliant blue to ensure equal protein loading between lanes. No difference in protein load was observed.

Mitochondrial DNA Deletions and normal mtDNA content. Mitochondrial DNA deletions were determined using XL-PCR as previously described (37). Briefly, DNA was extracted from approximately 25 mg of wet muscle using a PUREGENE® DNA

isolation kit, as per the instruction of the manufacturer (Gentra Systems, Minneapolis, MN). Two PCR primer sets were designed approximately 2000 bp apart, and all samples were run using both sets of primers. One set of primers were designed to begin amplification in the 16s rRNA region (3310L (30nt) – CCC ATG GCC AAC CTC CTA CTC CTC ATT GTA, 2690H (28nt) – TCT TGC TGT GTT ATG CCC GCC TCT TCA C) and a second pair was designed to begin amplification in the cytochrome b region (15148L (27nt) – GTG AGG CCA AAT ATC ATT CTG AGG GGC, 14841H (26nt) – TCT TGC TGT GTT ATG CCC GCC TCT TCA C). Methodological experiments determined that the optimal template concentrations for the 16s primers was 125 ng of total DNA, whereas the optimal template concentration for the cytb primers was 100 ng of total DNA. Amplification was achieved using the Expand Long Template PCR System (1 681 842, Roche Biochemicals, Mannheim, Germany), utilizing a final concentration of 500 μM of each dNTP, 300 nM of each primer, 2.25 mM of $MgCl_2$ in 10X Buffer 3, and 3.3 units of the Long Template Enzyme mixture. Mineral oil (30ul) was used to prevent evaporation. Optimal conditions for the reactions consisted of 2min hot-start at 92°, 10 cycles of 92° for 10 s to denature, 71° for 30 s for primer annealing, and 68° for 12 m and 30s for elongation. An additional 16 cycles were performed at these same temperatures however for each subsequent cycle after the initial 10 cycles the elongation time was increased by 20 s. At the end of a total of 26 cycles there was a final 7 m elongation step at 68°. All amplifications were performed on a Perkin Elmer thermal cycler. Ten ul of the final product was mixed with bromophenol blue and run on an agarose gel containing ethidium bromide for 1 hour at 100V. Gels were visualized on a

UV light gel documentation system, and analyzed using a commercially available software package.

Statistics. Statistical analysis was performed using the Statistica software package (Version 5.0, Tulsa, OK). All variables were statistically analyzed using a dependent t-test. Alpha was established at $p<0.05$, and all values were reported as mean ± standard deviation (SD).

RESULTS

Gender differences were investigated using a 2-way ANOVA with 1 within factor (pre, post), and 1 between factor (gender). All results were collapsed across gender since no gender differences were apparent following statistical analysis for any of the measured variables.

DNA Oxidation. Urinary 8-OHdG levels decreased significantly following training (N=26, Pre: 10783 ± 5856, Post: 8897 ± 4030 ng·g creatinine^{-1}; $P<0.05$). Results were expressed relative to creatinine to account for lean body mass, since skeletal muscle accounts for the vast majority of DNA degradation product (Fig 1).

Antioxidant Enzyme Content. Skeletal muscle protein content for CuZnSOD, MnSOD, and CAT were not different following the training period (N=26) (Fig 2).

Mitochondrial Enzymes. There was no difference following training for CS, complex I+III, and complex II+III activity, as compared to before training (N=18) (Table 2). However, complex IV activity was significantly increased following the training period (N=18, $P<0.01$; Table 2). In addition the complex IV/I+III ratio was significantly higher

following training as compared to before training ($P<0.05$: Table 2). Mitochondrial CK protein content was significantly higher following resistance training as compared to before (Pre – 109.7 ± 134.7, Post – 213.4 ± 166.6; $P<0.01$; Figure 3).

Mitochondrial DNA. Mitochondrial DNA deletions were apparent in 19/19 subjects before, and 19/19 after training. The number of visible deletion products were not different after training as compared to before (Pre – 2.1 ± 2.1, Post – 1.8 ± 1.8). Furthermore, resistance exercise training did not alter total XL-PCR amplifiable full-length mtDNA (Pre – 28045 ± 8609, Post – 27470 ± 8387 OD units) (Fig 4).

DISCUSSION

This paper represents the first examination of the effect of resistance training on antioxidant capacity, mtDNA deletions, mtDNA content and mitochondrial enzyme capacity in older adults. Fourteen weeks of progressive resistance exercise training resulted in a significant reduction in oxidative damage to DNA, with no change in antioxidant enzyme protein content. There was no increase in the number of mtDNA deletions, full-length mtDNA, or marker enzymes of mitochondrial mass (citrate synthase). Furthermore, ETC enzyme complex I+III, and II+III activity was unchanged yet complex IV activity and mtCK protein content increased. Together, these results suggest that specific components of mitochondrial function can adapt to the stress of resistance training without an increase in total mitochondrial mass. Furthermore, resistance training reduces oxidative stress, and does not induce mtDNA damage.

It is well established that aging is associated with a significant induction of oxidative stress (1;73;82). Oxidative stress is associated with damage to protein, lipids and DNA (49;53;54). Consistent with the results of others we found mtDNA deletions in 18/18 subjects, and we have previously found an increase in urinary 8-OHdG in older adults (Parise et al. - unpublished observations). A novel finding in the present study was a significant 17.5% decrease in urinary 8-OHdG following resistance training. This suggests that resistance training may have induced a more favourable cellular redox status. Endurance exercise training has been shown to lead to a reduction in oxidative DNA damage in rat skeletal muscle (60), however there is a paucity of data regarding oxidative DNA damage following resistance exercise. In fact, only one study has investigated oxidative stress following acute resistance exercise and reported an increase in malondialdehyde, a marker of lipid peroxidation (47). Furthermore, recent work has demonstrated that 6 months of resistance training in older adults (~68 years) resulted in a reduction in malondialdehyde and lipid hydroperoxides following an acute graded aerobic exercise challenge after training as compared to before training (79), confirming our results that resistance exercise training has protective effects against oxidative stress. In contrast, one study reported that 3 months of progressive resistance exercise training had no effect on urinary 8-OHdG in older adults (65-80 years). However, 8-OHdG levels in this latter study (62) changed 37.5% (24.8 nmol/day to 15.5 nmol/day) but were only measured in eight subjects. The fact that our study found a significant 17.5% decrease in 8-OHdG over 14 weeks with 26 subjects implies that the 8 subjects in the Rall et al. (2000) study would have resulted in the study being underpowered to detect a significant

difference. DNA, unlike proteins and lipids, possesses the ability to repair oxidatively modified bases through its own DNA repair system. Repair of modified bases is achieved through a process referred to as base excision repair (BER). Recent data has reported that human 8-oxoguanine DNA glycosylase (OGG1), the BER enzyme specific to 8-OHdG excision is up-regulated following a single bout of aerobic exercise (58;61). Thus, it is unclear in the present investigation if the reduction in urinary 8-OHdG excretion is the result of; 1. a decrease in ROS production, 2. an increase in the antioxidant capacity of the muscle through post-translational modifications, since our results do not support an increase in protein content of CuZnSOD, MnSOD, or catalase, 3. an up-regulation of glutathione peroxidase activity, or 4. an up-regulation of OGG1 and the BER pathway. Since a reduction in oxidative stress can be a result of either a decrease in the production of ROS or an increase in the antioxidant capacity we chose to examine the protein content of CAT, MnSOD, and CuZnSOD. In this study we demonstrate that 14 weeks of resistance exercise training had no effect on the protein content of any of the aforementioned antioxidant enzymes. To our knowledge, there have been no reports documenting changes in antioxidant enzymes following either acute or chronic resistance exercise. This is surprising given that resistance exercise is known to induce an inflammatory response (4;74) that includes a neutrophilic oxidative burst (2). This response lasts up to between 24 and 48 hours (4;74), and may be a significant source of ROS, which may shift the cellular redox status towards oxidation. In addition, O_2^- and H_2O_2 generated in the reaction of hypoxanthine to xanthine, catalyzed by the enzyme xanthine oxidase, may further contribute to cytosolic oxidative tress. This reaction is

normally catalyzed by the enzyme xanthine dehydrogenase, however in the presence of an elevated Ca^+ load, such as following exercise, a Ca^+ activated protease converts the dehydrogenase to an oxidase (34;48). Many reports have documented the effects of aerobic exercise on antioxidant enzymes, and show that MnSOD, CuZnSOD, CAT, and GPX increase in response to an acute bout of exercise (27-30;40;43;56;57;59;70), as well as in response to aerobic exercise training (24;25;27;31;32;39-42;51;55;56;69). The finding in the present study of a general lack of change in any of the antioxidant enzyme proteins does not necessarily mean that these systems do not respond to resistance exercise. For example Oh-Ishi and colleagues (1997) reported a significantly higher CuZnSOD enzyme activity in rat muscle following aerobic exercise training, despite no increase in CuZnSOD protein content, suggesting that these adaptations resulted from post-translational modifications to existing proteins, as opposed to the synthesis of new proteins. In support of this concept is the observation that SOD, CAT, and GPX are higher immediately following an acute bout of exercise (27;70). Since the exercise duration is relatively brief, the increased catalytic activity is likely a result of modifications to existing enzymes *via* allosteric and/or covalent mechanisms. Thus, antioxidant enzyme protein content did not change in the present investigation, however further analysis examining antioxidant enzyme activity is necessary to fully understand the role of antioxidant systems following resistance exercise training.

It has been suggested that ETC activity may decrease as a function of age. Reports on this issue have been equivocal, with some demonstrating a significant decrease in ETC function (5;9;13;78), whereas others have reported no change with aging (3;12;63;84).

Kwong and Sohal (2000) have suggested that a better measure of ETC dysfunction may be activity ratios, as opposed to the measurement of individual ETC enzymes. Theoretically, changes in activity ratios between complexes can disturb the flow of electrons through the ETC, and induce a greater production of ROS. In the present study we demonstrated a specific up-regulation of complex IV activity with no other changes in complex I+III, II+III, and CS activity observed, following 14 weeks of resistance exercise. In addition, the complex IV/I+III ratio increased significantly suggesting an improvement in ETC efficiency. Previously, a linear inverse relationship between the level of protein carbonyls, and the activity ratio of complex IV to complex I in rat brains of varying age were reported, suggesting that a decrease in the ratio of complex IV to complex I might lead to greater production of ROS (46). It has been hypothesized that a shift from the steady state condition of electron flow may lead to a loss of potential for generating the proton gradient, resulting in respiratory inefficiency (38). To the knowledge of the author's, there are no other reports examining ETC function following resistance exercise training in older adults.

Taken together, a reduction in oxidative damage to DNA, the lack of an increase in antioxidant enzyme protein content, and a significant increase in complex IV activity following training suggests that complex IV may have indirect antioxidant effects. Theoretically, an increase in the complex IV/I+III ratio may result in a greater driving force down the ETC, thus reducing the amount of electron "leak", resulting in a reduction in ROS production.

In addition to these findings, we demonstrate, for the first time, a significant increase in mitochondrial creatine kinase (mtCK) protein content following resistance exercise training. Mitochondrial CK is an important component of the phosphocreatine shuttle, located in the inter-membrane space, and serves to rephosphorylate free creatine during recovery from muscle contraction. Rephosphorylation of free creatine is known to be an aerobic process, and the rate of rephosphorylation is a reflection of ETC function. Presumably, the increase reported in the present study may reflect the stress associated with resistance exercise. During this rest period, free creatine will shuttle into the mitochondria and be rephosphorylated by a mtCK dependent process. Interestingly, CS activity did not change as a function of training, suggesting that mitochondrial volume did not change. Thus, the observed increase in mtCK represents a mitochondrial protein specific increase, similar to that of the increase in complex IV activity. Together, these results suggest that selected mitochondrial proteins may be up-regulated in response to metabolic stress.

Given the potential importance of mtDNA in the aging process we also examined the effect of resistance exercise on mtDNA deletions. In the present study we demonstrate that there was no effect of progressive resistance exercise training on normal length mtDNA or visible deletion products. The mitochondrial theory postulates that ROS result in damage to mtDNA leading to defective ETC proteins. Indeed, a progressive age-related increase in mtDNA deletions and mutations has been reported (14-17;33;35-37;44;45;50;68;72;80;81;83). To date, there have been no investigations examining the effect of exercise training on mtDNA deletions in the elderly. However, mtDNA

deletions have been quantified before and after resistance training in patients with mitochondrial disease (75). It was reported that following 12 weeks of concentric resistance exercise there was a significantly greater proportion of muscle fibres that stained positive for cytcochrome oxidase activity, suggesting a shift towards a more normal ETC function. More importantly, there was a shift in the genetic profile of mutant and normal mtDNA such that after training there was significantly greater wild-type mtDNA as compared to before the intervention (75). This phenomenon was coined "gene shifting", and was found to occur when satellite cells, which carry a much lower mutational burden than mature myofibers (22), proliferate and fuse donating their normal organelles, including mitochondria to damaged post-mitotic fibers (71;75). The lack of any effect on mtDNA in the present study may be due to the relatively low levels of mtDNA deletions in older adults as compared to the very high levels observed in patients with mitochondrial disease. Recent work has demonstrated that aerobic exercise training in patients with mitochondrial disease resulted in a decrease in the proportion of wild-type mtDNA (76). Importantly, we demonstrate that resistance training does not augment the number of mtDNA deletions, or cause a decrease in full-length mtDNA.

In conclusion, results from the present investigation suggest that resistance exercise training may be an effective means of inducing decreases in oxidative stress to DNA. In addition, electron transport chain activity, in particular an up-regulation of complex IV may be responsible for the reduction in oxidative stress by increasing the IV/I+III ratio and improving electron flux. Alternatively, a reduction in oxidative damage to DNA may be achieved through an up-regulation of DNA repair. Given this possibility, further work

is needed to elucidate the mechanism responsible for an exercise-induced decrease in 8-OHdG. In addition, the specific up-regulation of complex IV, and mtCK protein content, without changes in CS or mtDNA content suggest that specific components of mitochondrial function can adapt to the stress of resistance training, without changes in mitochondrial proliferation.

Figure 1. Lower oxidative damage to DNA following resistance exercise training. * denotes a significant difference from pre. Alpha was set at $P<0.05$.

Figure 2. Antioxidant enzyme protein content following resistance exercise training. Resistance exercise training did not alter antioxidant protein content.

Figure 3. Mitochondrial DNA following resistance exercise training. A. Representative image of a mtDNA profile. B. Resistance exercise training had no effect on normal length mtDNA, or, C. on mtDNA deletions.

Figure 1. Oxidative damage to DNA following resistance exercise.

