CHAPTER ONE:

INTRODUCTION

INTRODUCTION

Age-related physiological changes, such as degeneration of neurologic, musculoskeletal, cardiorespiratory and immune function, occur after maturation (Johnson, 1985). One of the dysfunctions experienced by the elderly is diminished exercise tolerance. Exercise intolerance is associated with strength decrements. In fact, strength is decreased by approximately 35% in both men and women by approximately the age of 80 years (Booth et al., 1994). Brooks et al. (1994) stated that muscle strength decreases approximately 8% per decade after the age of 45, with an overall decline of 30-40% from between strength observed at age 30 years and strength prior to the time of death.

Wasting or loss of muscle seems to contribute to the reduction in skeletal muscle function in aged individuals (Ermini, 1976). Sarcopenia is a generic term for the loss of skeletal muscle mass, quality and strength. Sarcopenia is correlated with a number of aging properties, i.e., changes in body mass and composition, bone density, muscle mass and muscle function (e.g., contractile properties, adenosine triphosphate (ATP) production, substrate utilization, thermogenic mechanisms) (Dutta & Hadley, 1995). It has been well documented that muscle atrophy increases with advancing age in both human and animal subjects (Rodgers & Evans, 1993). Sarcopenia appears primarily in limb muscles where the cross-sectional area of the muscle is decreased; while the length of the muscle remains constant. The onset of sarcopenia begins to develop at about the age of 40 years and is accelerated in inactive individuals.

One factor thought to contribute to the progression of sarcopenia is the production of reactive oxygen species (ROS). ROS can exist with one or more oxygen atoms which are determined by how and where they are molecularly generated. ROS are produced by radiation and are also the byproducts of aerobic metabolism. ROS appear to mediate various pathological conditions in a variety of tissues (de-Haan et al., 1995). ROS cause

oxidative damage to DNA, proteins, and lipids with increasing age, and have been presumed to be a major, but not the only, factor triggering endogenous tissue damage leading to aging (Jenkins, 1988). Also, ROS may play a role in decreasing muscular strength. This ROS theory of muscle decline was investigated by Zebra et al. (1990) in which they concluded that ROS mediated secondary or delayed onset of damage in muscle of aged mice which was greater than that of young and adult mice. This indicates that muscle from old mice is more susceptible to injury than muscle of young and adult mice, and that ROS may play a key role.

The sarcoplasmic reticulum (SR), a membranous organelle that surrounds each myofibril and runs parallel with the myofibril, is responsible for sequestering Ca^{2+} during muscle relaxation and for releasing Ca^{2+} during contraction. The Ca^{2+} ATPase pump is located in the longitudinal portion of the SR membrane. It is responsible for the uptake of Ca^{2+} during muscle relaxation and for maintaining low myoplasmic free Ca^{2+} in resting muscle. Energy for the pump is acquired via Ca^{2+} ATPase enzyme to hydrolyze adenosine triphosphate (ATP) yielding adenosine diphosphate (ADP) and inorganic phosphate (Pi). The Ca^{2+} release channel (ryanodine receptor) is located at the ends of the SR structure. Its function is to remove intracellular Ca^{2+} from the SR into the myoplasm producing relaxation of the muscle. If any one of these properities are disrupted, then normal function of the skeletal muscle will be modified, possibly preventing muscle contraction.

Hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) are two kinds of ROS which alter normal skeletal muscle Ca^{2+} homeostasis by disrupting normal sarcoplasmic reticulum (SR) function. H_2O_2 causes membrane damage by lipid peroxidation, leading to alterations of membrane structure and function by degradation of polyunsaturated fatty acids. This process changes the protein:lipid interactions forming breakdown-products which migrate to other cells, intensifying membrane damage and disturbance. ROS may disturb the level of intracellular Ca^{2+} homeostasis via activation of phospholipases (Glende

& Pushpendran, 1986) which can lead to a decline in glutathione levels which leads to H_2O_2 production. H_2O_2 will decrease protein-SH (sulfhydryl) (Pascoe et al., 1987) groups, signaling changes in the SR functional capacity which may trigger an increase in intracellular Ca^{2+}. A prolonged increase of intracellular Ca^{2+} will cause damage to the Ca^{2+} ATPase function (Braughler, 1988). H_2O_2 can then bind with chloride to form myeloperoxidase, an enzyme found in neutrophils. The end-product of this formation is HOCl (Stern, 1985). HOCl is a highly reactive ROS which primarily oxidizes -SH groups. These effects will lead to depletion of thiols and a decline in ATP which are both essential in maintaining cell integrity (Spragg, 1985). Cell death is believed to be the result of ROS intolerance.

Researchers have not established that ROS lead to skeletal muscle cell death, but many have documented the effects of ROS on SR function. For example, Favero et al (1995) showed that skeletal muscle SR was susceptible to ROS by using H_2O_2 to activate the Ca^{2+} release channel at 1mM concentration. However, in the presence of H_2O_2 concentrations greater than 10 mM, equilibrium binding (the amount of Ca^{2+} being released by ryanodine is equal to the amount of Ca^{2+} reuptake) was inhibited. Change in the equilibrium binding suggests that normal gating function has been affected by H_2O_2. This result was supported by the finding that Ca^{2+} release was inhibited in the presence of 10µM ruthenium red, a Ca^{2+} channel blocker. They concluded that H_2O_2 was acting on the release channel rather than the membrane. Ca^{2+} ATPase showed a decline in both the rate and amount of Ca^{2+} uptake when the SR membrane was pretreated with H_2O_2 [1-20mM]. Brotto & Nosek (1996) also investigated the affects of H_2O_2 on the Ca^{2+} channel from the SR of rat skeletal muscle. Their results showed that transient exposure to 1mM H_2O_2 for 5 minutes had no subsequent effect on Ca^{2+} uptake by the SR. However, H_2O_2 significantly depressed Ca^{2+} release. H_2O_2 appears to block the normal function of the Ca^{2+} channel. The method of exposure to H_2O_2, direct or transient,

appears to induce either an increase (Favero, 1995) or a depression (Brotto, 1996) in Ca^{2+} release, respectively.

