

Chapter 1

Introduction

Aging can be defined as the process by which organisms proceed through a physical deterioration of the body. Elderly individuals are the fastest growing segment of the population. In 1900, about 3.1 million persons, or roughly 4 percent of the population, were aged 65 years and older. By 1990, the number of elderly people had reached 31.2 million or 12.6 percent of the total population. "One out of every nine individuals in America is now age 65 or older" (Dychtwald, 1990). This aging trend is expected to continue, especially since the "baby boomer" generation has now passed age forty.

This graying is expected to continue until at least the year 2040, when the elderly portion of the total U.S. population could reach 22.6 percent (Clark & Weber, 1996). Expanding the longevity of life is no longer as great of a concern as is improving the quality of life (Russell, 1993). Most dramatic is the increase in the oldest segment of society, those needing the most care. While the 65 to 74 age group is expected to increase by 17 percent among 1980 and 2000, the population over age 85 will more than double during this period. The growth among the oldest old is one of the results of improved health care and disease prevention techniques used in the United States during the 20th century (Clark & Weber, 1996).

Researchers in the areas of muscle function and gerontology are concerned about the increasing number of aging individuals, primarily since as individuals age skeletal muscle function declines. The sarcoplasmic reticulum

(SR) is an organelle within muscle that is responsible for the sequester (uptake) and release of Ca^{2+} that initiates muscle contraction and relaxation (Williams & Klug, 1995). Since the SR is a key in regulation of muscle force production, the primary focus of this study centers on the relationship between reduced SR function and aging muscle.

Skeletal muscle is the type of muscle responsible for movement. It is considered to be a motor, since it has the ability to convert signals from the nervous system into mechanical action, such as the movement of an arm, in the external environment (Cormack, 1987). Skeletal muscle consists of several different tissues that collectively comprise the muscle trunk: epimysium, endomysium, and perimysium. The muscle trunk is surrounded by a layer of dense connective tissue called deep fascia. The muscle tissue is organized into subunits called fascicles. Each fascicle is surrounded by a sheath of connective tissue called the perimysium. Muscle fibers are the subunits of the fascicles.

The decline of physical ability that occurs with aging has been linked to reduced skeletal muscle function (Doherty, Vandervoort, & Brown, 1993). The deterioration process varies among individuals, and may be rapid or gradual. In theory, organisms age because of structural cell changes which result in reduced cell function, degeneration, and even death (Larsson, 1982). It has been theorized that Ca^{2+} uptake and release by the sarcoplasmic reticulum deteriorate with aging as well (Doherty, et. al., 1993).

The outer membrane of the muscle fiber known as the sarcolemma, encircles the myofilaments that comprise the muscle fiber. It is referred to as the

cell membrane, which is similar in appearance to the plasmalemma of other kinds of cells. The sarcolemma encloses the sarcoplasm, the muscle fiber equivalent of the cytoplasm of other cells. Within the muscle fiber, there is an elaborate system of membranes, vesicles, and tubules that collectively comprise the SR. The SR regulates the uptake and release of intracellular Ca. The actual physiological role of the SR is the release and sequester of Ca (Williams & King, 1995).

In order to generate movement, muscles must contract. For a muscle to contract, a nerve impulse from the Central Nervous System (CNS) must initiate the release of the neurotransmitter acetylcholine. The nerve impulse first reaches the motor end plate of the nerve and causes the release of acetylcholine into the neuromuscular cleft. Acetylcholine then diffuses across the neuromuscular cleft and initiates an impulse in the opposite muscle fiber. Once induced in the muscle fiber, the impulse proceeds over the surface of the fiber and passes down the transverse tubules (t-tubules) into the sarcolemma. The sarcolemma lies within the interior of the fiber within close proximity of the membranes of the SR.

The SR regulates the uptake and release of Ca^{2+} during the contraction-relaxation cycle, thus regulating the level of contractile apparatus activation (Williams & Klug, 1995). During muscle relaxation, Ca^{2+} is sequestered into the longitudinal portion of the SR. When the muscle is not relaxed, the SR releases the Ca^{2+} stored within the longitudinal portion into the myoplasm of the muscle.

A muscle that is not used diminishes in cross sectional area, a condition known as atrophy. Prior studies of mammalian aging skeletal muscle have shown that muscle atrophy is associated with weakness and prolongation of the contraction (Klitgaard, 1989). Muscle weakness and contraction prolongation impairment have been attributed to changes in the SR that regulates intracellular Ca^{2+} . Some data, although inconclusive, suggest that an age related process occurs.

Statement of the Problem

The growing number of elderly individuals has caused great concern for gerontology and muscle function researchers. As individuals age, they experience a decline in their ability to complete daily activities. This decline is mostly due to a reduction in muscle function. Russell (1993) concluded that “active life” is reduced greatly once individuals reach 65 years old.

Specific Aim

Aging trends show that the baby boom generation, which is the largest segment of the population, is growing older. As people age, their muscle function declines, and so does their quality of life. The specific cause of muscle function decline is not yet known. However, based on work with other models of atrophy, it is possible that a reduction in SR function may be a part of muscle decline. The specific aim of this study was to determine if SR function is altered as a result of aging. This was accomplished through measurement of the rate

of SR Ca uptake, and release in plantaris, soleus, and diaphragm muscles of young and aged rats.

Hypothesis

1. There will be no difference in the rate of SR calcium uptake release in the plantaris, soleus, and diaphragm muscles, between adult (approximately 12 months) and aged (approximately 24 months) Fisher 344 Brown x Norway Cross rats.

2. There will be no difference in the rate of SR calcium release in the plantaris, soleus, and diaphragm muscles, between adult (approximately 12 months) and aged (approximately 24 months) Fisher 344 Brown x Norway Cross rats.

Delimitations

In order to conduct the study, the following delimitations have been developed.

1. The subjects were limited to Fisher 344 Brown x Norway rats.
2. The independent variable of the study is age.
3. The dependent variable is the release and uptake of Ca^{2+} ions by the isolated sarcoplasmic reticulum vesicles.

Limitations

The following were limitations of the study.

1. Animals costs limited the number of animals purchased to conduct the study.

2. Three individuals used the equipment in the lab therefore time for using the equipment was shortened.

3. The muscle sample was limited to 3 muscles.

4. The variables measured were limited to Ca^{2+} uptake and release variables measured via the methods described.

5. The age of the animals was limited to 12 and 27 month old animals.

Basic Assumptions

1. It was assumed that 12 and 24 month old Fisher 344 Brown x Norway rats were a good model for the study because the SR obtained from their soleus, plantaris, and diaphragm is physiologically compatible to SR found in the soleus, plantaris, and diaphragm of a human being; thus, similarities could easily be drawn between humans and rats using the information obtained from the study.

2. It was assumed that the animals were free of known disease; hence, disease was not a contributing or inhibiting factor to the results.

3. It was assumed that the conditions used to isolate SR and to measure the uptake and release of Ca^{2+} and measure Ca^{2+} were adequate.

Definitions

ADULT FISHER 344 BROWN NORWAY RAT: approximately 12 months old.

AGING: biological process causing a reduction in an organisms functional capacity.

AGED FISHER 344 RAT BROWN NORWAY RAT: approximately 27 months old.

MUSCLE ATROPHY: the reduction of muscle mass, muscle cross sectional area, fiber number, and fiber cross-sectional area.

SARCOPLASMIC RETICULUM: structure within skeletal muscle responsible for relaxation and contraction via the regulation of Ca uptake and release.

SPECIFIC FORCE: amount of force created by a muscle fiber divided by its mass or cross-sectional area

V_{max}: the maximum shortening velocity of the muscle fiber

Organization of Thesis

Chapter 1 describes the problem, purpose, significance, limitations, and definitions of the study. Chapter 2 contains a review of related literature.

Chapter 3 includes a description of the methodology of the study. Chapter 4 presents the findings resulting from the study. Chapter 5 contains a discussion that provides a summary, discussion, conclusions, and implication based on research results. Chapter 5 also includes recommendations for educational efforts and future research.

Summary

Aging individuals are the fastest growing segment of the population. The aging process causes a concern for researchers in the areas of gerontology and muscle function primarily because as individuals age, skeletal muscle function declines. Skeletal muscle is responsible for overall movement. If the function of the skeletal muscle declines, so does the function to carry out the Activities of Daily Living.