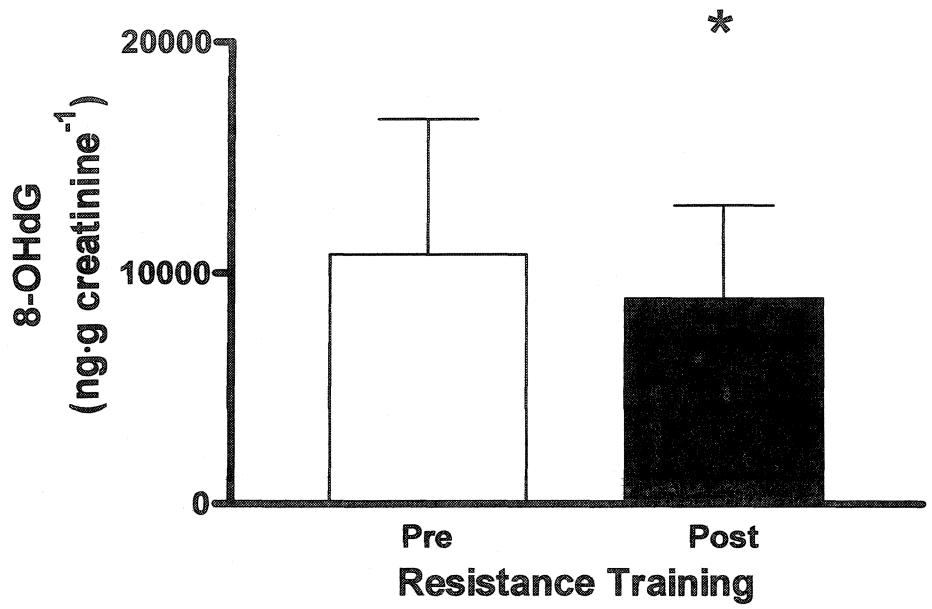
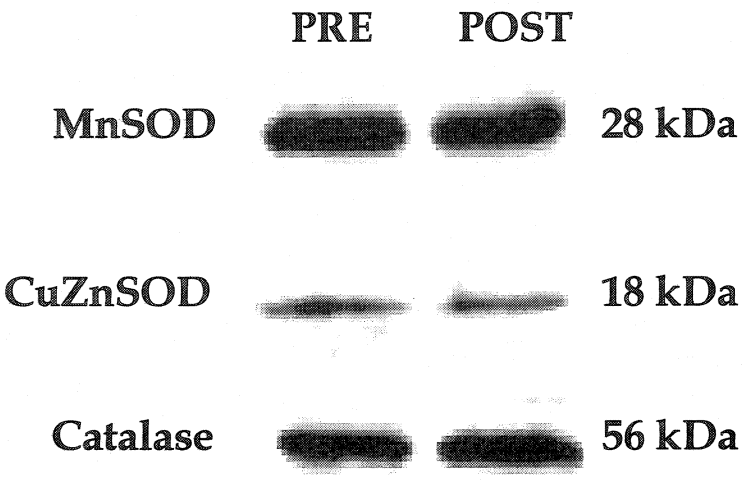


Figure 2. Antioxidant protein content following resistance exercise



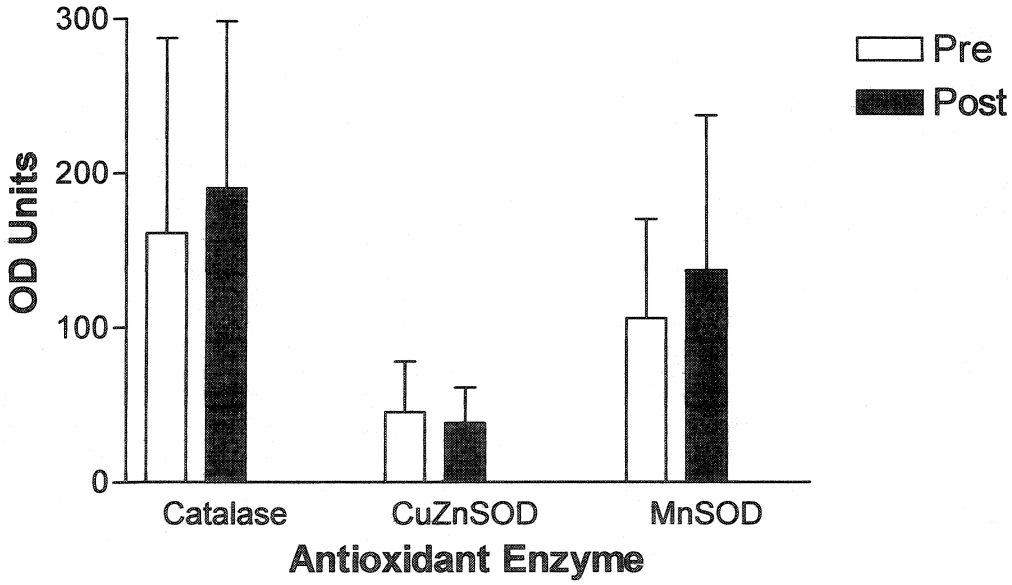
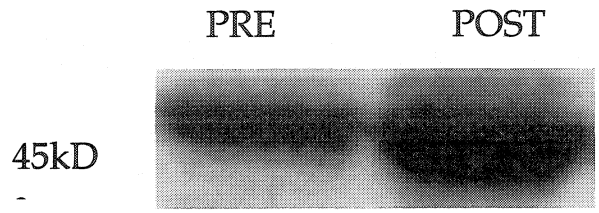


Figure 3. Mitochondrial CK Protein Content Following Resistance Exercise



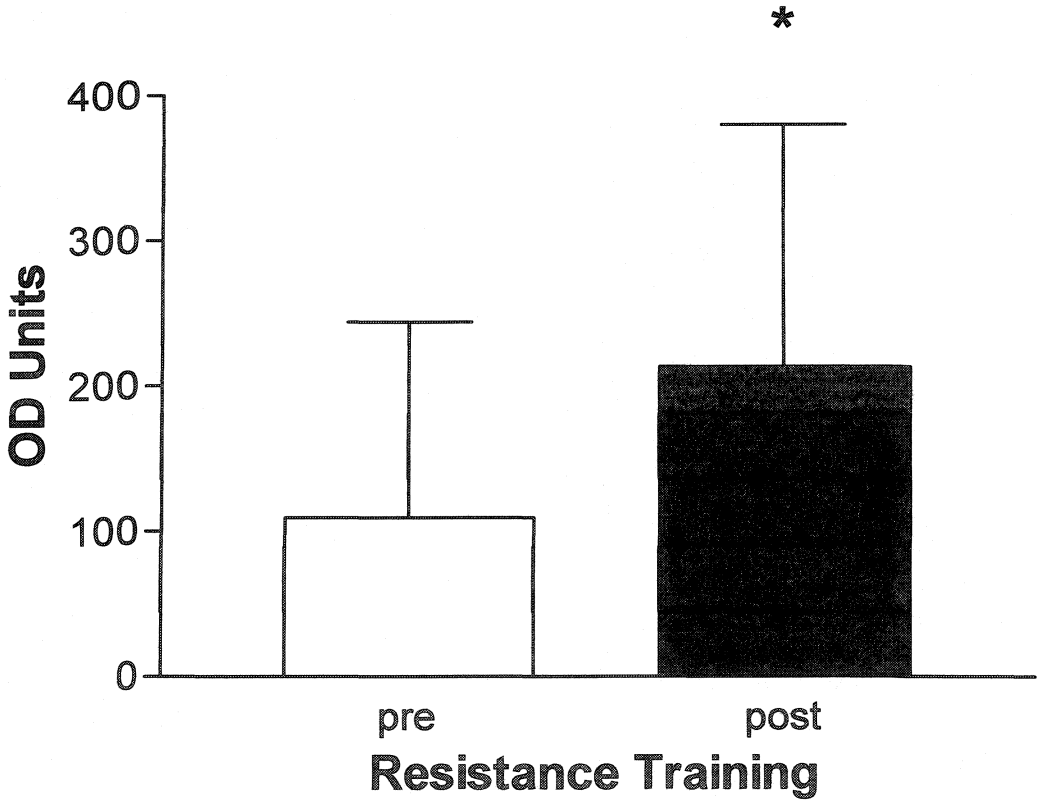
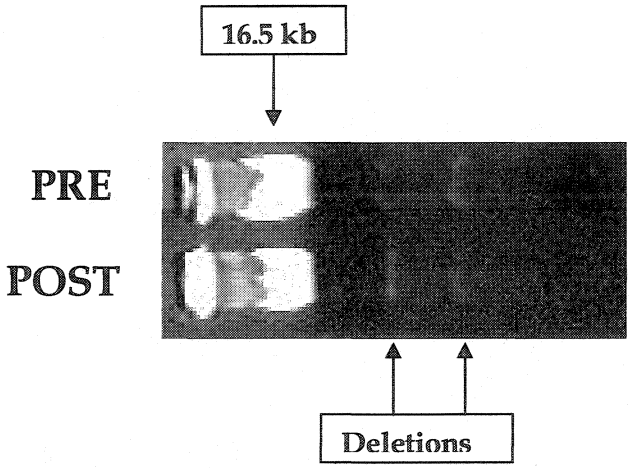
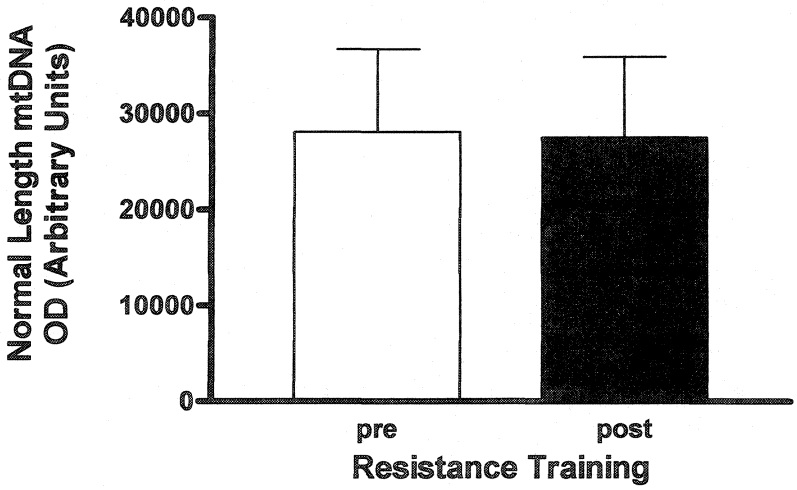


Figure 4. Mitochondrial DNA following resistance exercise training

A.



B.



C.

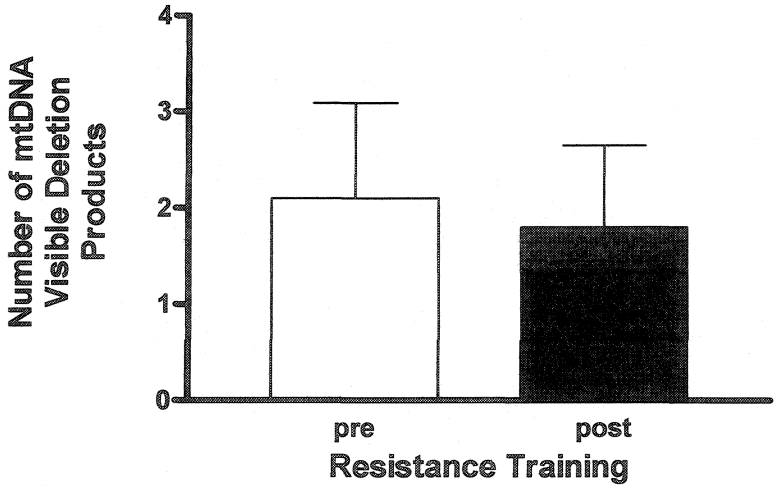


Table 1. Subject Characteristics.

Variable	
Age (years)	68.5 ± 5.1
Height (cm)	165.3 ± 8.2
Mass (kg)	73.8 ± 15.1
Body fat (%)	31.1 ± 10.5
LBM (kg)	43.8 ± 11.1
FM (kg)	21.2 ± 8.5
Leg Extension 1RM (kg)	46.0 ± 13.4

Table 1. Subjects characteristics. All subjects were healthy and recreationally active. All values are mean ± SD.

Table 2. Oxidative Enzymes Following Resistance Exercise

Enzyme	PRE	POST
Citrate Synthase	12.2(2.8)	13.2(3.2)
Complex I+III	1.9(0.6)	1.7(0.5)
Complex II+III	1.6(0.5)	1.8(0.8)
Complex IV	6.7(1.5)	8.6(2.8) *
Complex IV/I+III	3.9(1.8)	5.6(3.1)*

Table 2. Complex IV activity was significantly higher following resistance exercise training, as was the complex IV/I+III. * denotes a significant difference from pre. All values are mean ± SD and are expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww, except the complex IV/I+III, which is a ratio. Alpha was set at $P<0.05$.

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9.0 MANUSCRIPT 3

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THE AMERICAN JOURNAL OF PHYSIOLOGY

Antioxidant Enzyme Activity is Up-regulated Following Uni-lateral Resistance Exercise Training in Older Adults.

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Running Head: Antioxidant Enzymes and Resistance Exercise

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Keywords: oxidative stress, mitochondrial theory of aging, mtDNA, skeletal muscle, protein carbonyls, aging

Abstract - Resistance exercise is an effective therapy for attenuating, and in some cases reversing age-related deficiencies in skeletal muscle. Cellular antioxidant capacity and oxidative stress, both of which are implicated in the mitochondrial theory of aging, appear to be critical to the aging process. The effects of resistance exercise training on skeletal muscle oxidative stress, antioxidant capacity and mitochondrial deletions has not previously been examined in older adults. Muscle biopsies from both legs, were obtained from the *vastus lateralis* muscle of twelve men 71 ± 7 years old. Subjects performed progressive resistance exercise training with one leg for 12 weeks. The non-training leg underwent an acute bout of exercise at the same time as the last bout of exercise in the training leg. Muscle biopsies were collected from both legs 48hr following the final exercise bout. There was no significant effect of acute exercise on any variable measured. Training resulted in a significant up-regulation of CuZnSOD activity (pre - 7.2 ± 4.2, post – 12.6 ± 5.6 U·mg protein^{-1}; *P= 0.02*), and catalase activity (pre – 8.2 ± 2.3, post – 14.9 ± 7.6 μmol·min^{-1}·mg protein^{-1}; *P=0.02*), but not MnSOD. Muscle total protein carbonyl content did not change as a result of exercise training. There was also no change in oxidative enzyme activity, or mtDNA deletion profiles. In conclusion resistance exercise is an effective intervention for increasing cellular antioxidant capacity in skeletal muscle in older adults.

Aging is associated with losses in muscle strength, muscle mass, and function (62). Epidemiological studies suggest that regular exercise results in a decrease in morbidity (21), and mortality (59;66), suggesting that exercise may slow, and in some cases reverse age-related muscle dysfunction. In particular, resistance exercise has proven to be a potent stimulus for increasing strength (12;22), inducing muscle fiber hypertrophy (8;12;22), and improving function in older adults (19;20;30;67).

Despite an abundance of scientific evidence, the mechanisms underlying the aging process remain elusive. However, for the last 30 years, the mitochondrial theory of aging has been at the forefront of aging research. This theory suggests that aging is attributable to an increase in levels of oxidative stress. In turn, oxidative stress is thought to lead to damage to mtDNA, and as a result the electron transport chain (ETC) becomes dysfunctional, resulting in the inability to meet energy requirements, and exacerbating the production of reactive oxygen species (ROS) (26).

Indeed, human skeletal muscle aging has been associated with an increase in oxidative damage to protein, lipids, and DNA (17;54;60;61). Furthermore, an accumulation of mtDNA deletions and mutations have been associated with aging (14-16;18;36;38;40;41;48;49;56;68;70;77-79), and ETC dysfunction has been reported in many investigations (6;9;10;27;74;77). Taken together, and with recent data demonstrating a relationship between mtDNA deletions, ETC deficiency, and muscle atrophy in single fibers (7;9;39;77), there is strong evidence in support of the mitochondrial theory of aging in skeletal muscle, particularly as a multi-focal stochastic process.

Antioxidant enzyme capacity is a key variable in determining cellular redox status and survival. For example, administration of antioxidants has been reported to significantly lengthen lifespan in *C. elegans* (55). In human and animal skeletal muscle, it is generally accepted that antioxidant enzyme activity increases with age (33;42;44;45;51;60;61;75), presumably in order to adapt to an increase in oxidative stress. In spite of marked improvements in skeletal muscle function, there is no information regarding the effects of resistance exercise on antioxidant enzyme activity, and limited information regarding the antioxidant response to aerobic exercise training in old age. Two reports have suggested that gluthathione peroxidase increases following treadmill training in rats (24;32), however another report concluded there was no adaptation in antioxidant enzymes (45). There are several reasons why resistance exercise may lead to an increase in antioxidant enzyme activity. Secondary sources of ROS through neutrophil and macrophage infiltration following exercise-induced muscle damage is known to peak around 24 hr following an exercise bout in young men and women (4;71). The respiratory burst associated with neutrophil and macrophage infiltration results in the production of O_2^-, H_2O_2, and the OH· radical (3). In addition, an increase in the intracellular calcium concentration, known to occur following exercise-induced muscle damage (2), is a known stimulus for the conversion of xanthine dehydrogenase to xanthine oxidase. Xanthine oxidase produces O_2^- and H_2O_2 as byproducts in the reaction of hypoxanthine to xanthine (37;53).

To date, there has been little evidence examining the effects of resistance exercise on indices of oxidative damage. One study has reported no effect on basal levels of lipid

peroxidation after resistance training as compared to before (76). A separate study reported a non-statistical 40% decrease in urinary 8-OHdG following resistance exercise training in older adults (65). No studies have reported on the levels of oxidative damage to proteins following a resistance exercise intervention.