Hypochlorous acid (HOCl) effects on SR Ca^{2+} ATPase activity were investigated by Eley et al. (1991). They found that 100μM HOCl significantly decreased Ca^{2+} uptake and Ca^{2+} ATPase activity in SR microsomes prepared from isolated rat hearts perfused with HOCl for 60 minutes. The inactivation of the SR Ca^{2+} ATPase activity in skeletal muscle SR has also been shown by Favero et al. (unpublished data). Complete inhibition of Ca^{2+} transport was noted using 3mM HOCl. Also, binding of the fluorescent probe, flurescein isothiocyanate (FITC), (method to assess the structure of the ATP binding region) to the ATPase protein was reduced. The data seem to suggest that HOCl may cause structural damage to the ATPase enzyme.

STATEMENT OF THE PROBLEM

According to the free radical theory, aging may be associated with the progressive accumulation of ROS damage (Emerit & Chance, 1992). If ROS does indeed damage aged tissue, then it is possible that (1) aged muscle produces more ROS, (2) aged muscle cannot adequately buffer ROS production, and/or (3) aged muscle is more susceptible to adverse effects of ROS. If any one of these problems is perpetuated then hypothetically, the rate in which one ages could accelerate age-related physiological changes. These changes would directly impact an older adult's longevity and lessen his/her ability of independent living. By the year 2030, there will be about 70 million older people making independent living crucial in society. Also, persons 65 years old and over will present 13% of the population in the year 2000 but will be 20% by 2030. The number of persons utilizing formal care (nursing homes) and informal care (mainly care at home) will escalate due to social, economical, and poor health. Accordingly, a rise in those participating in entitlement programs such as Security and Medicare will occur. Therefore, mimimizing

ROS damage may possibly impact older adults (65+) overall health and decrease intrinsic contractile properties of muscle.

SIGNIFICANCE OF THE STUDY

Some studies have shown that ROS cause damage to the Ca^{2+} ATPase function and diminishes cell integrity possibly resulting in cell death. However, researcher have not adequately examined the effects of ROS with advancing age. Therefore, the purpose of this investigation was to determine whether the SR of muscle from aged rats are more susceptible to the deleterious effects of ROS. This was accomplished by measuring the effect of H_2O_2 on Ca^{2+} release and the effect of HOCl on Ca^{2+} uptake by skeletal muscle SR in young and old Brown Norway-Fischer 344 (F344BNF1/Nia) hybrid rats.

RESEARCH HYPOTHESES

Ho: H_2O_2 does not cause greater SR Ca^{2+} release in 12 than in 27 month old rats.

Ho: HOCl does not cause greater depression of SR Ca^{2+} uptake in the 12 month old than in 27 month old rats.

DELIMITATIONS

The following are known delimitations to this study:

1. The investigation was delimited to Brown Norway-Fischer 344 (F344BNF1/Nia) hybrid rats skeletal muscle (gastrocnemius) SR.
2. Two age groups of 12 and 27 months old.
3. The investigation was delimited to the use of H_2O_2 and $AgNO_3$ as Ca^{2+} release agents.
4. The investigation was delimited to the use of HOCl and ATP as Ca^{2+} uptake agents.
5. The investigation was delimited to the use of H_2O_2 and HOCl as ROS.

LIMITATIONS

The limitation of the study was generalized for the specific group in questioned:

1. Animal research (*in vitro*) may not translate well into human subjects.

BASIC ASSUMPTIONS

The following assumptions were made prior to the start of the investigation:

1. All specimens possed normal anatomy and were free of disease.
2. Subjects were properly anesthetized prior to muscle removal.
3. Brown Norway-Fischer 344 rats are excellent aging research models.

DEFINITIONS AND SYMBOLS

Sarcopenia	loss of muscle mass or cross-sectional area
ROS	Reactive Oxygen Species
H_2O_2	Hydrogen peroxide
HOCl	Hypochlorous acid
Ca^{2+}	Calcium
SR	Sarcoplasmic reticulum

SUMMARY

The production of ROS may be a contributor to the progression of sarcopenia. ROS are produced by radiation and are also the byproducts of aerobic metabolism. ROS have been found to mediate various pathological conditions in a variety of tissues, cause oxidative damage to DNA, proteins, and lipids with age and is presumely a major factor leading to aging (Jenkins, 1988). ROS may play a role in other physiological aspects of aging such as, a decrease in muscular strength. This decline in muscular strength by the affects of ROS, indicates that skeletal muscle is highly susceptible to injury (Zebra et al., 1990). ROS, H_2O_2 and HOCl, alter normal skeletal muscle Ca^{2+} homeostasis by disrupting normal sarcoplasmic reticulum function (Favero et al., 1995; Eley et al., 1991).

ROS also cause damage to the Ca^{2+} ATPase function and may later result in cell death. However, the effects of ROS with advancing age have yet to be investigated. Therefore, this study ventured to document damage , if any, between adult and aged rats.