CHAPTER 2

Literature Review

Overview

A good literature review will have a set of expectations that data can confirm or refute and it makes the investigator the master, not the captive of previous scholarship, (McCracken, 1988). It searches the conscious and unconscious assumptions of scholarly enterprises and determines how these assumptions force the definitions of problems and findings. The two main topics for investigation for this study are aging and muscle function.

This literature review was designed to summarize key research on the aforementioned topics. A rationale for rat study is also included.

Aging

Introduction

As individuals age, their health becomes a great concern. Thanks to advances in today's medical research, individuals are able to live longer. Consequently, as people live longer they become concerned with the quality of their health. Individuals are living longer; however, this does not mean they are living healthier. For many, it means growing old with disabilities and illness. "Active life," defined as the number of years remaining before people lose their ability to perform the activities necessary for daily life, such as bathing or dressing, is very limited (Dychtwald, 1990). Thus, it may be concluded that the

length of life may be increasing, but the quality of life is not necessarily improving. Dychtwald (1990) gives an example that old age does not necessarily have to be that of physical disabilities. He gives the following as an example of an individual, Jack LaLanne, being fit at an older age.

The myth that age automatically means decrepitude leaves little room for such people as diet and exercise guru Jack LaLanne. Throughout his lifetime, LaLanne has celebrated aging by demonstrating that with proper care and physical activity, the human body has remarkable powers of resilience, even in the years after youth. He marked his 40th birthday by swimming across the Golden Gate underwater, wearing 140 pounds of scuba gear. At 41, he did 1,033 pushups in 23 minutes on television. Every year the feats of strength got wilder.

In 1974, on his 60th birthday, LaLanne swam from Alcatraz to Fisherman's Wharf again, handcuffed. And with his ankles shackled. Towing a half-ton boat. On his 62nd birthday, he completed a mile and a half long swim in Long Beach Harbor, handcuffed and shackled. towing 13 boats filled with 76 cheering children from the local Y. When he was done, his pulse was a mere 76. He repeated the feat on his 70th birthday, this time hauling 70 boats full of friends and reporters.

At 75, LaLanne still follows the routine he has followed for half a century. He rises at 4:30 in the morning, and by 5:00 he is pumping iron. After two hours, he heads to the pool for an hour of swimming and water exercises. LaLanne is far from alone. he merely shows what the body can do when treated right. (Dychtwald, 1996).

Aging

Another definition of aging is the process by which all members of a given species, should they survive to experience aging, proceed through a physical deterioration of the body (Garthwaite, Cheng, Bryan, Craig, & Holloszy 1986).

The deterioration process may be rapid or gradual. The ultimate result is death.

Aging does not necessarily kill organisms, but it does make them more susceptible to a decline in their body process such as muscle function. Very little is known about the intrinsic details of aging. According to Gerber, Wolff,

Klores, and Brown, (1989), experts in gerontology generally agree that 120 years is about the longest life span humans can expect under ideal conditions.

Elderly Americans are the fastest growing segment of the American population, and by the year 2050 one in every twenty people will be 85 or older and one in every five will be retired. The fastest growing segment of the US population will be the “oldest old” people 85 and over. The numbers are expected to double to 7 million by 2020. When the baby boom generation starts turning 65 in 2011, it will have an explosive effect on all facets of society.

Health Issues

The impairment of mobility and physical strength is common in older adults. Of the 27.9 million elderly persons in the United States, 3.6 million (approximately 12%) have difficulty with a least one task of ADL (Schultz, 1995). Difficulty with bathing affects 2.5 million (8.9%), difficulty with walking 2.2 million (7.7%), and difficulty with bed or chair transfers 1.6 million (5.9%) (Schultz, 1995). The rate of these problems increases progressively after age 65.

In a Canadian study of the elderly, researchers found that elderly individuals were disabled for 80 percent of the extra time they gained as life expectancy rose (Gerber, Wolff, Klores, & Brown, 1989). Other researchers have noted that at least 16 percent of people aged 65 or older need assistance with at least one of their daily activities, including bathing, dressing, getting around outside, preparing meals, and or doing housework (Russell, 1993). Most of the inability to perform ADL is related to the loss of mobility and physical

strength due to the muscle atrophy that occurs with aging and a decline of the functional capacity of muscle contraction as well.

Physical Activity and Muscle Function

Physical activity typically declines with age, a fact related to the age related functional changes that occur within muscle. Marcus (1995) conducted a cross-sectional study to observe the actual effect physical activity had upon the functional changes within muscle that occur with aging. In his study, Marcus compared the physical functional capacity of a group of aging athletes to an aging sedentary control group. Basically, in his findings, Marcus showed that there was a functional decline within all subjects; however, the athletes had less of a decline in comparison to the sedentary control group. Therefore, this suggests that a continuation of physical activity shows the decline in the functional capacity of muscle.

Mobility impairments in older adults are common. One of the most serious problems of mobility impairment is the tendency of older adults to fall. Quetelet (1835) and Cathcart (1935), for example, recognized long ago the decline in strength that accompany aging. In overview, they found that the order of magnitude of these declines, between ages twenty-five and sixty-five, is one-third. Actual strength values vary widely because they depend on many factors, particularly on the characteristics of the subjects in whom strengths are measured.

Declines with age in physical performance abilities probably cannot be explained as due to only physical inactivity or lack of motivation. Declines in

these activities can be seen among even highly motivated and highly trained athletes.

Muscle mass, force, and power decrease normally with decreases in physical activity, which may occur at any age (Faulkner, Brooks, Zebra, 1994). Although there is muscle atrophy, weakness, and fatigability associated with decreased physical activity, highly trained and untrained individuals show similar rates to decline with aging. It can be hypothesized that the loss in muscle mass, force, and power do not solely result from the decrease in physical activity commonly associated with age, but are due to age related changes in muscles and in muscle fiber that appear to be immutable and irreversible.

The primary mechanism to explain the decreased strength of older vs. young adults appears to be reduced muscle mass stemming from a loss of functioning units such as the SR (Doherty, Vandervoort, & Brown, 1993). Additionally, muscle atrophy due to decreased activity that occurs with aging has been shown to cause a reduction in SR function whereas on the other hand, muscle hypertrophy tends to replenish the reduction in SR function (Schulte, Javier, & Kandarian, 1993).

Muscle Function

Introduction

Significant alterations in skeletal muscle properties are known to occur with aging, including reductions in mitochondrial enzyme activity, muscle mass, and, Type II fiber number as well as Type II fiber cross-sectional area. These changes are associated with alterations in skeletal muscle contractile properties, such as reduced specific force, prolonged contraction, and one-half realization time (Gosselin, Johnson, & Sieck, 1994). It is important to note that a reduction in muscle mass is indicative of a decline in muscle fiber number, fiber size, and other organelles such as the SR (Faulkner et. al., 1995).

Skeletal Muscle

Skeletal muscle is considered to be a transformer that converts signals from the nervous system into actions in the external environment. Some general functional properties of muscle include: contractility and growth/regeneration. The functional properties of skeletal muscle can change in response to increased or decreased muscle activity.

Alterations in skeletal muscle are important because the condition of muscle is important in an individual's ability to perform the activities of daily living. Muscle consists of muscle fibers grouped into bundles called fascicles. The entire muscle is surrounded by a fibrous layer of connective tissue. The connective tissue provide a resilient system to withstand the stress incurred during active muscle contraction.

The muscle fibers cause skeletal movement by contraction. The fibers can shorten by two-thirds of their resting length to cause a contraction (Dulbecco, 1991). A group of muscle fibers is supplied with a twitch from a motor nerve each time an impulse is sent down from the spinal cord. A blending of all the twitches results in a smooth contraction of the entire muscle. There are three muscle fiber types: slow twitch fiber, fast twitch fiber, and intermediate twitch fiber (Larsson & Salviati, 1989). The soleus muscle, located in the distal area of the leg is primarily composed of slow twitch fibers. The plantaris is primarily composed of fast twitch fibers. The diaphragm is composed primarily of intermediate fibers (Gosselin et. al., 1994).