Mitochondrial DNA is a central component to the mitochondrial theory of aging, and there is an abundance of data demonstrating an age-associated increase in mtDNA deletions and mutations (14-16;18;36;38;40;41;47;50;56;68;70;77-79). Although there has not been any data reported on the effects of resistance exercise on mtDNA in older adults, a novel concept, referred to as "gene shifting' was recently proposed to describe the effect of resistance training in patients with mitochondrial disease (72). Resistance training resulted in a significant increase in wild-type mtDNA in patients with mitochondrial disease, presumably achieved by the fusion of satellite cell-derived myoblasts, thought to carry a smaller mutational burden than mature myofibers, with existing myofibers. In doing so, a ratio of normal to mutant mtDNA is maintained above a critical threshold, after which cellular energy requirements could not be sustained. Although this phenomenon was described in patients with mitochondrial disease, there are striking similarities between mitochondrial disease and aging, so much so that there have been suggestions that aging itself may be the most common mitochondrial disease of all (25).

Finally, ETC activity has been proposed to decrease with advanced age in human skeletal muscle (6;13;74). There have not been any examinations on the effects of resistance exercise on oxidative enzyme activity in older adults. However, based on the concept of

"gene shifting", there is the potential that resistance exercise training may have effects on oxidative enzyme activity. Taivassalo and colleagues (1999), reported that there was a significant reduction in the number of fibers that stained negatively for cytochrome oxidase activity following resistance exercise. Furthermore, it has been shown that when satellite cells are activated by muscle damage, all regenerating muscle fibers are shown to stain positive for cytochrome oxidase activity, suggesting that regenerating muscle fibers possess normal ETC activity.

The purpose of the present investigation was to examine the effects of resistance exercise training on oxidative stress, antioxidant enzyme activity, mitochondrial enzymes, and mtDNA in older adults.

MATERIALS AND METHODS

Subjects. Twelve men (71.2 ± 6.5 years) volunteered to participate in a 12 week uni-lateral leg resistance training program (Table 1). All subjects underwent a thorough screening process before being admitted into the study. Subjects were first screened by telephone, and were then subject to a medical evaluation. Consent from their family physician was required, and then all potential subjects underwent a resting electrocardiogram, and a sub-maximal graded exercise test on a bicycle ergometer. Exclusion criteria included: evidence of coronary hear disease; congestive heart failure; uncontrolled hypertension; chronic obstructive pulmonary disease; diabetes mellitus; renal failure; major orthopaedic disability; and smoking. None of the subjects had ever participated in a structured exercise program. After subjects were advised of the benefits

and risks of participation, subjects gave their written informed consent. The study was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board and conferred to the principles of the declaration of Helsinki.

Exercise Training. Resistance training was performed three times weekly on non-consecutive days (Monday, Wednesday, and Friday) for 12 weeks, under strict supervision. Prior to and after each training session subjects were required to perform passive stretching. Resistance exercise for each session consisted of 3 sets of 10 repetitions for each of leg press and leg extension. Training progressed from one set of each exercise at 50% of the initial 1 repetition maximum (1RM) to 3 sets at 80% of 1RM over the training period. Training logs were kept to record the volume and intensity of each session. The 1RM was re-evaluated every 2 weeks, and the training load was adjusted accordingly. All exercises were performed on universal strength training equipment (Universal Gym Equipment, Inc., Cedar Rapids, Iowa).

Muscle Biopsy. A muscle biopsy was taken from the *vastus lateralis* muscle of both legs before as well as after the training period, 20 cm proximal to the knee joint using a modified Bergström needle (5 mm diameter) with suction modification. The biopsy specimen was dissected of fat and connective tissue and immediately frozen in liquid nitrogen. All samples were stored at -80 °C for subsequent analysis.

All subjects were required to abstain from strenuous physical activity for 48 hours prior to the testing session. The non-trained leg performed an acute bout of exercise at the same relative intensity of the training leg to allow for the determination of the effect of training and the effect of acute resistance exercise.

Protein Carbonyls. Protein carbonyls were determined by measuring the reactivity of carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH)as previously described (Levine et al., 1990). Briefly, the tissue was homogenizes in ice cold Hepes buffer (pH 7.2 – 7.4), containing leupeptin (0.5 $\mu g \cdot ml^{-1}$), pepstatin (0.7 $\mu g \cdot ml^{-1}$), aprotinin (0.5 $\mu g \cdot ml^{-1}$), and phenylmethylsulfonyl fluoride (40$\mu g \cdot ml^{-1}$). The soluble protein fraction was separated with centrifugation and then treated with strepatmycin sulfate to remove nucleic acids. The protein sample was pipetted into two glass borosilicated tubes and precipitated with trichloroacetic acid (TCA). DNPH (10 mM in 2 M HCl) was added to one tube, and HCl (2 M) to the other. The tubes incubated at room temperature for 1 hour, with vortexing every 10 min. TCA (10% final concentration) was added to both tubes and pelletes were recovered via centrifugation. Pellets were washed 3 x with ethanol-ethyl acetate to remove free DNPH. The precipitated protein was redissolved in 6 M guanidine solution, and the absorbance of both solutions was measured at 370 nm . All samples were analyzed in duplicate on a spectrophotometer (Delaware City, DE. HP - UV Visible Chemstation 8453).

Antioxidant Enzymes. Muscle catalase activity was determined by measuring the kinetic decomposition of H_2O_2. Muscle catalase activity was measured by combining 970 ul of K_2HPO_4 buffer (50 mM with 50mM EDTA, and 0.01% Triton X-100, pH 7.2-7.4), with 30 ul of muscle homogenate. 10 ul of H_2O_2 (1 M) was added to the cuvette and mixed by inversion to initiate the reaction. Absorbance was measured at 240 nm every 15 s for 2 min in a spectrophotometer (Delaware City, DE. HP – UV spectrophotometer Visible

Chemstation 8453). Catalase activity was calculated and reported in μmol·min^{-1}·mg protein^{-1}. Intra-assay CV was 8.2% determined by analyzing triplicates for each sample. Muscle total SOD activity was determined by measuring the kinetic consumption of O_2^- by superoxide dismutase in a competitive reaction with cytochrome c. Muscle total SOD activity was measured by combining 955 ul of K_2HPO_4 buffer (50 mM with 0.1 mM EDTA, pH 7.8) containing cytochrome c (25 mg/100 ml) and Xanthine (5 μmol). 30 ul of muscle homogenate was added to the cuvette and mixed by inversion. 15 ul of xanthine oxidase (0.2 U/ml) was added to initiate the reaction, and absorption at 550 nm was observed every 15 s for 2 min. One unit of SOD activity was defined as the amount of enzyme which caused a 50% inhibition of the reduction of cytochrome c. Activity was calculated and expressed in U·mg protein^{-1}.

In a separate cuvette, the same sample was analyzed under identical conditions with the addition of 10 ul of 0.2 M KCN (pH 8.5-9.5) for determination of Mn SOD activity. KCN is known to inhibit CuZn SOD activity as described by Higuchi and colleagues (1985). CuZn SOD was approximated by subtracting Mn SOD activity from total SOD activity, and was expressed in U·mg protein^{-1}.

ETC Enzymes and Citrate Synthase. Tissue preparation and analysis were performed as previously described (11). Breifly, approximately 30 mg of wet muscle tissue was homogenized using a glass homogenizer. Assays were preformed on fresh homogenates. Citrate synthase (CS) activity was determined by adding 10 μl of muscle homogenate to a solution of 1 ml of Tris buffer plus 2 μl of acetyl CoA (30 mM in H_2O), and 10 μl of oxaloacetate in a cuvette warmed to 37°C. Absorbance was recorded at 412 nm every 30

s for 3 min. CS activity was calculated and reported as $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 3.1%.

Complex I+III activity was measured by the reduction of cytochrome c in the presence of NADH, thus measuring the flux of electrons from Complex I through complex III to cytochrome c. This was achieved by adding 20 µl of muscle homogenate to 940 µl of potassium phosphate (KPi) , 30 µl of oxidized cytochrome c, and 10 µl of NADH in a cuvette warmed to 37°C. Absorbance was measured at 550 nm every 30 s for 3 min. In a separate cuvette, the same sample was analyzed under identical conditions, with the addition of rotenone (0.1 mM). The difference between the two cuvettes represents complex I activity. Enzyme activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 6.6%.

Complex II+III activity was measured by the reduction of cytochrome c in the presence of succinate, thus measuring the flux of electrons from complex II through complex III to cytochrome c. 15 µl of muscle homogenate was added to 930 µl of KPi buffer 30 µl of oxidized cytochrome c, and 25 µl of succinate (0.4 M) in a cuvette warmed to 37°C. Absorbance at 550 nm was recorded every 30 s for 3 min. Enzyme activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 5%.

Complex IV was determined by measuring the oxidation of cytochrome c, by the flow of electrons through cytochrome oxidase. Stock cytochrome c (oxidized), was reduced by sodium ascorbate and KPi (10 mM). 15 µl of muscle homogenate was added to 950 µl of KPi, and 30 µl of reduced cytochrome c to a cuvette warmed to 37°C. Absorbance was

recorded at 550 nm every 30 s for 3 min. Complex IV activity was calculated and reported in $\mu mol \cdot min^{-1} \cdot mg\ w.w^{-1}$. Intra-assay CV was 4.5%.

All samples were analyzed in duplicate on a spectrophotometer (Delaware City, DE. HP - UV Visible Chemstation 8453).

Mitochondrial DNA Deletions. Mitochondrial DNA deletions were determined using XL-PCR, as previously described (41). Briefly, DNA was extracted from approximately 25 mg of wet muscle using a PUREGENE® DNA isolation kit, as per the instruction of the manufacturer (Gentra Systems, Minneapolis, MN). Two PCR primer sets were designed approximately 2000 bp apart, and all samples were run using both sets of primers. One set of primers were designed in to begin amplification in the 16s rRNA region (3310L (30nt) – CCC ATG GCC AAC CTC CTA CTC CTC ATT GTA, 2690H (28nt) – TCT TGC TGT GTT ATG CCC GCC TCT TCA C) and another pair was designed in the cytochrome b region (15148L (27nt) – GTG AGG CCA AAT ATC ATT CTG AGG GGC, 14841H (26nt) –TCT TGC TGT GTT ATG CCC GCC TCT TCA C). Sequences were kindly shared by Dr. Anthony Linnane's laboratory. Methodological experiments determined that the optimal template concentrations for the 16s primers was 125 ng of total DNA, whereas the optimal template concentration for the cytb primers was 100 ng of total DNA. Amplification was achieved using the Expand Long Template PCR System (1 681 842, Roche Biochemicals, Mannheim, Germany), utilizing a final concentration of 500 μM of each dNTP, 300 nM of each primer, 2.25 mM of $MgCl_2$ in 10X Buffer 3, and 3.3 units of the Long Template Enzyme mixture. Mineral oil (30ul) was used to prevent evaporation. Optimal conditions for the reactions consisted of 2min

hot-start at 92°, 10 cycles of 92° for 10 s to denature, 71° for 30 s for primer annealing, and 68° for 12 m and 30s for elongation. An additional 16 cycles were performed at these same temperatures however for each subsequent cycle after the initial 10 cycles the elongation time was increased by 20 s. At the end of a total of 26 cycles there was a final 7 m elongation step at 68°. All amplifications were performed on a Perkin Elmer thermal cycler. Ten ul of the final product was mixed with bromophenol blue and run on an agarose gel containing ethidium bromide for 1 hour at 100V. Gels were visualized on a UV light gel documentation system, and analyzed using LabWorks.

Statistics. All statistics were performed using the Statistica software package (Statsoft. Version 5.1, Tulsa, OK). All variables were analyzed using a two - way ANOVA [2 factors: leg (2 levels: trained and untrained) and time (2 levels: pre and post training), all within]. In addition, we hypothesized, a priori, that significant results would only be observed in the trained leg. Therefore, we performed paired t-tests on each leg to examine the effects of training, as well as the effects of acute exercise, independent of each other. Alpha was established at $p<0.05$, and all values were reported as mean ± standard deviation (SD). All reported results reflect results calculated using the paired t-test analysis.

RESULTS

No differences were observed from baseline in the acutely exercised leg. Therefore, for the remainder of the manuscript the acute exercise leg will be referred to as the "untrained

leg". All figures in the text contain data from the trained leg only, however data from the untrained leg is represented in the text of the results section.

Protein Carbonyls. There was no significant effect observed in either the trained (N=8, pre – 0.78 ± 0.54, post – 0.89 ± 0.34 nmol·mg protein^{-1}; $p>0.05$: Fig 1) or the untrained leg after the 12 week period (N=8, pre – 0.65 ± 0.50, post – 0.63 ± 0.22 nmol·mg protein^{-1} ; $p>0.05$).

Antioxidant Enzymes. There was a significant increase in catalase activity as a function of training (N=9, pre – 8.2 ± 2.3, post – 14.9 ± 7.6 µmol·min^{-1}·mg protein^{-1}; $p = 0.02$), however there was no change in the untrained leg (N=9, pre – 9.6 ± 3.5, post – 10.9 ± 5.6 µmol·min^{-1}·mg protein^{-1}). Total SOD activity was unchanged in the trained leg (N=7, pre - 12.9 ± 4.5, post – 18.1 ± 6.3 U·mg protein^{-1}), as well as in the untrained leg (N=7, pre – 17.0 ± 3.9, post – 18.9 ± 9.6 U·mg protein^{-1}). However, CuZnSOD activity increased significantly from baseline in the trained leg (N=7, pre - 7.2 ± 4.2, post – 12.6 ± 5.6 U·mg protein^{-1};$p = 0.02$), whereas there was no significant change observed in the untrained leg (N=7, pre – 9.5 ± 4.9, post – 14.5 ± 8.8 U·mg protein^{-1}). MnSOD on the other hand did not change in either the trained (N=7, pre – 5.7 ± 3.2, post – 5.5 ± 3.0 U·mg protein^{-1}) or the untrained leg (N=7, pre – 7.2 ± 2.8, post – 5.3 ± 2.3 U·mg protein^{-1}) over the 12 week period. See figure 2.

ETC Enzymes and Citrate Synthase. No significant changes were observed in either the trained leg (N=9) or the untrained leg (N=9) over the 12 week period for any of the enzymes analyzed. See Table 2.

Mitochondrial DNA. No significant change was observed for visible mtDNA deletion products in the trained leg (N=8, pre – 1.5 ± 1.0, post – 1.6 ± 0.9) or the untrained leg (N=8, pre – 1.4 ± 0.5, post – 1.0 ± 0.9). In addition there was no change in the amount of normal length mtDNA for the trained (N=8, pre – 31453 ± 3305, post – 29870 ± 4377 OD units) or the untrained leg (N=8, pre – 33901 ± 5453, post – 34098 ± 6062 OD units). See Fig 3.

DISCUSSION

Given the known beneficial effects of regular exercise on muscle function and longevity, the present investigation examined the effect of resistance exercise on variables of the mitochondrial theory of aging. We report that muscle protein carbonyls did not change as a function of acute or chronic resistance exercise and the significant increase in several antioxidant activities may have prevented an increase. In addition, there was no change in oxidative enzyme activity or mtDNA content or deletions following the resistance exercise protocol. Results from the present study suggest that resistance exercise is a sufficient stimulus for increasing antioxidant enzyme activity in older adults, and this may attenuate aging and/or exercise induced increases in ROS.

We report that 12 weeks of unilateral leg resistance training resulted in an 82.5% increase in catalase activity, and a 75% increase in CuZnSOD activity, with no apparent changes in MnSOD in the trained leg. There is an abundance of data documenting increases in SOD (28;31;45;46;58;63;69), catalase (29;31;57), and glutathione peroxidase (34;35;43-46;58;63;69), activity following aerobic exercise training in young rodents. However, the

data in old animals is minimal and equivocal. It has been reported that treadmill training in rats resulted in a significant increase in glutathione peroxidase activity (24;32), however Leeuwenburgh and colleagues (1994) reported no significant increases in any antioxidant enzymes in old rats following treadmill training . The present study represents the first time that antioxidant enzyme adaptation has been measured in response to resistance exercise, particularly in older adults. Based on our observations of an increase in catalase and CuZnSOD we could assume that resistance exercise resulted in a cytosolic oxidative stress. There are two significant potential sources of ROS in the cytosol. First, following muscle damage induced by eccentric exercise it has been demonstrated that neutrophils and macrophages peak approximately 24 hr following the exercise bout (4;71). Lysosomes are associated with a respiratory burst resulting in the generation of many potent ROS including O_2^-, H_2O_2, and the OH· radical. It is conceivable that repeated bouts of resistance exercise over a 12 week period resulted in a chronic elevation of cytosolic oxidative stress. A second potential source of cytoslic ROS production is via the xanthine oxidase reaction. Under normal conditions, xanthine dehydrogenase functions to convert hypoxanthine to xanthine with H_2O as a byproduct. However, under conditions of elevated intracellular Ca+ concentration, a calcium activated protease cleaves the enzyme forming xanthine oxidase. Xanthine oxidase serves the same function as xanthine dehydrogenase, however produces O_2^-, and H_2O_2 as byproducts. There is convincing evidence to suggest that exercise results in a loss of Ca+ homeostasis, and there are reports that damage to myofibrils are associated with elevated Ca^+ concentrations, however the underlying mechanisms of this damage remains

unknown (2). Therefore, resistance exercise may be associated with at least two sources of ROS production derived in the cytosol. MnSOD is compartmentalized in the mitochondrial inner-membrane, and the lack of adaptation following resistance exercise training may simply be related to the fact that resistance exercise is generally not associated with mitochondrial stress, to the same extent as endurance exercise. A second possibility may be related to the notion that older adults may be limited by a ceiling effect with respect to adaptation of MnSOD. We have previously observed that older adults have significantly higher levels of MnSOD in skeletal muscle as compared to young adults, whereas there was no difference between young and old with respect to CuZnSOD (Parise et al., 2003 - unpublished observation). In addition, Pansarasa and colleagues (1999, 2000), demonstrated that although there was an age-related increase in catalase and MnSOD, Total SOD decreased, suggesting a reduction in CuZnSOD activity. It is possible that older adults have reached an upper-limit to their adaptability in MnSOD as a function of age, and may require a greater intensity of exercise to provoke a training effect. Conversely, there is no apparent age-associated adaptation in CuZnSOD and thus CuZnSOD may be more responsive to the stresses of exercise.

Despite significant increases in antioxidant enzymes indicative of oxidative stress, there were no changes in protein carbonyls following the resistance exercise period. One other study found that resistance exercise training for 6 months in older adults had no effect on basal levels of lipid peroxidation (76). Interestingly however, there was a decrease in malondialdehyde and hydroperoxides following an acute aerobic challenge as compared to before training (76). One would expect that an increase in antioxidant capacity would

result in a reduction of damaged macromolecules. In fact, in a previous study we demonstrated a significant reduction in 8-OHdG, a marker of oxidative damage to DNA (Parise et al. - unpublished observation). In addition, a 40% decrease in urinary 8-OHdG was reported following resistance exercise training in older adults, however this did not achieve statistical significance, likely due to low subject numbers (65). The mechanism underlying the decrease in 8-OHdG is unclear, however it is well documented that oxidatively modified bases undergo base excision repair, and it was recently demonstrated that the OGG1 pathway is up-regulated following an acute bout of endurance exercise (64). In contrast to DNA, the only recourse for oxidative damage to protein is degradation, via the protease pathway, and synthesis of new proteins. It is also possible that an up-regulation of antioxidant enzymes simply serves to maintain levels of oxidative stress for physiological purposes, and prevent further damage from occurring with excessive ROS generation.

Progressive resistance exercise had no effect on mtDNA. Although, the traditional belief is that resistance training does not increase mitochondrial content (1;52), a concept referred to as "gene shifting" has been proposed in patients with mitochondrial disease, which may be relevant to older adults. This concept is based on the idea that muscle satellite cells have a low mutational burden (23). In other words, because satellite cells are known to remain quiescent unless induced, it is theorized that these cells do not accumulate somatic mtDNA mutations and deletions (*via* ETC flux and subsequent oxidative stress) at the same rate as mtDNA from mature myofibers. It is well known that satellite cells are activated in response to muscle injury (5). Upon activation, satellite

cells proliferate, giving rise to myogenic precursor cells that fuse with mature myofibers. During the fusion process the organelles of the satellite cell, such as nuclei, mitochondria, and molecular machinery are donated to the existing myofiber. Hence, the process of muscle damage and regeneration may aid in maintaining the ratio of wild-type to mutant mtDNA above the threshold at which the metabolic demands can no longer be met. Taivassalo and colleagues (1999) demonstrated a significant increase in wild-type mtDNA following resistance exercise training. In addition, there was a significant decrease in muscle fibers that stained negative for cytochrome oxidase, suggesting a shift towards a more normal ETC function (72). Given that aging has been referred to as the most common acquired mitochondrial disease (25), there is potential that resistance exercise may have similar effects in a population of older adults. In the present study, all the subjects tested had pre-existing visible mtDNA deletion products, and resistance exercise training did not reduce the number of deletions. Interestingly, it has recently been suggested that endurance exercise training in patients with mitochondrial disease may actually decrease the proportion of wild-type mtDNA (73). In the present study, however resistance exercise training did not change the proportion of normal length mtDNA, suggesting from a genetic standpoint that resistance exercise is a safe treatment option for older adults.

In the present investigation we demonstrated no significant increase in any of the oxidative enzymes measured, using biochemical techniques. To date, there are no other known investigations to examine the effects of resistance exercise on oxidative enzymes in older adults. The study by Taivassalo and colleagues (1999) demonstrated a significant

decrease in the number of negatively stained cytochrome oxidase fibers in patients with mitochondrial disease. In addition, we have previously demonstrated that resistance exercise training increased complex IV maximal enzyme activity following 14 weeks of whole-body resistance training in older adults (Parise et al., 2003 – unpublished observation). Exactly why we could not reproduce this finding is not completely clear. However, differences in the exercise protocol, duration of training, and sample size may be contributing factors. Finally, the traditional notion that in order to induce mitochondrial adaptation one must perform exercise that stresses the mitochondria, such as endurance exercise, may not be completely true. Here we report ***no*** change in oxidative enzyme activity. However, in the face of muscle fiber hypertrophy, mitochondrial biogenesis must occur or we would expect a relative reduction in enzyme activity. Therefore, although resistance exercise did not result in an increase in enzyme activity, it is probable that mitochondrial biogenesis was stimulated by resistance exercise, since there was no decrease in enzyme activity in the face of a significant increase in muscle fiber cross-sectional area (unpublished observations).

In conclusion, we report that 12 weeks of resistance exercise training may be a sufficient stimulus to increase muscle antioxidant capacity. In addition, there appears to be no detrimental effect of resistance exercise on normal length mtDNA or mtDNA deletions.

Figure 1. Muscle total protein carbonyl content was not different following exercise training. Alpha was set at $P<0.05$.

Figure 2. Antioxidant enzyme capacity increased as a function of resistance training. A. * denotes a significant up-regulation of muscle catalase activity following exercise training ($P<0.05$). B. * denotes a significant up-regulation of CuZnSOD activity following exercise training ($P<0.05$). C. No up-regulation of MnSOD activity was observed after training as compared to before.

Figure 3. A. represents a typical mtDNA profile before and after resistance training. B. Normal length mtDNA was not different after training as compared to before. C. The number of visible deletion products were not different after training as compared to before.

Figure 1. Total carbonyls following resistance exercise training.

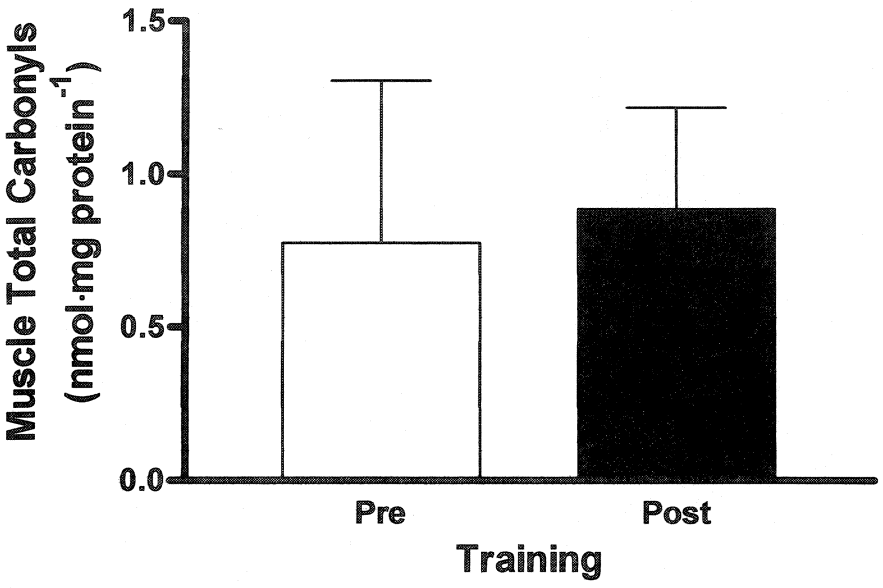
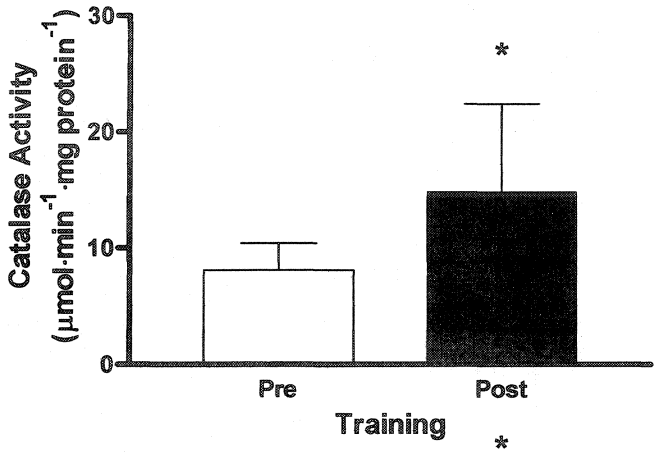
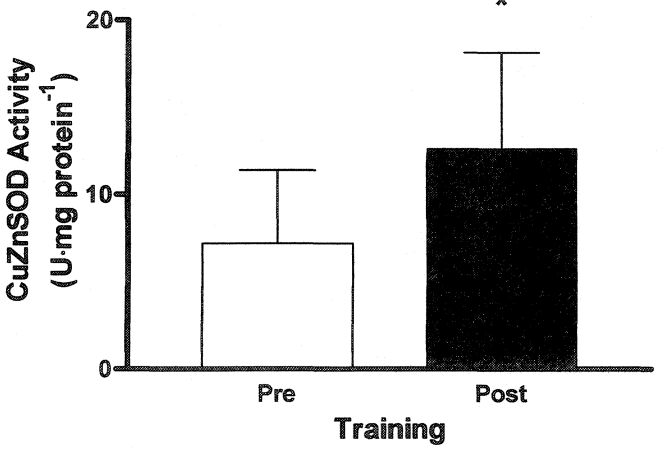


Figure 2. Antioxidant enzyme activity following resistance exercise training.

A.



B.



C.

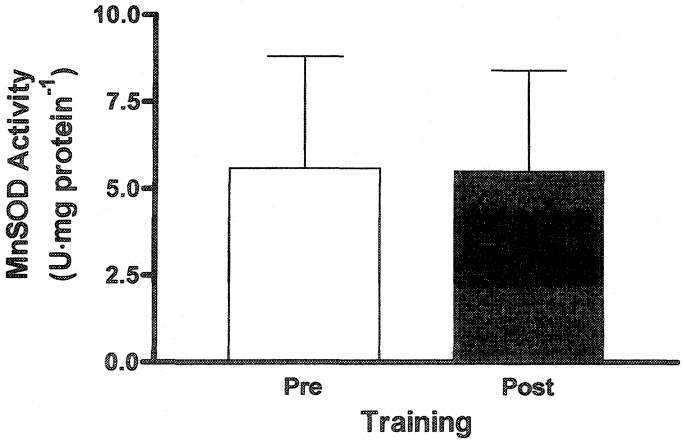
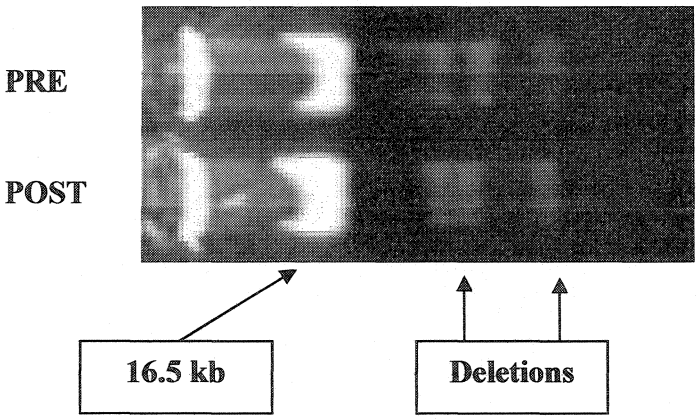


Figure 3. Mitochondrial DNA following resistance exercise training.

A.



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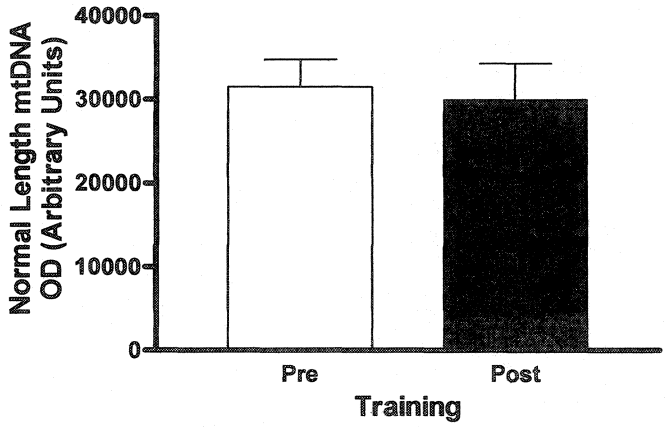

C.

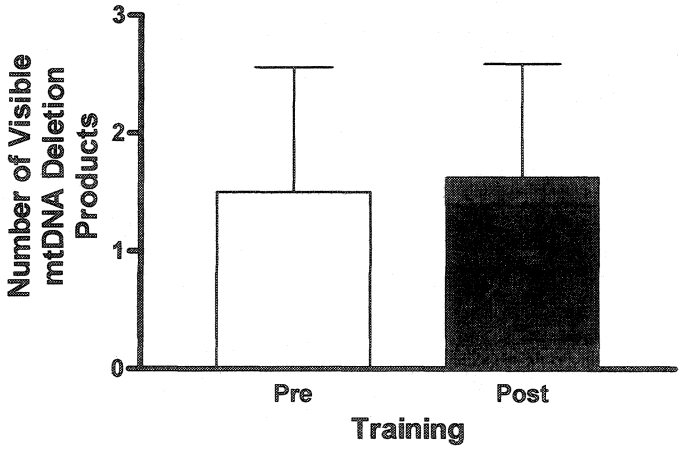

Table 1. *Subject Characteristics.*

Age (years)	71.2 ± 6.5
Mass (kg)	88.8 ± 11.3
Height (cm)	174.7 ± 4.8
Body Fat (%)	21.4 ± 3.6

Table 1. Typical subject characteristics are reported. All subjects were healthy, recreationally active older adults. Values are mean ± SD.

Table 2. *Oxidative enzymes following resistance exercise training.*

	Training Leg		Untrained Leg	
	Pre	Post	Pre	Post
Citrate Synthase	13.1 ± 5.1	12.7 ± 4.1	13.7 ± 4.5	12.8 ± 2.9
Complex I+III	2.4 ± 0.7	2.7 ± 1.4	2.7 ± 0.7	2.8 ± 0.5
Complex II+III	1.9 ± 0.6	1.9 ± 0.9	2.1 ± 1.1	2.0 ± 0.5
Complex IV	6.7 ± 1.5	6.4 ± 2.2	6.8 ± 1.5	5.7 ± 1.0

Table 2. None of the oxidative enzymes measured were significantly changed following resistance exercise training. Values are mean ± SD, and expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww. Alpha was set at $P<0.05$.

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10.0. General Discussion.

The mitochondrial theory of aging has been at the forefront of aging research for the last thirty years (Harman 1972). This theory was proposed based on the assumption that mitochondria are the primary producers of ROS. Indeed, the last thirty years of research has established that, in fact, mitochondria are responsible for the production of the majority of all ROS (Harman 1972). The mitochondrial theory of aging postulates that the production of ROS increase in an age-dependent manner, and that subsequent damage to mitochondria, and other cellular constituents ultimately leads to cellular aging. A vicious cycle of events has been proposed to describe the events associated with the mitochondrial theory, which include: 1. An increase in oxidative stress, 2. mtDNA damage, 3. Defective ETC proteins, and 4. ETC deficiency (Fig. 1). Where this vicious cycle begins and ends is difficult to elucidate, however, an increase in oxidative stress is thought to be at the heart of the matter.