Profound morphological and functional changes such as muscular atrophy, a decrease of muscle strength, and prolongation of the contraction isometric twitch, occur with aging in skeletal muscle (Larsson, 1982). The biochemical basis for these modifications has not been clarified. Studies of aging human muscle shows a selective atrophy of Type IIA and IIB (fast) fibers, together with the absence of modifications of the fiber type composition (Grimby and Saltin, 1983). Since Types IIA and IIB fibers produce force output faster, it can be concluded that the atrophy of these fibers will reduce the force output of the muscle, thus reducing the overall muscle strength (Grimby et. al., 1983).

As skeletal muscle ages, contractile function declines, and fiber area are lost. Muscle mass tends to also decline with aging. The rate of decline is variable according to the muscle type and it's function (Grimby and Saltin, 1983; Fitts et al., 1984; Faulkner et al., 1990; Holloszy et al., 1991). Postural muscle

appears to be affected more than non-postural muscle in rats (Gutman et al., 1971; Fitts et al., 1984; Hollozy et al., 1991). For example, the soleus muscle, which is primarily composed of slow muscle fibers, has been reported to show a larger loss of mass with age in comparison to the EDL muscle of the rat (Brown et al. 1993). The plantaris that is primarily made of fast muscle fibers, shows less mass loss with age in comparison to the gastrocnemius; however, the plantaris has been shown to have a larger loss of mass in comparison to the soleus (Klitgaard, Ausoni, & Damiani, 1989).

Fiber Type

When speaking of the rate of muscle contraction, one must note the muscle fiber type that comprises the muscle in question. There are five muscle fiber types. These are Type IIA and Type IIB (fast-twitch), Type I (slow-twitch), and Type I-IIA and Type IIA-IIB (intermediate) (Bottinelli, Canepari, Pellerino, & Reggiani, 1996). Each fiber type has a different rate of twitch and V_{max} . V_{max} is the maximal shortening velocity of a muscle fiber. The rate of twitch refers to the speed in which a muscle fiber can generate a movement. V_{max} is dependent upon the upon the myosin heavy chain isoform and fiber composition of the muscle. In a study conducted by Bottinelli et. al. (1996), researchers found that V_{max} was significantly lower in Type I (slow) than in the Types IIA and IIB (fast). The V_{max} values and specific force tensions of Types I and IIA fibers in young human muscle are significantly higher in young individuals than in older individuals, even older physically active individuals (Larsson & Li, 1997). It can be concluded that this information provides evidence of qualitative

changes in contractile properties of human skeletal muscle in old age, which probably plays an important role in the age-related impairment of skeletal muscle function (Larsson, et. al., 1997).

Numerous studies have shown that normal aging results in a loss of skeletal muscle fibers and atrophy of fast-twitch (Type II) fibers in humans (Proctor, Sinning, Walro, Sieck, & Lemon, 1995). Proctor (1995) observed atrophy, due to aging, of Type II fibers in lower limb muscle of healthy human subjects. This atrophy is hypothesized to not relate only to aging per se, but also to a reduction in physical activity that accompanies aging as well. The changes observed with aging human skeletal muscle are thought to contribute to the decline in exercise performance and the ability to perform activities of daily living in older individuals.

As muscle ages the composition of its fiber type changes. Type I fibers (slow-twitch) tend to increase in composition. Whereas, Type II fibers (fast-twitch) tend to decrease. Lexell (1988), reported that while the total numbers of Type I and Type II muscle fibers are lower in older adults compared to young adults, atrophy of Type II muscle fibers is also characteristic of aging muscle.

Several studies have shown a selective atrophy of Type II fibers together with other neuropathic signs in the aging human skeletal muscle. These findings suggest that denervations occur to the nerve endings of Type II fibers that is followed by a reinnervation of these nerve ending into Type I motorneurons (Larsson, 1982). The impulse to the motorneuron from the CNS initiates the fiber to produce a force. The impulse to Type I motorneurons initiates a slow

force production, whereas the impulse to Types IIA and IIB fibers initiates a fast force production. Therefore, when the nerve endings of Types IIA and IIB fibers are reinnervated into Type I fiber motoneurons, the fiber will no longer initiate a fast force production, instead, it will initiate a slow force production. In theory, Type IIA and IIB fibers become Type I fibers. This underlies, at least in part, the aging of skeletal muscle. From these findings, it can be assumed that the force produced by Type IIA and IIB fibers becomes similar to the force produced by Type I fibers due to the reinnervation of their nerve endings. Klitgaard et al., (1989) found that within humans, the relative area of Type I fibers increased with aging. There was a marked atrophy of Type IIA and Type IIB fibers within the old control group as compared to the young control group.

Soleus

The soleus is a flat broad muscle of the calf. It is located just beneath the gastrocnemius muscle. The soleus arises from the upper portions of the tibia and fibula, the bones of the lower leg, and then joins with the gastrocnemius to attach via the Achilles tendon at the heel. It's major action is flexion of the ankle joint.

The soleus is primarily composed of slow-twitch fibers (Type I). It's composition is also made of some fast-twitch fibers. Due to the majority of the soleus muscle's composition being slow twitch fibers, the soleus is considered a slow muscle regardless of the few fast-twitch fibers it contains.

The soleus has been reported to show a larger loss of mass with age than other muscles (Brown et al., 1993). In a study conducted by Larsson (1982), the

slow twitch soleus fibers capacity for Ca^{2+} uptake by the SR was the same in the young and old animals. However, Larsson (et al., 1987) observed an age related decrease in the speed of contraction in fast twitch motor units and in the slow twitch soleus muscle, and also a decreased proportion of fast twitch fibers in the soleus muscle were confirmed in Larsson's study.

Plantaris

The plantaris is a calf muscle located in the calf muscle. Unlike the soleus, it is composed primarily of fast-twitch (Type II) muscle fibers. It's composition is also made of some slow-twitch fibers. Due to the majority of the plantaris muscle's composition being fast-twitch fibers, the plantaris is considered a fast muscle irrespective of the few slow-twitch fibers it contains.

Diaphragm

The diaphragm is found only in mammals. It lies in the thorax between the chest and abdominal cavities. The diaphragm is the muscle involved in breathing, and it's motions are controlled through a nerve center in the brain stem. It is a dome shaped muscle lying horizontally below the lungs and above the abdominal cavity. Contraction of the diaphragm greatly increases the volume of the thorax, allowing air to be drawn into the lungs with each inspiration. The diaphragm is primarily composed of fast twitch motor units (Type II fibers); it is also composed of slow twitch motor units (Type I fibers) as well. Thus, the diaphragm is known as an intermediate muscle. Within the diaphragm, the fast twitch fibers generate approximately two times the specific force of its slow twitch fibers (Sieck, 1991).

Only recently, the changes in diaphragm muscle morphologic, biochemical, and contractile properties with aging have been studied (Gosselin et al, 1994). Tolep, Higgins, Muza, Criner, & Kelsin, (1995), conducted a study to test the hypothesis that aging is associated with the reduction in the diaphragm's force generating capacity. The researchers conducted this study by measuring and comparing the maximum transdiaphragmatic pressure (P_{dimax}) obtained during voluntary maximal inspiratory efforts in nine young (19-28 yr.) and ten elderly (65-75 yr.) subjects. Tolep et. al. (1995) found that the diaphragm strength is reduced in elderly individuals in comparison to young individuals. This age related decrease in diaphragm strength may predispose elderly patients to diaphragm functional impairment. An age related reduction in specific force of diaphragm from golden hamsters has been reported (Zhang, 1990). Additionally, Gosselin et. al. observed age related increase in the relative contribution of slow myosin heavy chain isoform in diaphragm muscle from female F344 rats. This finding supports the idea that diaphragm muscle undergoes age related changes.

Sarcoplasmic Reticulum

Skeletal muscle cells contain a reticular system, which is important for the control of free Ca^{2+} concentration in the cell. The SR is an important element concerning the excitation-contraction coupling of muscles. Previous studies have shown muscle atrophy in aging muscle, as well as a prolongation of twitch. This functional impairment of aging skeletal muscle has been attributed to changes in the membrane system that regulates intracellular Ca^{2+} , i.e. SR (Larsson, 1982). Data available in literature pertaining to the structural and functional changes of SR due to aging are limited.

In striated muscle, the SR forms longitudinal tubules near the transverse tubules (T Tubules). SR is a tubular network surrounding the myofibrils, forming lateral sacs known as the terminal cisternae (TC). Terminal cisternae contain “feet-like” projections that connect the SR to the plasma membrane and the T Tubules. One of these “feet-like” structures is identified as the calcium release channel of SR.