Irrespective of the underlying mechanism(s) of aging, there is a need to identify effective therapies to reduce or reverse age-related losses in skeletal muscle function. One such therapy that has been shown to be effective is progressive resistance exercise training. Resistance training represents a non-pharmacological approach, which has been associated with many beneficial outcomes such as improvements in strength (Frontera et al., 1988, Charette et al., 1991), increases in muscle fiber hypertrophy (Frontera et al., 1988, Charette et al., 1991, Brown et al., 1990), and most importantly an improvement in function (Fiatarone et al., 1990, Fiatarone et al., 1994, Hunter et al., 1995, Schlicht et al.,

2001). Epidemiological studies have identified that regular exercise is associated with a reduction in morbidity (Fries 1996), as well as mortality (Paffenbarger et al., 1993, Sandvik et al., 1993). These observations suggest that resistance exercise induces positive cellular adaptations that may disturb the processes responsible for aging. Assuming the mitochondrial theory of aging is valid, then it stands to reason that resistance exercise may stop or slow the progression of the so-called vicious cycle. Thus, this dissertation set out to accomplish two objectives: 1. To conduct a comprehensive examination of the mitochondrial theory of aging in skeletal muscle; and 2. To examine the effects of regular resistance exercise on indices of the mitochondrial theory of aging.

10.1. Antioxidant Enzymes and Aging

Antioxidant capacity is an important factor in determining cellular redox status. In fact, oxidative stress is defined as an imbalance between ROS production and the ability to quench ROS. Thus, oxidative stress can be induced by either an over production of ROS or a decrease in antioxidant capacity. Age-related oxidative stress has been demonstrated in some tissues to be related to a reduction in antioxidant capacity (Ji 1993), however studies in animals and humans have reported that antioxidant enzyme activity increases as a function of age in skeletal muscle (Leeuwenburgh et al., 1994, Pansarasa et al., 2000, Pansarasa et al., 1999, Lammi-Keefe et al., 1984, Vertechy et al., 1989, Ji et al., 1990, Lawler et al., 1993, Luhtala et al., 1994). Indeed, in our hands we also report a significant age-related higher MnSOD activity, and catalase activity with no change in CuZnSOD (Table 1.). The compartmentalization of changes in the present study lends support to

age-related mitochondrial oxidative stress, an integral component of the mitochondrial theory of aging. MnSOD is the mitochondrial isoform of SOD, and thus an elevated activity reflects a mitochondrial derived oxidative stress. CuZnSOD on the other hand is the cytosolic isoform of SOD and does not appear to change with advancing age. Interestingly, catalase is also found in the cytosol, however it was significantly higher in older adults as compared to young, unlike CuZnSOD. The apparent discrepancy may be explained by the function of each of these enzymes. The SOD enzymes are known to detoxify O_2^- to H_2O_2. Hydrogen peroxide is a relatively stable ROS, and possesses the ability to freely cross membranes and travel great distances within a cell (Matsuo and Kaneko 2000). Superoxide, on the other hand is short-lived and cannot freely cross-membranes, rather requires an anion channel for it to be transported (Lynch and Fridovich 1978). This presents the distinct possibility that O_2^- becomes trapped in the mitochondria and with aging requires an up-regulation of MnSOD. Once O_2^- is detoxified to H_2O_2, H_2O_2 can then cross the inner and outer mitochondrial membrane and enter the cytosol. In the cytosol, mitochondrial-derived H_2O_2 can be detoxified by catalase to O_2 and H_2O. A large flux of H_2O_2 out of the mitochondria may have caused an up-regulation of catalase activity. Therfore, similar to previous studies (Leeuwenburgh et al., 1994, Pansarasa et al., 2000, Pansarasa et al., 1999, Lammi-Keefe et al., 1984, Vertechy et al., 1989, Ji et al., 1990, Lawler et al., 1993, Luhtala et al., 1994), we report that antioxidant enzyme activity is increased as a function of age in skeletal muscle, and thus a reduction in their activity cannot be the source of age-related oxidative stress.

Table 1. Antioxidant Enzymes and Aging

	YOUNG	OLD
Catalase ($\mu mol \cdot min^{-1} \cdot g^{-1}$ protein)	6.2 ± 2.4	8.5 ± 2.0*
MnSOD ($U \cdot mg^{-1}$ protein	4.4 ± 1.7	6.6 ± 2.6*
CuZnSOD ($U \cdot mg^{-1}$ protein)	10.0 ± 4.5	9.9 ± 4.3

10.2. Antioxidant Enzymes and Resistance Exercise

In addition, progressive resistance exercise training was sufficient to induce an up-regulation of CuZnSOD and catalase activity, however had no effect on MnSOD activity (Table 2.). There are several possible explanations for the compartmentalized response to resistance exercise training. First, it is possible that the source of ROS production in response to resistance exercise was localized to the cytosol. The inflammatory response, in particular neutrophils and macrophages, peak approximately 24 to 48 hr following an eccentric exercise bout (Beaton et al., 2002, Stupka et al., 2000). Part of the normal function of phagocytic cells is an associated respiratory burst resulting in the production of O_2^-, H_2O_2, and $OH\cdot$ by the enzyme NADPH oxidase (Babior 1978). In addition, loss of Ca^+ homeostasis during exercise may result in the transformation of xanthine dehydrogenase to xanthine oxidase. Both of these enzymes catalyze the reaction of hypoxanthine to xanthine, however xanthine oxidase produces O_2^- and H_2O_2 as byproducts (Kondo et al., 1993, McCord 1985). Thus repeated bouts of muscle damage and repair, induced by regular resistance exercise, may have caused a chronic oxidative stress eliciting an adaptive response in the cytosolic antioxidant enzymes, but not the mitochondrial isoform of SOD due to similar reasons as those discussed above.

Alternatively, it is possible that MnSOD activity has reached a ceiling as a function of aging, and thus may require an exercise stress above and beyond that elicited in the present study. Interestingly, two investigations reported that endurance exercise training in old animals resulted in an up-regulation of GPX activity (Hammeren et al., 1993), whereas Leeuwenburgh and colleagues (1994) reported no changes in antioxidant enzymes following exercise training. These authors also suggest that the lack of an exercise induced increase in antioxidant capacity may be related to a ceiling effect, induced by aging.

Based on our data it appears that the changes in antioxidant enzyme activity are attributable to post-translational mechanisms. In study 2 we reported no increase in the protein content of MnSOD, CuZnSOD, or catalase (Table 3), however in study 3 we reported significant increases in the antioxidant enzyme activity of CuZnSOD, and catalase following exercise training. Being cognizant of the inherently less sensitive or reproducible nature of western blots, together the results suggest that antioxidant adaptations occurred through an increase in post-translational mechanisms. In support of these findings, a significantly higher CuZnSOD enzyme activity in rat muscle, without an increase in CuZnSOD protein content was reported following exercise training (Oh-ishi et al., 1997). In addition, antioxidant enzymes are thought to possess the ability to rapidly respond to stress evidenced by up-regulation immediately following an acute exercise bout (Leeuwenburgh and Ji 1996, Ji et al., 1990, Lawler et al., 1993, Sen et al., 1994, Quintanilha and Packer 1983, Radak et al., 1995, Ji and Fu 1992, Ji et al., 1992,

Quintanilha 1984, Jenkins 1993). Thus it is generally accepted that antioxidant enzymes are inducible through post-translational mechanisms.

Table 2. Antioxidant Enzyme Activity and Resistance Exercise

	PRE	POST
Catalase ($\mu mol \cdot min^{-1} \cdot g^{-1}$ protein)	8.1± 2.3	14.8 ± 7.6*
MnSOD ($U \cdot mg^{-1}$ protein	5.6 ± 3.2	5.5 ± 2.9
CuZnSOD ($U \cdot mg^{-1}$ protein)	7.2 ± 4.2	12.6 ± 5.5*

Table 3. Antioxidant Enzyme Protein Content and Resistance Exercise

	PRE	POST
Catalase	161.4 ± 126.8	190.4 ± 107.8
MnSOD	106 ± 64	137 ± 100
CuZnSOD	44.5 ± 32.9	37.5 ± 23.3

Values are arbitrary OD units.

10.3. Aging and Oxidative Damage

Age-related increases in antioxidant enzymes infer the development of an age-related oxidative stress. Indeed, 8-OHdG, and muscle total protein carbonyls were significantly higher in old vs. young adults (Table 4). Animal studies commonly report a significant age-associated accumulation in oxidative damage to lipids, proteins, and DNA in a variety of tissues (Sohal and Dubey 1994, Sohal et al., 1993, Hamilton et al., 2001, Leeuwenburgh et al., 1994, Xia et al., 1995, Butterfield et al., 1997, Ji et al., 1991). More recently, data from human skeletal muscle suggests that aging is associated with an elevation in lipid peroxides, protein carbonyls, and 8-OHdG (Fano et al., 2001, Pansarasa

et al., 2000, Pansarasa et al., 1999, Miro et al., 2000, Mecocci et al., 1999). These findings represent one of the major events postulated by the mitochondrial theory of aging, as they definitively demonstrate oxidative stress in advancing age.

Table 4. Oxidative Damage and Aging

	YOUNG	OLD
8-OHdG (ng·g creatinine^{-1})	5333 ± 1191	7714 ± 1402*
Protein Carbonyls (nmol·mg protein^{-1})	0.26 ± 0.14	0.72 ± 0.43*

10.4. Oxidative Damage and Resistance Exercise

The effects of resistance exercise on markers of oxidative damage remain unclear. In study 2 we demonstrated a significant 17.5% reduction in levels of 8-OHdG following exercise training, and in study 3 we demonstrate that resistance training had no effect on levels of muscle total protein carbonyls (Table 5). We chose not to examine levels or urinary 8-OHdG in study 3 since a uni-lateral leg model was utilized and not likely to induce changes given the relatively small muscle mass stressed, whereas the protocol in study 2 was a full-body exercise program resulting in muscle damage to a relatively large muscle mass. Irrespective of difference in study design, it stands to reason that if resistance training induced a reduction in the production of ROS, or an increase in antioxidant enzymes then decreases in oxidative damage to both DNA and proteins would have been realized. Since we did not observe these changes, the reduction we observed in urinary 8-OHdG was likely related to something other than a reduction in oxidative stress.

The recourse for oxidative damage to DNA involves a process referred to as base excision repair (BER), which ultimately results in the repair of oxidatively damaged DNA, whereas the recourse for oxidative damage to protein is simply to tag and degrade the protein. This presents the possibility that the decreases we observed in 8-OHdG following resistance training may have been related to an up-regulation in the enzyme OGG-1, which is the enzyme in the BER process specific to the repair of 8-OHdG (Radak et al., 2002, Radak et al., 2003). Indeed, recent evidence demonstrates an up-regulation of this pathway following an acute bout of endurance exercise in humans (Radak et al., 2003). Although we report an increase in antioxidant enzyme activity with resistance training it is possible that the observed decrease in 8-OHdG was due to an up-regulation in the BER pathway. This may represent the critical adaptation to slowing or reversing some of the age-associated deficiencies in skeletal muscle. In the vicious cycle, proposed in the mitochondrial theory of aging, damage to mtDNA represents a critical event leading to deficiency of the ETC and an increase in ROS production. An up-regulation of OGG-1 following resistance exercise training may disturb the vicious cycle thus slowing the aging process.

Table 5. Oxidative Damage and Resistance Exercise

	PRE	POST
8-OHdG (ng·g creatinine^{-1})	10783 ± 5856	8897 ± 4030*
Protein Carbonyls (nmol·mg protein^{-1})	0.78 ± 0.53	0.89 ± 0.33

10.5. Oxidative Enzymes and Aging

Loss of ETC function represents a critical event in the mitochondrial theory of aging, which ultimately may lead to cell atrophy (Wanagat et al., 2001, Cao et al., 2001). Studies in humans report equivocal findings with some investigations reporting decreases in ETC activity (Boffoli et al., 1994, Trounce et al., 1989, Cooper et al., 1992, Cardellach et al., 1989), whereas others report no change associated with age (Rasmussen et al., 2003, Chretien et al., 1998, Barrientos et al., 1996, Zucchini et al., 1995). We report that citrate synthase, complex II+III, and complex IV activity were not different in old as compared to young adults (Table 6). We do report, however, that complex I+III activity was significantly higher in old vs. young individuals (Table 6). The lack of change in the aforementioned enzymes is consistent with several reports in human skeletal muscle (Rasmussen et al., 2003, Chretien et al., 1998, Barrientos et al., 1996, Zucchini et al., 1995). It is possible that with advancing age there is an attempt to maintain normal levels of ETC function, which may be achieved by mitochondrial proliferation. Aging has been shown to be associated with a proliferation of mitochondria, eventually progressing to a condition described histochemically as ragged-red fibers (Muller-Hocker 1990). Although aging may cause abnormalities in mtDNA, it is possible that proliferation of mitochondria act to compensate for abnormalities in mtDNA, resulting in the maintenance of ETC activity. However, proliferation beyond a threshold may become pathologic and lead to ragged-red fiber formation. To the authors knowledge, a higher complex I activity has never been reported in human or animal skeletal muscle consequent to aging. Mitochondrial proliferation is not likely responsible for this

observation since we would expect that all enzymes would be higher with such a non-specific response. Complex I is the largest complex of all the ETC enzymes composed of ~ 43 subunits. The specific up-regulation of complex I activity may be an attempt to compensate for mitochondrial deficiency as a function of age and may reflect a higher redox sensitivity than the other complex enzymes.

Table 6. Oxidative Enzymes and Aging

	YOUNG	OLD
Citrate Synthase	11.5 ± 2.6	13.2 ± 4.5
Complex I+III	1.9 ± 0.5	2.5 ± 0.5*
Complex II+III	1.9 ± 0.6	1.8 ± 0.6
Complex IV	5.7 ± 2.7	6.7 ± 1.0

Values expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww

10.6. Oxidative Enzymes and Resistance Exercise

In study 2 we reported that resistance exercise resulted in a specific up-regulation of complex IV activity (Table 7a). This finding was particularly interesting since complex IV activity has been implicated as being important in maintaining normal electron flow (Martinez et al., 1996). It has been suggested that a higher complex IV activity acts to draw electrons down the chain, therefore reducing the proportion of electrons that "leak" and become ROS by reacting with O_2 (Kwong and Sohal 2000). In further support of this notion, the complex IV/I+III ratio was significantly higher following resistance training as compared to before. Together, these results implicate complex IV as playing an indirect role as an antioxidant by improving the efficiency of the ETC. Unfortunately, we

were unable to duplicate these findings in study 3 (Table 7b). There may be several reasons for this including, different exercise protocols, a longer training period in study 2, and a lack of statistical power in study 3.

A second interesting finding related to oxidative phosphorylation was a significant increase in mtCK protein content (Table 8). Mitochondrial CK is found in the inter-membrance space of the mitochondria and is critical for the re-phosphorylation of free creatine. Following muscle contraction, free creatine is rephosphorylated by mtCK in an ATP dependent process. The increase in mtCk protein content likely represents an adaptation to exercise induced metabolic stress to maintain cellular homeostasis. More importantly, the increase in mtCK, like the increase in complex IV activity, appears to be a specific up-regulation of mitochondrial components without an increase in mitochondrial volume, as demonstrated by lack of change in citrate synthase activity.