The folds of the membranes of the SR divide the muscle fiber into many long, cylindrical subunits called myofibrils. The membranes of the SR extend throughout the muscle fiber, encircling each myofibril with a network of channels through which nutrients are delivered to the inner fiber. Mitochondria are suspended in the membranes to provide ATP during muscle contraction.

The passage of the impulse along the T tubule has an effect on the SR that makes it permeable to Ca^{2+} ions stored within the SR. Hence, when muscle fibers are stimulated by an initial nerve impulse the SR releases Ca^{2+} ions via

the Ca^{2+} release channel. The Ca^{2+} ions then diffuse into the fluid surrounding the thick and thin filaments of the sarcomere and trigger the mechanism that contracts the muscle.

The SR regulates the uptake and release of calcium. The actual physiological role of the SR is the release and sequester of calcium (Williams & Klug, 1995). Studies of aging skeletal muscle have shown as muscle ages, it will atrophy and its functional characteristics begin to decline. The decline in the functional characteristics has been attributed to physiological changes in the SR (Klitgaard et.al., 1989).

A major component of SR is an ATPase (CaATPase) that pumps Ca^{2+} out of the area. CaATPase can represent as much as 90% of the membrane protein in SR of skeletal muscle.

SR regulates intracellular Ca^{2+} of the muscle fiber by releasing Ca^{2+} ions stored in the lumen of the TC (Fleischer et al. 1985). The intracellular Ca^{2+} is actively localized in the extrajunctional SR.

Research has shown the physiological role of SR is to release and sequester Ca^{2+} during the contraction relaxation cycle, thus regulating the level of contractile apparatus activation (Williams & Klug, 1995).

After force production, Ca^{2+} is sequestered (uptake) into the longitudinal portion of the SR. The sequester action is activated by an intermembrane pump known as CaATPase. During the sequester action, Ca ions dissociate from troponin C and cross-bridges to weaken into a non-force generating state. ATP is hydrolyzed in order to remove Ca^{2+} from CaATPase.

Changes in the SR ability to release and/or sequester Ca^{2+} affect the rate and magnitude muscle fiber bundles put out force which is contingent upon the actual twitch of the muscle fiber.

Ca^{2+} Uptake and Release

Calcium uptake, also known as sequestration, occurs during relaxation (Dulbecco, 1991). Ham's Histology provides a sequence of the process in which Ca^{2+} is sequestered (Cormack, 1987). During relaxation, Ca^{2+} is sequestered into the longitudinal portion of the SR. Ca^{2+} is sequestered upon activation of an intramembrane pump. When Ca^{2+} is sequestered Ca ions dissociate from troponin C. Once Ca ions dissociate from troponin C, tropomyosin molecules cover actin sites, causing cross-bridges to weaken into a non-force generating state. Once tropomyosin molecules cover actin sites and cause cross-bridges to weaken into a non-force generating state, the cross-bridges are considered to be in a weak binding state. Consequently, once a weak binding state has been reached, crossbridges can no longer generate force.

Ca^{2+} release occurs during the force generating state. The release of Ca^{2+} by the SR, begins force generation. For instance, in an activated muscle, Ca ions released from the SR react with troponin-tropomyosin complexes. This interaction causes muscle fibers to shift position, exposing actin sites and allowing crossbridges to form; thus, allowing force to generate and contraction of the muscle (Cormack, 1987 et al).

Changes in SR Function with Aging

The function of the SR changes with aging. The impact of aging on skeletal muscle SR was investigated by Narayanan, Jones, Xu, & Yu in 1996. The investigators used SR vesicles isolated from the slow-twitch soleus muscle isolated from adult (6-8 months) and aged (26-28) Fisher 344 rats. The rates of Ca^{2+} uptake by the soleus muscle was markedly lower (approximately 50%) in the aged rats compared with adult rats. Additionally Narayanan et. al., (1996) found that the contraction duration was significantly prolonged in the age rats compared with adult rats, also suggesting that a correlation exists between the change in SR function and the contraction time of a muscle.

Taffet & Tate (1993) found the rate of Ca^{2+} uptake to be 30-40% slower in the SR isolated from the hearts of aged Fisher 344 male rats. In order to investigate changes in SR function, Taffet et. al., isolated cardiac SR from adult (11-12 months) and aged (22-24 months) male Fisher 344 rats. The rate of Ca^{2+} uptake by the homogenate and isolate SR was 28-44% slower in the aged group. In the isolated SR the calculated maximal velocity (V_{max}) was 20-30% lower in the aged rats (Taffet et. al., 1993).

Rationale for Rat Study

Human experimentation is limited; thus, human muscle research can benefit from animal studies. Human experimentation is limited because of the length of time necessary for longitudinal studies and limitations on performing invasive procedures in human beings (Cartee, 1995). Additionally, since the occurrence of events such as the Tuskegee Experiment, biomedical ethics have set strict standards regulating human experimentation (CNN, 1997).

Beginning in the 1930's, 399 men signed up with the US Public Health Service for free medical care. The service was conducting a study on the effect of syphilis on the human body and, at the time, the sexually transmitted disease was rampant in Macon, County Alabama.

The men were never told they had syphilis. They were told they had "bad blood" and were denied access to treatment, even for years after penicillin came into use in 1947.

Hundreds of African-American men thought they were getting free medical care, instead, their syphilis went untreated for decades so medical researchers could study how the disease progressed. By the time the study was exposed in 1972, 28 men had died of syphilis, 100 others were dead of related complications, at least 40 wives had been infected and 19 children had contracted the disease at birth.

The Tuskegee syphilis study, remains a low point for public health service. It and other experiments human experiments have caused individuals to distrust participating in human experimentation. (CNN, website)

Human studies can benefit from animal studies primarily because the similarities and differences in the age related changes in skeletal muscle found in humans compared to other species and the value of knowledge derived from models of experimentally induced muscle atrophy enhance the understanding of normal aging. Additionally it is important to note that electron microscope studies demonstrate that the overall architecture of SR of human skeletal muscle is similar to that of other mammalian species (Hayashi et al., 1987). Damiani

demonstrated that the sublocalization of Ca ATPase and the Ca release channel within extrajunctional SR and TC of human skeletal muscle is identical to that of the rabbit (Damiani et al., 1989).

The rat has been the species most frequently used for examining age-related changes in muscle. The relative magnitude of the loss in muscle by older rodents coincides with that typically reported for older humans. For instance, the human quadriceps muscle group has been studied frequently, and an approximate 25% reduction in muscle cross sectional area has been consistently observed when 70-75 year olds are compared to 20-30 year olds (Young et al., 1984; Klitgaard et al., 1990, Overend et al., 1992). In comparison to the rat, an approximate 30% reduction in quadriceps mass had been determined in rats at 28 months of age compared to rats aged 10-12 months (Garthwaite et al., 1986; Holloszy et al., 1991). Previous animal research using the rat, and the known cost and limitations of human research has provided the rationale for using the rat animal model for this study.

The male rat was chosen for this study primarily because the majority of previous studies use the male rat for the animal model. Additionally, the male rat has fewer variables regarding hormonal changes that may or may not effect this research.

Summary

America is aging rapidly especially with the aging of the largest segment of the population, the baby boomers - - those individuals born among 1946 and 1964 (Rabon, 1994). The boomers parents are living longer as well.

Demographers predict that this trend will continue until the year 2050.

Gerontologist and muscle function researchers are all concerned with the aging and their quality of life - - how well will they be able to perform the activities of daily life (ADL) and live independent lives.

CHAPTER 3

Methodology

Introduction

The purpose of this investigation was to determine if the rate SR uptakes and/or releases Ca^{2+} is altered as a result of aging in the following muscles: soleus, diaphragm, and plantaris. Data were collected by using Fisher 344 Brown x Norway rats. All experimental procedures were approved by the Virginia Tech Animal Care and Use Committee. A quantitative research method, two way analysis of variance (ANOVA) was used to analyze the data. This chapter contains a research design, data collection, data analysis, and summary.