Table 7a. Oxidative Enzymes and Resistance Exercise

	PRE	POST
Study 2		
Citrate Synthase	12.2 ± 2.8	13.0 ± 3.2
Complex I+III	1.9 ± 0.6	1.7 ± 0.5
Complex II+III	1.6 ± 0.5	1.8 ± 0.8
Complex IV	6.7 ± 1.5	8.6 ± 2.8*
Complex IV/I+III	3.9 ± 1.8	5.6 ± 3.1*

Values expressed µmol·min-1·mg-1 ww
Complex IV/I+III is a ratio

Table 7b. Oxidative Enzymes and Resistance Exercise

	PRE	POST
Study 3		
Citrate Synthase	13.1 ± 5.1	12.7 ± 4.1
Complex I+III	2.4 ± 0.7	2.7 ± 1.4
Complex II+III	1.9 ± 0.6	1.9 ± 0.9
Complex IV	6.7 ± 1.5	6.4 ± 2.2

Values expressed μmol·min-1·mg-1 ww

Table 8. Mitochondrial CK and Resistance Exercise

	PRE	POST
Protein Content	109.7 ± 134.8	213.4 ± 166.7*

10.7. Mitochondrial DNA and Aging

The most common observation in mtDNA as a result of normal aging is an increase in the number and proportion of mtDNA deletions, and a decrease in the proportion of normal mtDNA (Fayet et al., 2002, Linnane et al., 1990, Cortopassi and Arnheim 1990, Yarovaya et al., 2002, Cormio et al., 2000, Zhang et al., 1998, Liu et al., 1998, Kovalenko et al., 1997, Eimon et al., 1996, Melov et al., 1995, Schwarze et al., 1995, Simonetti et al., 1992, Katayama et al., 1991, Wanagat et al., 2001, Kovalenko et al., 1998, Kopsidas et al., 1998). Accordingly, we report a significant increase in the number of mtDNA deletions in older adults as compared to young (Table 9). In addition, we demonstrate a significant reduction in the proportion of normal mtDNA (Table 9). This is particularly interesting given that we did not observe a significant reduction in ETC activity. It may be that the nature of mitochondrial genetics, with many copies of mtDNA per mitochondrion, and hundreds to thousands of mitochondria per muscle fiber, allow for a large degree of

redundancy. Therefore a 37% decrease in normal mtDNA may not affect mitochondrial energetics. Results from cybrid cell lines lend support to this notion by demonstrating that there is no apparent phenotype associated with mtDNA deletions until the mutational burden has reached very high levels (~ 90%) (Attardi et al., 1995, Moraes and Schon 1996). In addition evidence suggests an increase in transcript stability with aging, thus protein content can be maintained in the face of decreasing full-length mtDNA by an increase in mitochondrial mRNA stability (Barazzoni et al., 2000). Consequently, lack of changes in ETC activity despite the low-level accumulation of mtDNA deletions, and the reduction of full-length mtDNA in muscle homogenates may not be a surprise after all. Recent evidence suggests that mtDNA deletions accumulate in a focal and stochastic manner in isolated muscle fibers (Brierley et al., 1998, Kopsidas et al., 2002, Wanagat et al., 2001). Electron transport chain deficiency and muscle fiber atrophy are associated with fibers containing deletions (Wanagat et al., 2001, Cao et al., 2001). It is hypothesized that the more susceptible muscle fibers eventually accumulate mtDNA deletions crossing a critical threshold where ETC function is lost and the fibers undergo pronounced fiber atrophy, and eventual cell death. It is possible that in frail adulthood ETC dysfunction manifests at the whole muscle level and impaired functional capacity becomes apparent, leading to sarcopenia. Thus, low-level mtDNA deletions such as those observed in the present studies may reflect isolated muscle fibers with a high level of deletions, and the observed decrease in full-length mtDNA may be offset by an increase in mitochondrial mRNA stability (Barazzoni et al., 2000). Hence, ETC dysfunction may not be apparent in whole muscle homogenates from healthy older adults.

Table 9. Mitochondrial DNA and Aging

	YOUNG	OLD
Full-length mtDNA (OD units)	27490 ± 5375	17293 ± 8569*
Number of Subjects with Deletion Products	0/8	6/8

10.8. Mitochondrial DNA and Resistance Exercise

Resistance exercise did not alter the level of mtDNA deletions, or the proportion of normal mtDNA in older adults (Table 10). We anticipated that resistance exercise would have a significant effect on mtDNA, given the recently described phenomenon of "gene shifting" (Taivassalo et al., 1999). Taivassalo and colleagues (1999) demonstrated that regular resistance exercise in patients with mitochondrial disease resulted in a significant increase in the proportion of normal mtDNA, which was associated with a significant decrease in cytochrome oxidase negative fibers suggesting a restoration of ETC function. Given the similarities in phenotype between advanced age and sporadic mitochondrial disease, we hypothesized that resistance exercise may be an effective therapy in reversing mtDNA abnormalities, however our results do not support this notion. In the present investigations we demonstrated that mtDNA deletions were present in almost all older adults. Interestingly, recent evidence has suggested that aerobic exercise training actually results in a decrease in the proportion of wild-type mtDNA (Taivassalo et al., 2001). In addition, acute treadmill running in rats has been shown to result in the induction of mtDNA deletions (Sakai et al., 1999). It is important to note that although resistance

exercise training did not decrease mtDNA deletions, results from the present studies suggest that there was no increase in the number of mtDNA deletion products, and did not decrease the amount of amplifiable full-length mtDNA.

Table 10. Mitochondrial DNA and Resistance Exercise

	PRE	POST
Study 2		
Full-length mtDNA (OD units)	28045 ± 8609	27470 ± 8387
Number of Visible Deletion Products	2.1 ± 0.9	1.8 ± 0.8
Study 3		
Full-length mtDNA (OD units)	31453 ± 3305	29870 ± 4377
Number of Visible Deletion Products	1.5 ± 1.1	1.6 ± 1.0

In conclusion, aging in human skeletal muscle does appear to be related to oxidative stress. This is evidenced by changes in oxidative damage to proteins and DNA, up-regulation of several key antioxidant enzymes, and an accumulation of mtDNA deletion products. However, an important point to be made is that these events did not have any effect on ETC activity, which may be related to the relative health of our subject population. Thus, we propose a model of aging that involves cellular adaptation, accumulated cellular abnormalities, and their combined effects on cell function (Fig. 2). We propose that through a degree of redundancy and adaptation, "normal" cellular function, for example, normal ETC enzyme activity, can be maintained in the face of accumulating cellular abnormalities, such as mtDNA deletions, and oxidatively modified biomolecules. However, once a threshold is crossed, adaptive potential is lost, abnormalities continue to accumulate and there is a loss of "normal" cellular function.

Therefore, the mitochondrial theory of aging may be apparent in human skeletal muscle despite reports from Rasmussen and colleagues (2003), and results from study 1 in this dissertation which demonstrate normal ETC enzyme activity. It is possible that subjects in these two reports are below the biological critical threshold whereby mitochondrial function fails. Studies in frail adults may reveal results more consistent with the mitochondrial theory. In addition, resistance exercise appears to be an effective therapy at interrupting the course of the vicious cycle of events associated with the mitochondrial theory of aging. This was evidenced by a reduction in 8-OHdG, an increase in antioxidant enzymes, and improvement in ETC activity. Therefore, regular resistance exercise may delay the point at which the critical threshold is crossed, by shifting cellular abnormalities to the right, and cellular adaptation to the left (in figure 2), allowing for "normal" cellular function to continue. This is consistent with the findings that regular exercise is a form of therapy known to reduce morbidity (Fries 1996) and mortality (Paffenbarger et al., 1993, Sandvik et al., 1993), and results from the present studies suggest that resistance exercise may induce adaptations that aim to deal with the cause and not just the symptoms of aging.

10.9. Limitations and Future Directions.

There are several limitations that can be identified in the present studies. First is the fact that all of our muscle measurements were made in whole-muscle homogenates. Recent literature suggests that mtDNA deletions/mutations accumulate to high levels in a stochastic manner in muscle, and are associated with ETC dysfunction and muscle fiber

atrophy (Brierley et al., 1998, Wanagat et al., 2001, Cao et al., 2001, Kopsidas et al., 2002). Thus, results from whole muscle homogenates may be diluted by "normal", functional muscle fibers.

Second, our measurement of antioxidant enzymes did not include GPX activity. GPX has previously been shown to be responsive to acute and chronic (Hammermen et al., 1993, Ji et al., 1993) aerobic exercise, and is present in the cytosol as well as the mitochondria. Thus, in the present studies we may not have realized the total extent of antioxidant adaptation to aging or resistance exercise.

Future studies should examine the concept of "gene shifting" in aging skeletal muscle by harvesting satellite cell derived myoblasts from aging muscle and examining mtDNA deletions in these cells and compare them to mtDNA deletions in mature myofibers. In addition, the effect of resistance exercise on variables associated with the mitochondrial theory should be examined in single fibers. Finally, a broader age-range should be examined to validate our proposed theory of aging. In particular frail adults should be examined to determine if the mitochondrial theory becomes more apparent in skeletal muscle in advanced age.

Figure 1. The Vicious Cycle.

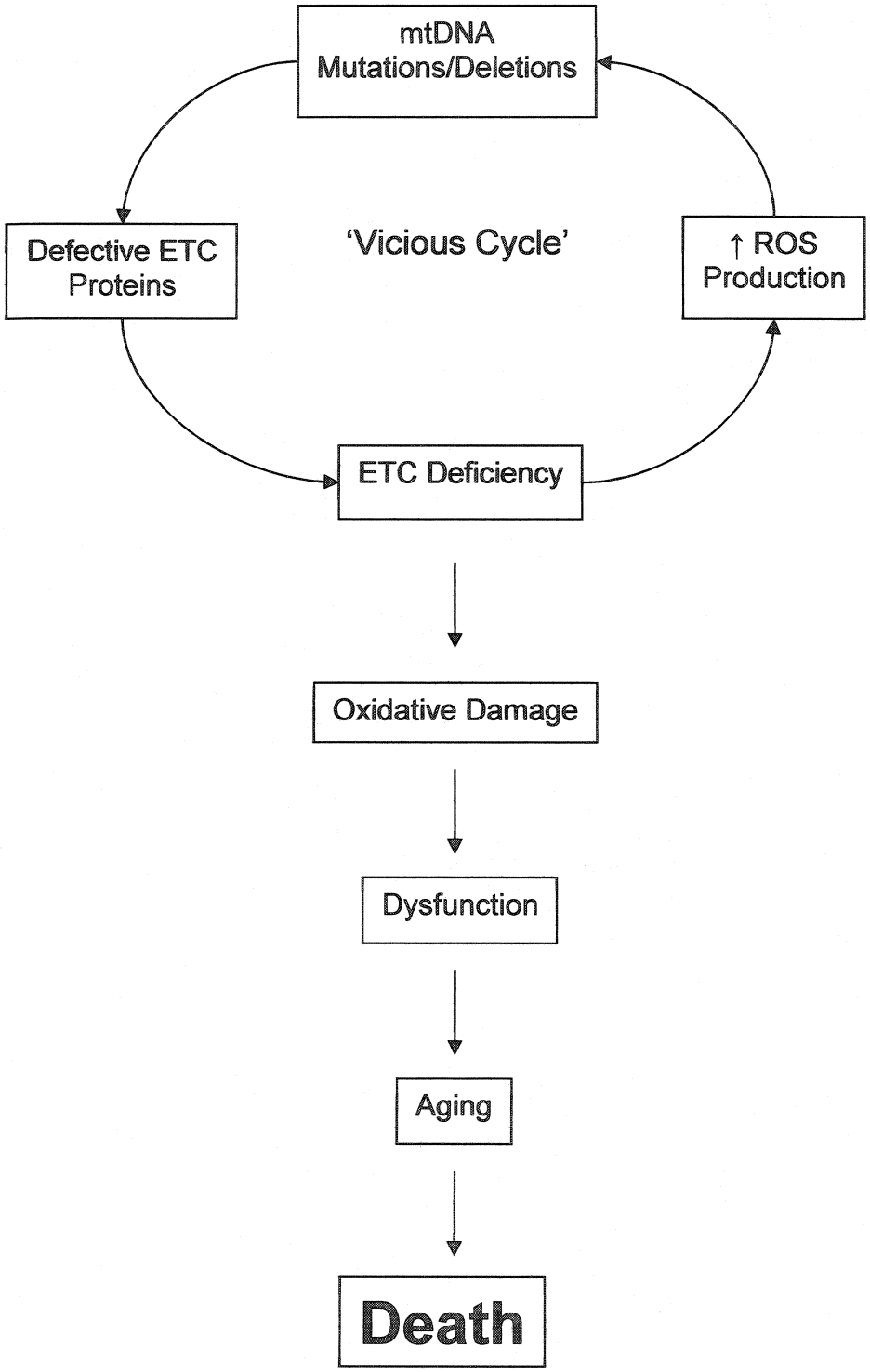
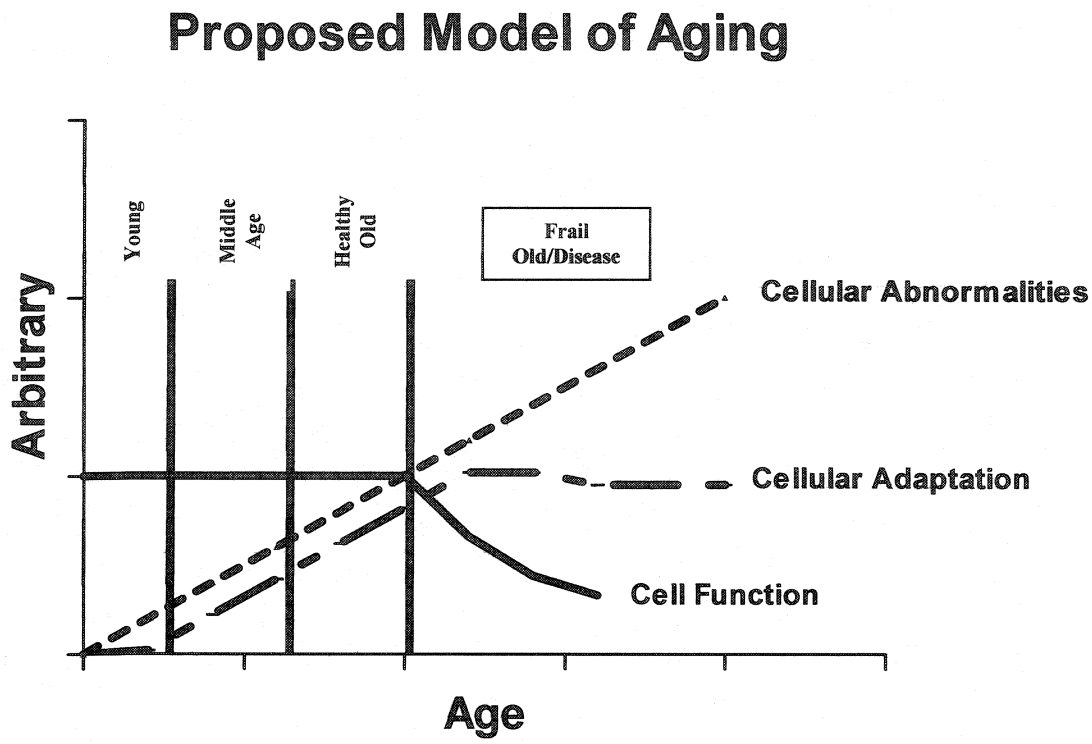


Figure 1.The vicious cycle as proposed by the mitochondrial theory of aging.

Figure 2. Proposed Model of Aging.



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APPENDIX 1

RAW DATA TABLES

STUDY 1: Old vs. Young

Table 1. Subject Characteristics

	AGE (YEARS)		MASS (KG)		HEIGHT (CM)		FFM (KG)		% BODY FAT	
	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD
	20.0	75.0	108.5	89.8	195.0	167.0	73.3	66.6	32.4	25.8
	25.0	71.0	69.1	107.1	156.0	180.0	45.8	81.6	33.6	23.8
	28.0	71.0	68.2	98.7	174.0	173.0	52.4	73.4	23.2	25.6
	19.0	69.0	81.8	86.5	175.0	174.0	64.6	67.9	21.0	21.5
	20.0	74.0	90.0	88.0	181.0	173.0	64.2	67.9	28.6	22.8
	26.0	68.0	85.5	89.1	173.0	173.0	63.2	71.2	26.1	20.1
	29.0	72.0	65.9	89.8	165.0	173.0	51.7	67.2	21.6	25.2
	22.0	70.0	79.5	81.4	170.0	171.0	56.6	65.4	28.9	19.6
	22.0	68.0	62.5	71.9	171.0	178.0	52.2	62.3	16.5	13.4
	23.0	68.0	70.9	71.4	178.0	170.0	50.8	58.8	28.3	17.7
	20.0	90.0	68.2	78.4	173.0	179.0	49.4	64.5	27.5	17.7
	20.0	66.0	70.0	106.0	180.0	185.0	53.1	73.5	24.1	23.4
Mean	22.8	71.8	76.7	88.2	174.3	174.7	56.4	68.4	26.0	21.4
SD	3.4	6.3	13.1	11.6	9.4	5.0	8.1	6.0	4.9	3.8

Table 2. Effect of Age on Urinary 8-OHdG

	OLD	YOUNG
	9527	4854
	7560	6960
	7715	4494
	7409	5266
	9927	4057
	9154	4275
	7005	6427
	5221	4141
	6288	6313
	7044	4606
	8014	7274
Mean	7715	5334
SD	1402	1192

Table 2. Effect of Age on Muscle Protein Carbonyls

	OLD	YOUNG
	0.36	0.18
	0.36	0.26
	0.31	0.17
	0.58	0.28
	0.67	0.05
	1.60	0.57
	1.19	0.34
	0.73	0.28
	0.68	0.24
Mean	0.72	0.26
SD	0.43	0.14

Table 3. Effect of Age on Muscle MnSOD Activity

	OLD	YOUNG
	8.07	7.17
	6.53	3.16
	6.77	7.09
	6.18	3.00
	7.50	4.88
	7.78	6.05
Mean	7.14	5.23
SD	0.75	1.86

Table 4. Effect of Age on Muscle CuZnSOD Activity

	OLD	YOUNG
	11.26	3.96
	9.56	19.42
	15.70	5.05
	13.00	8.05
	7.75	13.58
	9.96	14.92
Mean	11.20	10.83
SD	2.82	6.10

Table 5. Effect of Age on Muscle Catalase Activity

	OLD	YOUNG
	8.65	8.99
	5.98	6.12
	6.43	7.18
	9.05	5.87
	8.19	7.74
	10.05	1.00
	4.17	4.81
	11.90	9.62
	9.33	4.03
	9.29	7.60
	9.36	7.91
	9.62	3.90
Mean	8.50	6.23
SD	2.07	2.46

Table 6. Effect of Age on Muscle Complex I+III Activity

	OLD	YOUNG
	2.73	2.03
	3.28	2.34
	2.38	1.80
	2.12	2.04
	1.98	1.19
	2.86	2.68
	3.06	1.67
	2.05	2.27
	1.93	1.48
Mean	2.49	1.94
SD	0.51	0.46

Table 7. Effect of Age on Muscle Complex II+III Activity

	OLD	YOUNG
	1.32	1.43
	2.26	0.95
	2.55	2.55
	2.45	2.23
	3.14	1.05
	1.37	2.31
	1.60	1.77
	1.12	2.35
	1.62	1.29
Mean	1.94	1.77
SD	0.69	0.61

Table 8. Effect of Age on Muscle Complex IV Activity

	OLD	YOUNG
	6.65	6.08
	7.23	9.99
	8.26	2.54
	6.60	8.51
	6.92	5.59
	5.12	7.56
	6.91	3.40
	5.46	5.23
	7.25	2.32
Mean	6.71	5.69
SD	0.95	2.67

Table 9. Effect of Age on Muscle Citrate Synthase Activity

	OLD	YOUNG
	10.10	12.25
	12.36	8.06
	14.13	11.29
	14.05	13.85
	24.29	9.70
	9.72	11.62
	12.69	12.39
	9.77	16.15
	11.80	8.33
Mean	13.21	11.52
SD	4.49	2.59

STUDY 2: Whole-Body Resistance Exercise

Table 1. Subject Characteristics

Subject	Age	Height	Weight	%BF	LBM	FM	Leg extension 1RM
1	66	176	68.7				54.5
4	67	169	97.3				59.0
11	75	166	72.1				45.5
16	73	163	70.2	23.3	47.5	15.1	
17	70	181.5	103.6	26.0	66.7	24.6	50.0
26	60	174	97.3	31.7	58.1	28.2	66.0
30	65	169	76.3	23.7	53.6	17.5	59.0
32	70	177.5	87.6	30.1	53.8	24.3	43.0
12	65	170.5	90.3				
19	66	151	50.5	37.3	28.2	17.9	29.0
21	68	160	46.2	16.8	34.7	7.4	29.5
22	65	160	73.0	49.0	33.6	33.8	29.5
25	81	161	71.0	38.8	38.5	25.4	32.0
27	78	153	61.1	39.0	33.4	22.4	35.0
2	66	165.5	96.9				50.0
7	64	170	77.3				64.0
10	72	166	70.7				41.0
14	68	170.5	76.0				50.0
15	68	161.5	73.8				41.0
20	70	166.5	65.4	16.8	48.2	10.7	27.0
23	63	177	76.2	19.8	55.7	14.3	68.0
3	64	158	56.3				
9	72	157	82.7				
13	78	146	53.5				
18	72	170.5	85.9	46.7	39.8	37.0	
24	66	164	63.8	36.4	36.3	21.8	
29	61	163	70.6	41.7	36.4	27.9	
31	65	164	50.9	20.9	36.2	10.1	
Mean	68.5	165.4	73.8	31.1	43.8	21.2	45.9
SD	5.2	8.2	15.1	10.5	11.1	8.5	13.4

Table 2. Effect of Resistance Training on Urinary 8-OHdG

SUBJECT	PRE	POST
1	9040	8482
11	17227	10025
12	16188	8486
16	7828	8621
17	9444	8634
19	12939	10314
21	25730	20280
22	9268	8755
25	4337	3796
26	10725	10225
27	8162	7746
30	7222	4088
32	6327	3878
2	4277	6325
7	17913	6022
9	22378	14485
10	4630	4324
13	6156	8400
14	11894	9676
15	21349	18994
18	9694	7910
20	8830	6855
23	8079	12450
24	8639	8636
29	5630	7238
31	6472	6686
Mean	10784	8897
SD	5856	4030

Values expressed as $ng \cdot g^{-1}$ creatinine

Table 3. Effect of Resistance Training on Citrate Synthase Activity

SUBJECT	PRE	POST
25	13.6	14
9	14.6	12
14	14.2	17.6
16	13.8	15.6
18	10.6	10.6
19	8.4	14
20	12.4	12.8
21	10.2	12.4
23	10.8	10.8
24	7	5.6
26	14	14
27	11.8	8.2
29	10.8	17.8
3	9.4	11.6
30	15	12.8
31	15.4	18.6
4	14.6	15.8
8	14.2	11.8
Mean	12.2	13.2
SD	2.4	3.2

Values Expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww

Table 4. Effect of Resistance Exercise on Complex I+III Activity

SUBJECT	PRE	POST
21	2.2	2.3
3	2.1	1.9
31	1.14	1.0
20	3.36	1.1
29	1.8	1.8
9	1.71	1.20
18	2.1	2.1
25	2.4	1.8
23	2.45	2.7
4	1.74	1.4
27	1.62	1.66
30	1.0	1.59
14	1.62	1.0
26	1.8	1.28
16	1.0	1.2
12	2.0	2.4
24	2.7	2.4
19	1.8	2.1
Mean	1.9	1.7
SD	0.6	0.5

Values Expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww

Table 5. Effect of Resistance Exercise on Complex II+III Activity

SUBJECT	PRE	POST
21	1.22	3.38
20	1.88	1.64
16	1.96	1.90
3	1.28	1.30
31	1.78	2.80
29	1.10	0.80
26	1.76	2.00
12	0.48	1.06
24	0.72	0.9
19	1.02	1.86
27	1.6	0.82
4	2.32	1.52
23	1.76	1.84
30	1.34	0.98
14	1.9	1.78
13	1.56	1.34
18	2.72	3.86
9	2.26	2.38
Mean	1.59	1.78
SD	0.48	0.86

Values Expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww

Table 6. Effect of Resistance Exercise on Complex IV Activity

SUBJECT	PRE	POST
25	5.8	7.8
9	4.4	6.2
14	8.4	10.2
16	6.4	9.9
18	5.3	6.4
19	6.5	16.5
20	8.8	8.0
21	5.8	8.5
23	6.8	9.6
24	4.3	5.2
26	7.6	6.0
27	7.3	11.4
29	7.8	4.2
3	4.8	7.4
30	7.1	12.0
31	9.9	9.7
4	6.1	9.6
9	8.4	8.5
12	5.6	7.0
Mean	6.7	8.6
SD	1.5	2.8

Values Expressed as $\mu mol \cdot min^{-1} \cdot mg^{-1}$ ww

Table 7. Effect of Resistance Exercise on IV/I+III

SUBJECT	PRE	POST
25	2.6	3.4
9	2.1	3.3
14	7.4	10.2
16	1.9	9.0
18	2.9	3.6
19	3.8	13.8
20	4.2	3.8
21	2.4	4.7
23	2.8	3.6
24	2.5	3.7
26	4.7	3.6
27	7.3	7.2
29	4.8	4.2
3	2.7	5.8
30	7.1	10.0
31	5.0	4.0
4	2.3	4.0
9	3.1	3.3
Mean	3.9	5.6
SD	1.8	3.1

Table 8. Normal Length mtDNA Following Resistance Exercise

	16S rRNA		CYTB PRIMERS	
SUBJECT	PRE	POST	PRE	POST
12	21617	18934	18383	15845
4	28739	23409	21767	15790
13	29604	38601	23101	13750
25	21059	19248	13090	27638
14	37203	35208	20057	35106
32	8174	35574	14864	14211
18	31432	31944	5447	18257
29	33009	37659	16351	668
20	34567	33158	10033	12826
19	31967	23464	11571	12499
21	6023	6347	6316	5567
22	34183	31492	4895	5830
23	35026	30327	17550	10016
24	27749	28223	36925	29092
26	40405	37557	9775	8113
31	27904	19533	7544	10510
27	24947	22165	15581	5707
30	28042	26606	10601	6506
9	30989	18616	24714	13851
3	28270	31342	8720	22436
Mean	28045	27470	14864	14211
SD	8610	8388	7888	14129

Table 9. Number of Visible Deletion Products

	16S rRNA		CYTB	
SUBJECT	PRE	POST	PRE	POST
12	2	2	0	1
4	2	1	1	1
13	3	2	2	1
25	2	2	1	0
14	3	1	1	0
32	2	2	1	0
18	2	1	0	0
29	2	1	0	0
20	2	3	0	2
19	1	4	1	1
21	1	1	3	3
22	1	1	1	0
23	1	1	0	0
24	2	2	4	2
26	3	2	3	1
31	4	2	3	3
27	4	3	3	3
30	1	1	0	0
9	3	2	3	2
3	1	2	2	3
Mean	2	2	1	1
SD	2	2	2	1

Table 10. Effect of Resistance Exercise on Catalase Protein Content

SUBJECT	PRE	POST
1	63.4	57.1
2	18.0	165.7
3	22.4	81.6
4	70.9	16.8
7	75.5	124.1
9	81.8	112.7
10	90.4	327.1
11	31.6	44.3
12	51.5	144.4
13	169.0	170.9
14	31.5	88.8
15	43.4	278.7
16	223.6	222.3
17	119.8	82.7
18	74.2	107.2
19	177.9	177.2
20	369.4	312.1
21	119.2	126.0
23	374.6	281.6
24	291.2	271.2
25	207.5	273.2
27	254.9	345.2
29	345.0	368.7
30	394.9	213.4
31	334.2	367.1
Mean	161.4	190.4
SD	126.8	107.9

Table 11. Effect of Resistance Exercise on CuZnSOD Protein Content

SUBJECT	PRE	POST
1	19.3	35.9
2	25.8	12.9
3	19.1	20.0
4	18.7	17.5
7	126.8	73.7
9	86.0	50.2
10	104.4	56.3
11	37.2	19.1
12	73.1	19.8
13	17.7	15.8
14	11.8	8.2
15	12.3	10.3
16	71.2	47.7
17	56.6	17.7
18	20.2	82.9
19	51.2	73.1
20	25.4	42.5
21	16.9	30.2
23	12.2	17.6
24	27.1	37.7
25	15.6	15.5
27	68.3	60.1
29	86.1	40.3
30	36.7	56.6
31	73.8	77.0
Mean	44.5	37.5
SD	33.0	23.4

Table 12. Effect of Resistance Exercise on MnSOD Protein Content

SUBJECT	PRE	POST
16	190	84
17	110	60
18	68	154
19	122	230
20	86	212
21	142	76
23	122	46
24	80	80
25	8	14
27	38	26
29	252	316
30	80	240
31	80	240
Mean	106	137
SD	64	100

Table 13. Effect of Resisrance Exercise on mtCK Protein Content

SUBJECT	PRE	POST
1	73.9	131.9
2	26.7	73.2
3	22.2	119.9
4	112.2	384.2
7	345.9	594.6
9	148.7	367.8
10	190.5	189.3
11	31.0	167.0
12	56.9	215.8
13	174.5	117.2
14	14.8	538.8
15	118.9	320.9
16	441.5	419.0
17	51.4	219.6
18	27.6	81.6
19	23.3	91.8
20	52.0	114.3
21	121.4	278.5
23	516.6	500.0
24	14.7	46.2
25	26.8	71.8
27	33.0	69.5
29	26.1	18.8
30	33.9	74.0
31	57.6	129.3
Mean	109.7	213.4
SD	134.8	166.7

Table 1. Subject Characteristics

Subject	Age (years)	Mass (kg)	Height (cm)	%Body Fat
1	75	89.8	167	26
2	71	107.1	180	24
3	71	98.7	173	26
4	69	86.5	174	22
5	74	88	173	23
6	68	89.1	173	20
7	72	89.8	173	25
8	70	81.4	171	20
9	68	71.9	178	13
10	68	71.4	170	18
11	90	78.4	179	18
12	63	106	185	21
13	66	95.9	175	23
Mean	71.2	88.8	174.7	21.4
SD	6.5	11.3	4.8	3.6

Table 2. Effect of Training on Muscle Protein Carbonyls

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
1	0.41	0.399	0.3	0.71
3	0.44	0.54	0.28	0.68
5	0.31	1.3	0.3	0.13
8	0.47	0.84	0.69	0.54
7	0.68	0.84	0.59	0.65
10	1.4	0.9	1.8	0.72
11	1.8	1.4	0.58	0.7
12	0.69	0.87	0.69	0.87
Mean	0.78	0.89	0.65	0.63
SD	0.54	0.34	0.50	0.22

Values expressed as $nmol \cdot mg^{-1}$ protein

Table 3. Effect of Resistance Training on Muscle Catalase Activity

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
7	8.65	21.95	15.23	6.71
8	5.98	22.31	3.75	7.34
1	6.43	6.24	9.29	21.25
12	9.05	24.20	9.36	4.27
9	8.19	6.97	9.62	9.29
5	10.05	17.61	7.89	9.77
3	4.17	8.12	14.51	19.03
11	11.90	19.48	7.38	9.58
10	9.33	7.09	9.62	10.90
Mean	8.19	14.89	9.63	10.91
SD	2.34	7.62	3.50	5.62

Table 4. Effect of Resistance Exercise on Muslce CuZnSOD Activity

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
7	9.66	10.66	12.86	9.21
8	8.79	22.54	10.33	14.43
12	5.84	7.40	3.28	33.66
3	1.66	10.10	15.76	12.72
11	9.49	10.28	3.63	7.90
1	2.05	9.09	13.45	10.38
10	13.00	18.26	6.93	13.20
Mean	7.21	12.62	9.46	14.50
SD	4.21	5.56	4.94	8.76

Table 5. Effect of Resistance Exercise in Muscle MnSOD Activity

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
7	3.39	4.67	3.87	2.46
8	4.06	5.29	9.00	8.35
12	8.07	2.76	10.27	4.85
3	1.94	10.76	6.77	5.79
11	5.15	2.13	7.21	8.00
1	11.57	7.74	3.42	0.57
10	5.47	5.36	10.10	4.80
Mean	5.66	5.53	7.23	4.98
SD	3.23	2.95	2.79	2.80

Values expressed as $U \cdot mg^{-1}$ protein

Table 6. Effect of Resistance Training on Muscle Complex I+III Activity

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
7	1.93	1.45	2.63	2.96
8	2.82	2.43	2.97	3.48
1	3.58	4.79	3.64	2.84
12	2.65	2.06	2.12	2.45
9	2.40	3.63	1.84	3.49
5	2.19	4.96	1.77	1.91
3	2.83	1.64	2.90	2.66
11	0.76	1.60	3.34	2.51
10	2.47	1.53	2.65	2.79
Mean	2.40	2.68	2.65	2.79
SD	0.77	1.42	0.64	0.50