Research Design

Research design is an action plan for conducting a study. It is frequently argued that a problem well stated is half completed. It is reasonable to assume that the method of investigation constitutes a significant phase of what remains to be explained in the outline (Castetter & Heisler, 1980). The plan of investigation, generally referred to as the study design, is basically a preconceived notion of what information the investigator needs to generate to complete the study, why it is needed, how it is to be secured, and how it is to be refined and related to the statement of the problem. Thus, the design guides the investigator while collecting, analyzing, and interpreting observations

(McCracken, 1988). This section clarifies the details of the aforementioned that will be taken to test the hypotheses appearing in Chapter 1 of this document.

Data Collection

Subjects

The Fisher 344 Brown x Norway rats were the animals used in this study. This rat strain is widely accepted as an animal model for aging. They were obtained from the National Institute on Aging (NIA) division of the National Institutes of Health. The NIA rederived the animals to alleviate any mutations that may correlate with inbreeding. The NIA also monitored the animals on a regular basis to detect pathogens and genetic change that could result from contamination.

The animals were shipped to Virginia Tech from the NIA. They were then housed for two weeks in the Laboratory Animal Resource (LAR) prior to sacrifice.

The rats were divided into two groups. The control animals (n=6) were 12 month old adult animals. The aged animals (n=6) were 27 month old animals.

Schedule

Tissues were collected for three days. Set up was done one day prior to tissue collection. Tissue was harvested from four animals each day. Two animals from each group were sacrificed each day also. For instance, on day one, two control animals and two aged animals were anesthetized. Their diaphragm, soleus, and plantaris muscles were harvested and made into a

homogenate. Then, the animals were euthanized and returned to Laboratory Animal Resource (LAR) for proper disposal.

A protein analysis was made of each homogenate. There was a total of twelve homogenates, each divided into four tubes and frozen for later analysis. This same process took place on two additional days.

Five different days was used for the assaying the rate of Ca^{2+} uptake and Ca^{2+} release. The rate of Ca^{2+} uptake and Ca^{2+} release was analyzed in the using a sample from the homogenate and a Jasco CAF-110 fluometer.

Set Up

During the set up the isolation buffer was prepared. The isolation buffer consisted of 20 mM N-[2 Hydroxyethyl] piperazine-N-[z-ethanesulfonic acid] (Hepes), pH 7.5, 250 mM sucrose, 0.2% sodium azide, and 0.2 mM phenylmethylsulfonylfluoride (PMSF). This isolation buffer was used to keep the muscles viable once they were removed from the animals. The labware was cleaned such that all labware used would be readily available. Additionally all collection tubes were labeled.

Sample Preparation

The animals were anesthetized through sodium pentobarbital (50mg/kg, ip). Anesthetizing the animals ensured that they would not feel any pain as the muscles were being removed. After the animals were anesthetized, the diaphragm, soleus, and plantaris were surgically removed and each muscle was weighed and placed in 1:10 (w/v) dilution of cold isolation buffer. Muscles were minced and homogenized. The muscles were minced by being cut into small

pieces by scissors. The muscles were homogenized by using a homogenizer for 15 second bursts. Next, the homogenate was centrifuged in a small centrifuge at 1600 x g for 10 minutes at a temperature of 4°C. 90 minutes. The supernatant fraction was separated and stored at -80° for later analysis.

Protein Analysis

In order to determine the amount of sample to use in the Ca²⁺ uptake and Ca²⁺ release analysis, a total protein assay was done immediately following the preparation of the homogenate. In order to increase reliability, two cuvettes was used for each sample. A Bio-Rad solution was prepared using 1:4 ratio of Bio-Rad and distilled water. A standard curve was done on the Spectrophotometer using BSA. Appropriate dilutions were made for homogenate and concentrations were determined at 595λ nm. Once the standards were measured, a correction factor was made for each homogenate and the amount of sample to use for Ca²⁺ uptake and release analysis was determined.

Ca²⁺ Uptake and Ca²⁺ Release

SR Ca²⁺ uptake and release was assayed in the homogenate. Each homogenate sample was assayed four times in order to determine reliability. A Jasco CAF-110 fluorometer was used to measure the Ca²⁺ uptake and release, using Fura-2 as a extravessicular Ca²⁺ indicator. The fluorescence (excitation = 340 nm and 380 nm, emission = 500 nm) was sampled via microcomputer (2 Hz) and converted into free Ca²⁺ concentrations using the following equation: $[Ca^{2+}]_i = K_d \cdot B \cdot (R - R_{min}) \cdot (R_{max} - R)^{-1}$, where K_d is the dissociation constant for Ca²⁺ and Fura-2 (200 nM), B is the ratio of the fluorescence due to excitation at 340

nm in the presence of zero and 100 μM free Ca^{2+} R_{\min} and R_{\max} represent fluorescence ratios in the presence of zero and 100 μM free Ca^{2+} . Homogenate protein (250 μg) was suspended in 1ml of assay buffer (92.5 mM KCl, 7.5 mM pyrophosphate, 18.5 mM TRIS, 1-2 mM Mg^+ , 1 μM Fura-2 and 2 μM free Ca^{2+} (pH 7.0 and 37 $^{\circ}\text{C}$). Ca^{2+} uptake was initiated by the addition of 1.0 mM Mg ATP and continued until no change in extravascular free Ca^{2+} was observed. Ca^{2+} release was initiated by the application 25 μM AgNO_3 and continued until a plateau in extravascular free Ca^{2+} was observed. The rates of Ca^{2+} uptake and release were determined as the steepest negative (uptake) and positive (release) slopes of the extravascular free Ca^{2+} vs. time curve and normalized by the SR protein concentration. All rates and magnitudes of Ca^{2+} uptake were normalized by SR protein concentration.

Data Analysis

Quantitative inquiry seeks to learn how many in a population have a particular characteristic or group of characteristics. It is specifically designed to produce accurate and reliable measurements that permit statistical analysis. The core value of statistical methodology is its ability to assist one in making inferences about a large group (a population) based on observations of a smaller subset of that group (a sample). In this case, the aging population serves as the large group, and the rats serve as the subset. In order for this to work correctly, a couple of things have to be true: the sample must be similar to the target population in all relevant aspects; and certain aspects of the

measured variables must conform to assumptions which underlie the statistical procedures to be applied (Helberg, 1995).

Data were analyzed by using methods of the two-way ANOVA. The ANOVA has long enjoyed the status of being the most used statistical technique in research (Howell, 1992). The method was used in this study because ANOVA deals with differences between or among sample means and it imposes no restriction on the number of means. It also allowed the investigator to deal simultaneously with two or more independent variables.

The two-way ANOVA uses two independent variables known as factors. The age of the rats and the muscle serve as factors. There is no interaction between the two factors. The two factors affect the dependent variable. In this investigation, the dependent variables are the rate of Ca^{2+} uptake and Ca^{2+} release by the SR.

CHAPTER 4

Findings

Introduction

A common consequence of the aging process is a reduction in the ability to complete everyday tasks. It has been reported that the aging process is associated with a loss in muscle mass, (atrophy), and a loss in the force generating capacity of skeletal muscle. Since the functioning of the sarcoplasmic reticulum (SR) is critical in regulating force production in skeletal muscle, it was interesting to hypothesize whether altered SR function could explain the reduced force generating capacity in aged skeletal muscle. Evidence to support this hypothesis was reported by Lynch, Rodgers, & Williams (1993). These investigators show that with increasing age, the level of SR proteins decline.

Muscle Mass

The muscle masses in the soleus, plantaris, and diaphragm were lower in the aged animals in comparison to the control animals. The masses were 22, 23, and 15% lower respectively in the aged animals (Figure 1 in Appendix A). However, these differences were eliminated when masses were normalized by body mass (Figure 2 in Appendix A). The difference in muscle mass supports the theory of the occurrence of atrophy with aging.

Ca²⁺ Uptake and Release

In all three muscles examined, the rates of Ca²⁺ uptake were not significantly different between the young and old animals. The rates of Ca²⁺ release were significantly reduced by 30% in the plantaris and diaphragm of the old animals. However, no difference was found in the soleus muscle pertaining to the rates of Ca²⁺ release.

Statistical Analysis

The analyzed data showed that release rates were different in aged, regardless of muscle. Specifically plantaris, diaphragm rates were reduced and soleus rates were unchanged (See Table 5 in Appendix D). These data also showed that there is no effect of aging on Ca²⁺ uptake. However, uptake by the plantaris is greater than diaphragm and soleus, regardless of condition (See Table 6 in Appendix D).

Summary

The results of this investigation suggest that SR function is altered with aging. This is supported by the finding that the rates of Ca²⁺ release were reduced by 30% in the plantaris and diaphragm of the aged animals. Results suggest that SR function is altered in “fast” muscles of the rat. The difference in muscle mass supports the theory of the occurrence of atrophy with aging. Masses of the soleus, plantaris, and diaphragm were 22, 23, and 15% lower in the aged animals. Overall, the findings suggest the possibility that changes in SR Ca²⁺ release contribute to diminished muscle function. In making an

inference one could say that this in turn leads to declines in physical ability of older adults.

CHAPTER 5

Summary, Discussion, Conclusion, and Implication

Summary

As stated in Chapter 1 of this document, the aim of this study was to determine if sarcoplasmic reticulum (SR) function, specifically the rate of Ca^{2+} uptake and Ca^{2+} release, was altered as a result of aging. Using an experimental procedure, data were collected and then analyzed by using the two way ANOVA. Four figures and two tables that appear in the appendices summarize the findings. The findings were grouped under the following segments: muscle mass, Ca^{2+} uptake and release. These segments are discussed more in the discussion section. The discussion will focus on the findings as it relates to the specific aim of the study.

Discussion

Muscle Mass

The absolute masses of the soleus, plantaris, and diaphragm were 22, 23, and 15% lower, respectively, in the aged animals. The decline in muscle mass supports the theory that muscles atrophy with aging. Muscle atrophy means muscle decreases in size, particularly, fiber cross-sectional area. Klitgaard, Ausoni, & Damiani (1989) found that mammalian aging skeletal muscle shows atrophy as well as prolongation of contraction. Muscle atrophy and contraction prolongation have been associated with changes in SR which regulates intracellular Ca. Since the percentage of decrease in muscle mass is not similar for each muscle, the findings are support by other researchers who have found

the degree of atrophy to be variable according to muscle and muscle function (Grimby and Saltin, 1993; Fitts et. al., 1984; Faulkner et. al., 1990; Holloszy et. al., 1991). For instance, the soleus muscle has been reported to show a larger loss of mass with age in comparison to other muscles (Brown, Ross, & Holloszy, 1993). As with the results of this investigation, the soleus had a 7% greater decreased mass in comparison to the diaphragm, however, there was no significant difference shown in the statistics.

Ca²⁺ Uptake and Release

In all three muscles examined, the rates of Ca²⁺ uptake were not significantly different between the young and old animals. This does not support the findings by Narayanan, et. al. (1996). Narayanan et. al. (1996), found that the Ca²⁺ uptake by the soleus muscle, which is composed of Type I fibers, was approximately 50% lower in the aged rats compared with adult rats.

Narayanan's findings may differ from the findings of this study for several reasons. First of all, Narayanan used different animals, he used the Fisher 344 rat instead of the Fisher 344 Brown x Norway. Also, Narayanan did not measure using the homogenate, he used the actual SR enriched membrane vesicles. Most importantly, Narayanan used animals of ages different from the animals used in the present study. The animals were 6 and 26 months. Furthermore, the findings of this investigation do support the research conducted by Larsson (1982). He found that the rate for Ca²⁺ uptake by the SR was the same in young and old animals.

The rates of Ca^{2+} release were reduced by 30% in the plantaris and diaphragm of the old animals. No difference was found in the rates of Ca^{2+} release of the soleus muscle. The plantaris is composed of mostly Type IIA and Type IIB fibers (fast-twitch fibers). The diaphragm is composed of a mixture of fibers, and contains fast-twitch fibers as well. These results suggest that SR rate of Ca^{2+} release is altered in “fast” muscles of the rat. The depression of the release rates support the notion by Gosselin, Johnson, & Sieck (1994) that alterations in skeletal muscle contractile properties are associated with age. A conclusion can be drawn from these results that Type IIA and Type IIB contractile properties that are dependent upon Ca^{2+} release may be depressed with aging.

Since the Ca^{2+} release rates were depressed in the plantaris and diaphragm, but not in the soleus, the present results support Kiltgaard’s findings. Klitgaard et. al., (1989), found that Type I fibers increased with aging, and Type IIA and Type IIB fibers undergo an atrophy process with aging as well as a decline in functional ability. The findings of this investigation also support Larsson. In 1997, Larsson & Li reported finding a related decrease in the speed of contraction, which is dependent upon Ca^{2+} release, in fast twitch fibers.

A depression in SR Ca^{2+} release may also result in decreased force output. The depression of the rates of Ca^{2+} release by the diaphragm supports research conducted by Tolep, Higgins, Muza, Criner, & Kelsen (1985). They conducted a study to test the hypothesis that aging is associated with the reduction in the diaphragm’s force generating capacity. The findings from

Tolep's study suggest that diaphragm strength is reduced in elderly individuals in comparison to young individuals. It is also supported by Gosselin et. al. who also observed age related increases in the relative contribution of slow myosin heavy chain isoform in the diaphragm muscle from female F344 rats. This supports the idea that diaphragm muscle undergoes age related changes such as a depression in the rate of Ca^{2+} release.

Conclusion

The results of this investigation suggest that SR function is altered in some muscles but not in others, with aging. This is supported by the findings that the rates Ca^{2+} release were reduced by 30% in the plantaris and diaphragm of the old animals. The results suggest that SR function is altered in "fast" muscles of the rat. The difference in muscle mass supports the theory of the occurrence of atrophy with aging. Masses of the soleus, plantaris, and diaphragm were 22, 23, and 15% lower respectively in the aged animals. Overall, the findings suggest that it is possible that changes the SR Ca^{2+} release may contribute to diminished muscle function and leads to a decline in physical ability of older adults.

Implications

Gerontologist and muscle function researchers will benefit from the findings in this study. The implications for each are discussed below.

Data pertaining to the actual structural and functional changes of SR due to aging are limited. Consequently, the SR functional changes that occur within muscle remains an unsolved research area for researchers. However, it has been theorized that Ca^{2+} uptake and release by the SR is altered with aging leading to the decline of reduced skeletal muscle function and decline of physical activity. In this investigation, atrophy was noted to occur in aging muscle as well as a depression in the rates of Ca^{2+} release by the plantaris and diaphragm muscles. As stated by Larrison (1982) it can be inferred that if we relate these changes in rat muscle to changes in human muscle, it can be assumed that atrophy and a depression in Ca^{2+} release rates occurs in some human muscles as well. Since, very little research has been using humans relating to SR function, this area is a vast one for future research.

The research should be of interest to gerontologist because, people are living longer than ever before (Clark & Weber, 1996). The aging population represent a growing segment of the US population for whom educational programs and interventions are needed (Burbaker & Roberto, 1993). Once additional research has been done on human SR, muscle function researchers in the area of gerontology will be able to take the information and begin to research that may help to decrease some of the SR functional changes.