Values expressed as µmol·min-1·mg-1 ww

Table 7. Effect of Resistance Training on Muscle Complex II+III Activity

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
7	1.32	1.17		
8	2.02	2.17	2.49	1.99
1	2.87	3.58	2.23	2.67
12	2.67	2.14	2.23	2.10
9	1.88	2.90	4.39	2.67
5	2.14	1.32	0.61	1.59
3	1.57	1.41	1.63	2.03
11	0.84	1.23	1.40	1.13
10	1.62	0.93	2.26	2.08
Mean	1.88	1.87	2.16	2.03
SD	0.64	0.90	1.10	0.51

Values expressed as µmol·min-1·mg-1 ww

Table 8. Effect of Resistance Training on Muscle Complex IV Activity

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
7	6.65	6.05		
8	6.63	3.00	7.82	6.08
1	9.22	8.67	7.31	7.01
12	5.10	7.97	8.11	4.69
9	8.06	10.54	5.78	5.27
5	6.44	5.45	3.79	6.88
3	7.63	6.00	6.19	4.93
11	4.34	5.52	6.57	4.43
10	5.95	4.80	8.56	6.44
Mean	6.67	6.44	6.77	5.72
SD	1.49	2.26	1.47	1.05

Values expressed as μmol·min-1·mg-1 ww

Table 9. Effect of Resistance Training on Muscle Citrate Synthase Activity

SUBJECT	TRAINED LEG		UNTRAINED LEG	
	PRE	POST	PRE	POST
7	10.10	11.41		
8	11.99	10.84	12.73	13.19
1	13.10	19.55	15.17	12.68
12	13.71	12.78	14.39	13.57
9	26.02	18.83	22.56	18.74
5	12.86	10.13	6.58	12.33
3	10.34	13.94	15.04	11.52
11	9.01	7.62	10.53	8.34
10	10.66	9.12	12.94	11.89
Mean	13.09	12.69	13.86	12.91
SD	5.10	4.13	5.13	2.58

Values expressed as μmol·min-1·mg-1 ww

Table 10. Normal Length Mitochondrial DNA Following Resistance Exercise

	16S rRNA Primers			
Subject	Trained Leg		Untrained Leg	
	Pre	Post	Pre	Post
1	30587	28233	35000	26013
12	38275	38364	43938	42066
9	26873	24372	27032	31129
5	32668	30587	34451	33615
3	30999	29870	31236	33005
11	32518	26070	30600	38808
6	29483	28220	38955	41006
4	30225	33250	29997	27147
Mean	31454	29871	33901	34099
SD	3305	4378	5454	6063

Table 11. Normal Length Mitochondrial DNA Following Resistance Exercise

	Cytb Primers			
Subject	Trained Leg		Untrained Leg	
	Pre	Post	Pre	Post
1	31674	20982	35820	24090
12	28402	31780	29163	24417
9	24787	24468	31610	40197
5	22962	25625	12803	21610
3	34885	39839	35690	34885
11	22163	18243	38999	3560
6	43616	42741	38996	14328
4	35519	36134	10224	32250
Mean	30501	29977	29163	24417
SD	7379	9018	11419	11740

Table 12. Number of mtDNA Visible Deletion Products

	16 S rRNA			
Subject	Trained Leg		Untrained Leg	
	Pre	Post	Pre	Post
1	1	1	1	1
12	0	2	1	1
9	3	3	2	1
5	2	3	1	3
3	1	1	2	1
11	3	1	2	0
6	1	1	1	0
4	1	1	1	1
Mean	2	2	1	1
SD	1	1	1	1

Table 13. Number of Visible Deletion Products

	CYTB			
Subject	Trained Leg		Untrained Leg	
	Pre	Post	Pre	Post
1	0	0	0	1
12	0	0	0	0
9	1	1	4	1
5	1	1	0	1
3	0	0	1	2
11	2	0	0	0
6	0	0	0	0
4	0	0	1	1
Mean	1	0	1	1
SD	1	0	1	1

APPENDIX 2

STATISTICS TABLES

STUDY 1: Old vs. Young

Independent t-tests

Table 1. Effect of Age on Urinary 8-OHdG

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	YOUNG	OLD				YOUNG	OLD	YOUNG	OLD		
8-OHdG	5333.517	7714.943	-4.29129	20	0.000356	11	11	1191.968	1402.427	1.384305	0.616765

Table 2. Effect of Age on Muscle Protein Carbonyls

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	OLD	YOUNG				OLD	YOUNG	OLD	YOUNG		
Carbonyls	0.719333	0.263466	3.044379	16	0.007729	9	9	0.426293	0.141687	9.052258	0.005351

Table 3. Effect of Age on Muscle Catalase Activity

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	YOUNG	OLD				YOUNG	OLD	YOUNG	OLD		
Catalase	6.231298	8.5005	-2.44395	22	0.023007	12	12	2.462037	2.069696	1.415063	0.574502

Table 4. Effect of Age on Muscle MnSOD Activity

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	OLD	YOUNG				OLD	YOUNG	OLD	YOUNG		
MNSOD	7.138323	5.225904	2.33875	10	0.041428	6	6	0.751338	1.856714	6.106879	0.068867

Table 5.Effect of Age on CuZnSOD Activity

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	OLD	YOUNG				OLD	YOUNG	OLD	YOUNG		
CUZNSOD	11.20492	10.82999	0.136608	10	0.894052	6	6	2.815428	6.104955	4.701928	0.114572

Table 6. Effect of Age on Citrate Synthase Activity

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	OLD	YOUNG				OLD	YOUNG	OLD	YOUNG		
Citrate Synthase	13.21212	11.51577	0.982273	16	0.340586	9	9	4.485896	2.591989	2.995243	0.141663

Table 7. Effect of Age on Complex I+III Activity

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	OLD	YOUNG				OLD	YOUNG	OLD	YOUNG		
Complex I+III	2.487778	1.94484	2.378251	16	0.030197	9	9	0.507414	0.459989	1.216833	0.788072

Table 8. Effect of Age on Complex II+III Activity

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	OLD	YOUNG				OLD	YOUNG	OLD	YOUNG		
Complex II+III	1.936449	1.770218	0.542894	16	0.594685	9	9	0.685712	0.611225	1.258583	0.752812

Table 9. Effect of Age on Complex IV Activity

VARIABLE	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	p
	G_1:1	G_2:2				G_1:1	G_2:2	G_1:1	G_2:2	variancs	variancs
Complex IV	5.692752	6.7112	-1.07974	16	0.296265	9	9	2.666423	0.947291	7.923027	0.008341

STUDY 2: Whole-Body Resistance Exercise

Dependent T-tests

Table 1. Effect of Resistance Training on Urinary 8-OHdG

VARIABLE 8-OHdG	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	10783.79	5856.403						
PST	8897.389	4030.368	26	1886.397	3611.033	2.663719	25	0.013329

Table 2. Effect of Resistance Exercise on Muscle Citrate Synthase

VARIABLE Citrate Synthase	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	12.2	2.4						
POST	13.1	3.3	18	-0.84444	2.831383	-1.265	17	0.222817

Table 3. Effect of Resistance Exercise on Muscle Complex I+III

VARIABLE Complex I+III	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	1.91	0.59						
POST	1.71	0.53	18	0.200556	0.624777	1.361901	17	0.191005

Table 4. Effect of Resistance Exercise on Muscle Complex II+III

VARIABLE Complex II+III	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	1.59	0.57						
POST	1.78	0.86	18	-.19444	0.730441	-1.1294	17	0.274415

Table 5. Effect of Resistance Exercise on Muscle Complex IV

VARIABLE Complex IV	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	6.68	1.54						
POST	8.63	2.8	19	-1.9447	2.84397	-2.9806	18	0.008016

Table 6. Effect of Resistance Exercise on Complex IV/I+III

VARIABLE Complex IV/I+III	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	3.8	1.8						
POST	5.6	3.1	18	-1.7558	2.82154	-2.64014	17	0.017189

Table 7. Effect of Resistance Exercise on Full-Length mtDNA

VARIABLE CYTB	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	14864.25	7888.57						
POST	14210.9	8769.695	20	653.35	8973.169	0.325623	19	0.748268

Table 8. Effect of Resistance Exercise on Catalase Activity

VARIABLE CATALASE	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	161.432	126.8197						
POST	190.404	107.8799	25	-28.972	90.36809	-1.603	24	0.122016

Table 9. Effect of Resistance Exercise on CuZnSOD Activity

VARIABLE CuZnSOD	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	44.54	32.97316						
POST	37.544	23.35127	25	6.996	27.75103	1.260494	24	0.219609

Table 10. Effect of Resistance Exercise on MnSOD Activity

VARIABLE MnSOD	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	106	63.44026						
POST	136.7692	99.69132	13	-30.7692	92.18293	-1.20348	12	0.251997

Table 11. Effect of Resistance Exercise on Mitochondrial CK Protein Content

VARIABLE MtCK	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	109.684	134.7688						
POST	213.4	166.6527	25	-103.716	123.9609	-4.18342	24	0.000331

STUDY 3: Unilateral Resistance Exercise
Two-way ANOVA: 2 fatcors with 2 levels (2 legs X 2 time points) all within

Table 1. Effect of resistance exercise on citrate synthase activity

1-LEG, 2-TIME	df Effect	MS Effect	Df Error	MS Error	F	p-level
1	1	3.81E-05	7	5.257485	7.24E-06	0.997928
2	1	2.936412	7	6.430667	0.456626	0.520896
12	1	1.003706	7	6.758434	0.148512	0.711405

Table 2. Effect of resistance exercise on complex I+III acivity

1-LEG, 2-TIME	df Effect	MS Effect	Df Error	MS Error	F	p-level
1	1	0.29033	8	1.148469	0.252798	0.628659
2	1	0.380797	8	0.566314	0.672414	0.43596
12	1	0.042033	8	0.568344	0.073956	0.792548

Table 3. Effect of resistance exercise on complex II+III activity

1-LEG, 2-TIME	df Effect	MS Effect	Df Error	MS Error	F	p-level
1	1	0.161427	7	0.569822	0.283294	0.611019
2	1	0.004373	7	0.073535	0.059474	0.814323
12	1	0.079746	7	0.440968	0.180842	0.683418

Table 4. Effect of resistance exercise on complex IV activity

1-LEG, 2-TIME	df Effect	MS Effect	Df Error	MS Error	F	p-level
1	1	1.709978	7	3.256418	0.52511	0.492175
2	1	2.684299	7	1.254714	2.139371	0.186959
12	1	1.768097	7	3.002919	0.588793	0.467973

Table 5. Effect of resistance exercise on CuZnSOD activity

1-LEG, 2-TIME	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	29.84084	6	46.70499	0.638922	0.454572
2	1	190.9284	6	32.73872	5.831882	0.052222
12	1	0.233882	6	49.6662	0.004709	0.94752

Table 6. Effect of resistance exercise on MnSOD activity

1-LEG, 2-TIME	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	1.806659	6	16.36131	0.110423	0.750966
2	1	10.01011	6	8.986462	1.113911	0.331866
12	1	7.905375	6	4.906808	1.611104	0.251348

Table 7. Effect of resistance exercise on catalase activity

1-LEG, 2-TIME	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	14.60733	8	56.45758	0.258731	0.624721
2	1	142.9958	8	10.87728	13.14628	0.006726
12	1	65.99389	8	31.46363	2.097466	0.185575

Table 8. Effect of resistance exercise on protein carbonyls

1-LEG, 2-TIME	df Effect	MS Effect	df Error	MS Error	F	p-level
1	1	0.292421	7	0.145826	2.005281	0.199675
2	1	0.013571	7	0.136733	0.099254	0.76191
12	1	0.03913	7	0.083697	0.467519	0.516119

T-tests for the trained and acute exercised leg

Table 1. Effect of Resistance Exercise Training on Muscle Protein Carbonyls

VARIABLE Carbonyls	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	0.775	0.53615						
POST	0.886125	0.337461	8	-0.11113	0.462344	-0.67982	7	0.518463

Table 2. Effect of Acute Resistance Exercise on Muscle Protein Carbonyls

VARIABLE Carbonyls	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	0.65375	0.495117						
POST	0.625	0.219675	8	0.02875	0.476548	0.170638	7	0.869336

Table 3. Effect of Resistance Exercise Training on Catalase Activity

VARIABLE Catalase	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	8.19316	2.33608						
POST	14.88708	7.617484	9	-6.69392	7.132168	-2.81566	8	0.022645

Table 4. Effect of Acute Resistance Exercise on Muscle Catalase Activity

VARIABLE Catalase	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	9.627063	3.500476						
POST	10.9052	5.623621	9	-1.27814	5.814981	-0.6594	8	0.528157

Table 5. Effect of Resistance Exercise Training on Muscle MnSOD Activity

VARIABLE MnSOD	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	5.664207	3.229931						
POST	5.531078	2.953052	7	0.133129	4.705525	0.074854	6	0.942764

Table 6. Effect of Acute Resistance Exercise on Muscle MnSOD Activity

VARIABLE MnSOD	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	7.870845	2.4357						
POST	5.710889	2.20758	6	2.159957	2.586863	2.045254	5	0.096223

Table 7. Effect of Resistance Exercise Training on Muscle CuZnSOD Activity

VARIABLE CuZnSOD	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	7.212653	4.214881						
POST	12.61803	5.560026	7	-5.40538	4.779127	-2.99245	6	0.024243

Table 8. Effect of Acute Resistance Exercise on Muscle CuZnSOD Activity

VARIABLE CuZnSOD	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	9.460139	4.942133						
POST	14.49994	8.761869	7	-5.0398	11.91511	-1.11909	6	0.305892

Table 9. Effect of Resistance Exercise Training on Muscle Citrate Synthase Activity

VARIABLE Citrate Synthase	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	13.08651	5.09811						
POST	12.69208	4.129893	9	0.394427	3.881642	0.304841	8	0.768267

Table 10. Effect of Acute Resistance Exercise on Muscle Citrate Synthase Activity

VARIABLE Citrate Synthase	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	13.74241	4.552497						
POST	12.78235	2.894057	8	0.960056	3.060771	0.887178	7	0.404433

Table 11. Effect of Resistance Exercise Training on Complex I+III Activity

VARIABLE Complex I+III	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	2.40392	0.771604						
POST	2.677955	1.417782	9	-0.27404	1.308577	-0.62824	8	0.547356

Table 12. Effect of Acute Resistance Exercise on Complex I+III Activity

VARIABLE Complex I+III	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	2.651867	0.644816						
POST	2.789223	0.498434	9	-0.13736	0.746286	-0.55216	8	0.595923

Table 13. Effect of Resistance Exercise Training on Complex II+III Activity

VARIABLE Complex II+III	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	1.881345	0.636354						
POST	1.871984	0.896449	9	0.009361	0.630168	0.044564	8	0.965547

Table 14. Effect of Acute Resistance Exercise on Complex II+III Activity

VARIABLE Complex II+III	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	2.155692	1.095006						
POST	2.03247	0.510647	8	0.123222	0.806797	0.431985	7	0.678746

Table 15. Effect of Resistance Exercise Training on Complex IV Activity

VARIABLE Complex IV	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	6.668571	1.49165						
POST	6.444663	2.259051	9	0.223908	2.066426	0.325066	8	0.753469

Table 16. Effect of Acute Resistance Exercise on Complex IV Activity

VARIABLE Complex IV	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	6.766613	1.540142						
POST	5.717238	1.013487	8	1.049375	1.941809	1.528513	7	0.17023

Table 17. Effect of Resistance Exercise Training on Full-Length mtDNA

VARIABLE 16S	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	31453.5	3305.223						
POST	29870.75	4377.814	8	1582.75	2664.345	1.680222	7	0.136803

Table 18. Effect of Resistance Exercise Training on Full-Length mtDNA

VARIABLE CYTB	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	30501	7379.443						
POST	29976.5	9017.633	8	524.5	4953.709	0.299475	7	0.773274

Table 19. Effect of Acute Resistance Exercise on Full-Length mtDNA

VARIABLE 16S	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	33901.13	5453.934						
POST	34098.63	6063.004	8	-197.5	5130.755	-0.10888	7	0.916356

Table 20. Effect of Acute Resistance Exercise on Full-Length mtDNA

VARIABLE CYTB	Mean	Std.Dv.	N	Diff.	Std.Dv. Diff.	t	df	p
PRE	29163.13	11418.5						
POST	24417.13	11739.63	8	4746	18827.97	0.712967	7	0.498938