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APPENDIX A:
FIGURES

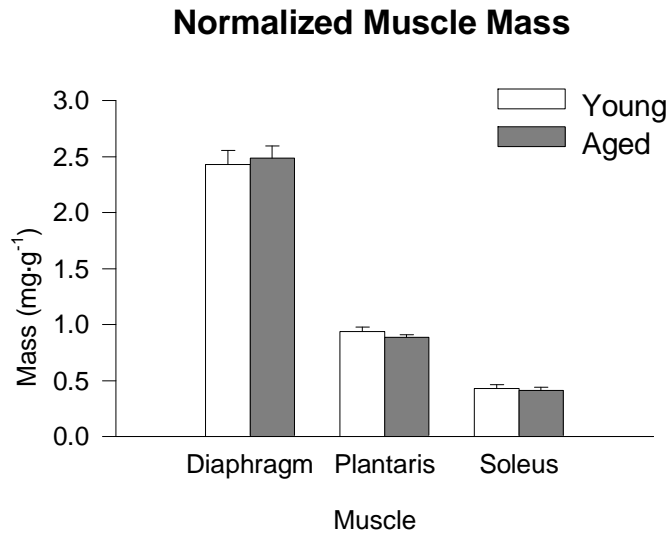
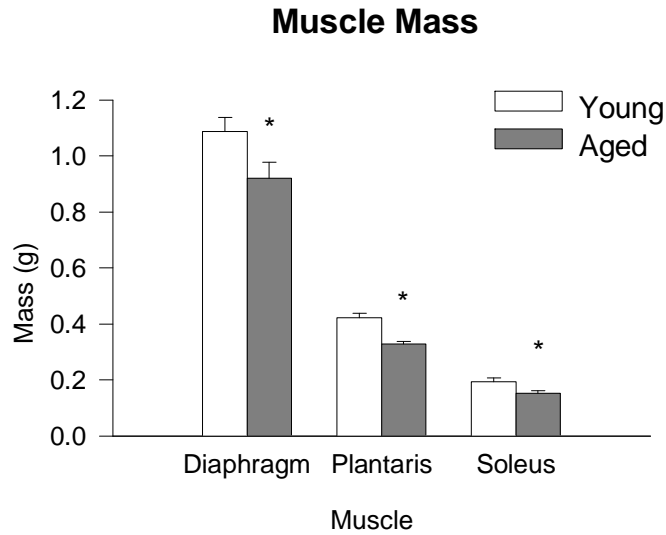
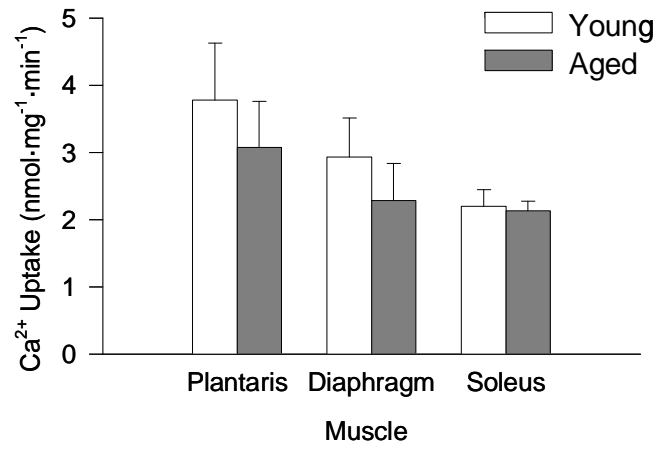


Figure 1. and Figure 2. shows the difference in muscle mass and normalized muscle mass respectively. The muscle mass in the soleus, plantaris, and diaphragm was lower in the aged animals in comparison to the control animals. The masses were 22, 23, and 15% lower in the aged animals. However, these differences were normalized by body mass. The difference in muscle mass supports the occurrence of aging. * Significant difference ($p < .05$)

Ca²⁺ Uptake



Ca²⁺ Release

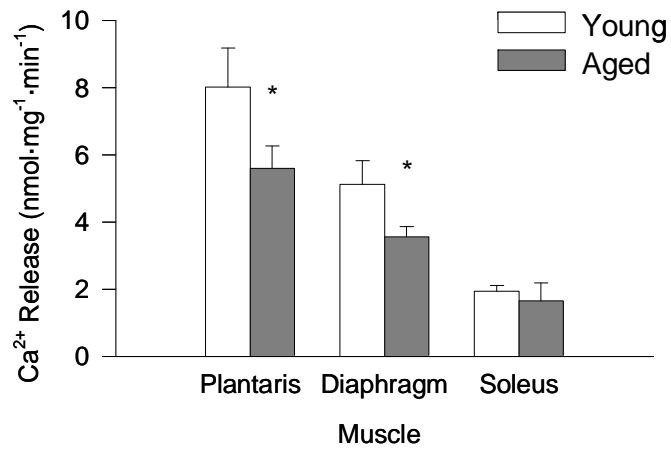


Figure 3. and Figure 4. Figure 3. shows the difference in the rate of Ca²⁺ uptake and figure 4. shows the difference in the rate of Ca²⁺ release. * Significant difference (p<.05)

APPENDIX B:
DESCRIPTIVE DATA

Descriptive Data

Table 1. This table shows the demographics of the adult animals in the control group.

Control Group (12 months)				
Subject	Body Wt.	Soleus	Diaphragm	Plantaris
C-1	431 g	0.38 g	1.20 g	0.81 g
C-2	476 g	0.39 g	1.02 g	0.85 g
C-3	449 g	0.38 g	0.91 g	0.74 g
C-4	447 g	0.50 g	1.71 g	0.98 g
C-5	445 g	0.39 g	1.20 g	0.86 g
C-6	448 g	0.28 g	1.03 g	0.83 g

Table 2. This table shows the demographics of the aged animals.

Aged Group (27 months)				
Subject	Body Wt.	Soleus	Diaphragm	Plantaris
A-1	401 g	0.32 g	1.10 g	0.69 g
A-2	342 g	0.36 g	0.90 g	0.57 g
A-3	373 g	0.25 g	0.72 g	0.96 g
A-4	369 g	0.34 g	0.69 g	0.83 g
A-5	342 g	0.27 g	0.71 g	0.63 g
A-6	385 g	0.28 g	1.02 g	0.63 g

APPENDIX C:
RAW DATA

Raw Data

Table 3. This table shows the SR homogenate values for the aged (27 months) animals.

SR Homogenate Function in Aged Rats

**Raw
Data**

Animal #	<i>Diaphragm</i>			<i>Plantari s</i>			<i>Soleus</i>		
	Ca Up.	Ca Rel.	Free Ca	Ca Up.	Ca Rel.	Free Ca	Ca Up.	Ca Rel.	Free Ca
Control									
1	3.48	6.16	1.53	1.45	3.83	1.91	1.16	1.76	2.6
1	1.89	5.12	1.72	1.3	5.19	2.14	1.14	1.12	2.39
1	2.14	4.8	1.83	1.47	2.11	5.8	4.84	2	2.39
1	2.49	3.7	1.89	1.8	5.65	2.28	1.39	1.24	2.64
Mean	2.50	4.95	1.74	1.51	4.20	3.03	2.13	1.53	2.51
SD	0.60	0.88	0.14	0.18	1.38	1.60	1.57	0.36	0.12
SEM	0.30	0.44	0.07	0.09	0.69	0.80	0.78	0.18	0.06
2	2	3	1.8	1.95	6.91	2.3	2.45	2.17	2.44
2	1.44	2.19	2	2.24	6.08	2.66	2.22	2.26	2.44
2	1.26	2.29	1.96	3.14	4.71	2.26	2.3	1.88	2.47
2	1.04	2.06	1.88	2.48	6.42	2.53	2.27	2.35	2.5
Mean	1.44	2.39	1.91	2.45	6.03	2.44	2.31	2.17	2.46
SD	0.36	0.36	0.08	0.44	0.82	0.16	0.09	0.18	0.02
SEM	0.18	0.18	0.04	0.22	0.41	0.08	0.04	0.09	0.01
3	4.4	7.65	1.95	6.79	12.85	1.76	1.24	1.82	1.72
3	3	7.5	1.88	6.61	14.86	2.23	1.26	1.64	1.08
3	2.83	5.28	1.68	6.78	9.68	1.92	2.14	1.32	2.22
3	2.34	6.26	1.67	7.23	10.39	2.1	2.2	1.2	2.09
Mean	3.14	6.67	1.80	6.85	11.95	2.00	1.71	1.50	1.78
SD	0.77	0.97	0.12	0.23	2.05	0.18	0.46	0.25	0.44
SEM	0.38	0.48	0.06	0.11	1.03	0.09	0.23	0.12	0.22
4	2.35	6.04	1.7	3.24	8.3	2.12	2.87	2.49	2.13
4	2.3	4.78	1.71	3.68	10.56	2.67	3.68	3.34	2
4	2.56	4.6	1.7	3.21	10.6	2.45	1.99	2.2	2.04
4	2.71	5.28	1.53	3.57	11.67	2.26	1.53	2.67	1.87

(Table 3. Continued)

Mean	2.48	5.18	1.66	3.43	10.28	2.38	2.52	2.68	2.01	
SD	0.16	0.56	0.08	0.20	1.23	0.21	0.83	0.42	0.09	
SEM	0.08	0.28	0.04	0.10	0.61	0.10	0.41	0.21	0.05	
	5	2.46	4.61	1.91	3.77	6.12	1.81	2.89	1.95	2.31
	5	3.67	5.19	1.8	2.74	6.77	1.99	2.89	1.62	2.24
	5	4.97	5.69	2.08	2.89	6.91	2.09	2.35	1.57	2.23
	5	3.2	4.98	1.93	2.89	4.23	1.54	2.72	1.92	2.58
Mean	3.58	5.12	1.93	3.07	6.01	1.86	2.71	1.77	2.34	
SD	0.91	0.39	0.10	0.41	1.07	0.21	0.22	0.17	0.14	
SEM	0.46	0.20	0.05	0.20	0.53	0.10	0.11	0.09	0.07	
	6	5.64	8.2	2.09	1.74	2.77	1.08	2.28	1	2.28
	6	5.98	5.91	1.96	5.95	7.99	1.87	2.59	1.74	2.6
	6	5.3	6.25	1.89	5.45	7.17	1.76	2.08	3	2.49
	6	6.77	7.6	2.32	6.03	8.16	2.19	3.57	3.27	2.48
Mean	5.92	6.99	2.07	4.79	6.52	1.73	2.63	2.25	2.46	
SD	0.55	0.94	0.16	1.78	2.20	0.40	0.57	0.93	0.12	
SEM	0.27	0.47	0.08	0.89	1.10	0.20	0.29	0.46	0.06	

Table 4. Thus table shows the SR homogenate values for the adult (12 months) animals.

Animal #	<i>Diaphragm</i>			<i>Plantari s</i>			<i>Soleus</i>		
	Ca Up.	Ca Rel.	Free Ca	Ca Up.	Ca Rel.	Free Ca	Ca Up.	Ca Rel.	Free Ca
Aged 1	1.59	3.87	1.73	0.99	2.25	2.23	2.33	2.14	2.6
1	1.34	3	1.56	1.46	6.04	2.11	1.52	2.25	2.69
1	1.43	3.02	1.92	2.16	5.45	2.26	2.54	2.27	2.43
1	1.42	2.43	1.75	1.54	4.68	2.25	1.65	1.84	2.55
Mean	1.45	3.08	1.74	1.54	4.61	2.21	2.01	2.13	2.57
SD	0.09	0.51	0.13	0.42	1.44	0.06	0.43	0.17	0.09
SEM	0.05	0.26	0.06	0.21	0.72	0.03	0.22	0.09	0.05
2	1.31	1.91	1.9	2.39	3.09	2.66	1.06	1.16	2.48
2	1.44	2.19	2	3.09	3.94	2.81	2.48	0.99	2.33
2	0.86	2.09	1.83	1.71	5.92	2.32	2.47	1.34	2.83
2	1.28	1.66	1.83	1.69	5.51	2.81	2.39	0.83	2.48
Mean	1.22	1.96	1.89	2.22	4.62	2.65	2.10	1.08	2.53
SD	0.22	0.20	0.07	0.58	1.15	0.20	0.60	0.19	0.18
SEM	0.11	0.10	0.03	0.29	0.57	0.10	0.30	0.10	0.09
3	1.27	2.59	1.77	2.27	8.25	2.42	2.07	1.46	2.2
3	0.93	2.67	1.69	1.87	8.12	2.42	1.47	1.35	2.05
3	1.04	2.8	1.78	3.32	8.12	2.54	0.76	1.2	2.11
3	0.95	2.55	1.66	1.93	5.42	2.2	1.72	1.4	2.12
Mean	1.05	2.65	1.73	2.35	7.48	2.40	1.51	1.35	2.12
SD	0.13	0.10	0.05	0.58	1.19	0.12	0.48	0.10	0.05
SEM	0.07	0.05	0.03	0.29	0.59	0.06	0.24	0.05	0.03
4	3.86	5.23	1.78	7.63	14.28	3.08	1.88	0.97	1.81
4	4.4	4.08	1.56	6.45	14.68	2.36	2.5	1.03	2.02
4	1.82	4.26	1.75	4.97	14.25	2.27	2.01	1.66	2.23
4	1.73	4.96	1.55	5.29	10.61	2.63	1.93	1.19	2.03
Mean	2.95	4.63	1.66	6.09	13.46	2.59	2.08	1.21	2.02
SD	1.19	0.48	0.11	1.05	1.65	0.31	0.25	0.27	0.15
SEM	0.60	0.24	0.05	0.52	0.83	0.16	0.12	0.14	0.07
5	2.79	4.38	1.71	5.15	6.56	2.11	2.64	1.77	2.65
5	4.41	5.45	2.2	3.79	5.66	1.93	2.66	1.43	2.45

(Table 4. Continued)

	5	5.22	6.61	2.19	3.21	3.13	1.73	1.24	1.26	2.53
	5	4.24	5.49	2.31	2.97	2.18	1.81	1.12	0.96	2.07
Mean		4.17	5.48	2.10	3.78	4.38	1.90	1.92	1.36	2.43
SD		0.88	0.79	0.23	0.85	1.79	0.14	0.74	0.29	0.22
SEM		0.44	0.39	0.12	0.42	0.89	0.07	0.37	0.15	0.11
	6	1.65	3.7	1.7	4.73	6.48	2.19	2.74	2.67	2.73
	6	2.51	4.58	2.38	2.46	2.41	1.36	2.5	1.62	2.69
	6	2.81	3.24	1.97	3.4	5.13	2.24	2.82	3.42	2.68
	6	5.23	6.17	1.89	3.63	4.88	2.11	3.28	2.67	2.75
Mean		3.05	4.42	1.99	3.56	4.73	1.98	2.84	2.60	2.71
SD		1.33	1.12	0.25	0.81	1.47	0.36	0.28	0.64	0.03
SEM		0.66	0.56	0.12	0.40	0.73	0.18	0.14	0.32	0.01

APPENDIX D:
ANOVA DATA ANALYSIS

Table 5. Two Way repeated measure ANOVA results for Ca²⁺ release.

Dependent Variable: Release

Source of Variance	DF	SS	MS	F	P
Condition	1	17.82	17.82	6.80	0.014
Muscle	2	143.66	71.83	27.39	<0.0001
Condition x Muscle	2	6.54	3.27	1.25	0.3022
Residual	29	76.05	2.62		
Total	34	250.25	7.36		

Power of performed test with $\alpha = 0.0500$: for Condition : 0.645

Power of performed test with $\alpha = 0.0500$: for Muscle: 1.000

Power of performed test with $\alpha = 0.0500$: for Condition x Muscle: 0.0823

Table 6. Two-Way repeated measures ANOVA results for Ca²⁺ uptake.

Dependent Variable: Uptake

Source of Variance	DF	SS	MS	F	P
Condition	1	1.988	1.988	1.035	0.3171
Muscle	2	9.894	4.947	2.576	<0.042
Condition x Muscle	2	0.726	0.363	0.189	0.8288
Residual	30	57.62	1.921		
Total	35	70.23	2.007		

Power of performed test with alpha = 0.0500: for Condition : 0.0512

Power of performed test with alpha = 0.0500: for Muscle: 0.297

Power of performed test with alpha = 0.0500: for Condition x Muscle: 0.0500

VITA

VITA

Karma Melisa Rabon was born in Sylvester, Georgia and attended Natchez High School in Natchez, Mississippi. After graduation, she attended Alcorn State University in Lorman, Mississippi. After the completion of her freshmen year, she transferred to Virginia Polytechnic Institute and State University in Blacksburg, Virginia and received a B.S. degree in Health Education.

The majority of Karma's professional experience has related to academic excellence. In 1992-1995, Karma worked as a Biology Tutor for the Office of Academic Enrichment at Virginia Tech. In 1995-1996, Karma worked as the graduate assistant for the Valuing Diversity Project in the College of Human Resources at Virginia Tech. As the graduate assistant for the Valuing Diversity Project, Karma was responsible for the development and implementation of several diversity programs including, several student forums, and a film festival.

Beginning in the Spring of 1997, Karma became the graduate assistant for the Office of Minority Engineering Programs (OMEP). As a graduate assistant for OMEP, Karma was responsible for the Young Scholars Program and the implementation of several conferences. Additionally, Karma interned as Exercise Leader with the Cardiac Therapy and Intervention Center at Virginia Tech.

Karma is a member of Delta Sigma Theta Sorority, Inc. She has served as a Big Sister through BOOM (Black Organization of Mentors). She has

developed a monthly health day held at St. Paul AME Church and she has served as a volunteer at the Adult Day Care Center.

Karma has received many honors throughout her college career. In 1992, Karma wrote and presented a research paper at the Minority Biochemistry Research Symposium (MBRS). In 1993-1995, Karma was recognized for her academic achievement by being on the Dean's List. In 1994, she received the Distinguished African American Award at Virginia Tech.

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