MOTOR UNIT PROPERTIES IN HUMAN LIMB MUSCLES: CHRONIC AND ACUTE PERTURBATIONS

BY

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ABSTRACT

The objective of this thesis was to determine whether, and to what extent the adapted states of age, Addison's Disease (AD), or creatine monohydrate (Cr) supplementation alter the mechanical (contractile properties) and excitation (motor unit firing rates) components of the motor unit (MU). As well, it was assessed whether the components of the MU change independently or in tandem, and how these alterations might affect muscle strength.

Study one investigated quadriceps function in nine AD women and nine age-matched controls (C). Results indicated that in AD maximal voluntary strength (MVC) was similar to C, but central activation was less (~ 7%), and muscle contractile properties were slowed (~ 40%). During fatigue, the rate of force decline did not differ between groups. However, the AD patients did not persist with the fatigue task for a similar length of time as the C group. Within the first minute of fatigue, maximal and submaximal IEMG in the AD group were significantly elevated compared to the C group. Results indicated that contractile properties slowed, and endurance time decreased, but MVC did not change with AD.

The purpose of **study two** was to assess six younger and six older men to determine whether age-related alterations in MU firing rates and contractile properties were similar in an agonist - antagonist muscle pair in the upper limb. For both muscles MVC decreased ~45%, but contractile properties were not slowed with age. The MU firing rates showed a greater age-related decrease in the biceps compared to the triceps. Thus, alterations in the excitation (MU firing rates) and

mechanical (contractile properties) components of the MU occurred independently and this may affect force control of the arm.

Fourteen younger and 13 older men were assessed in **studies three and four**, respectively. The effect of acute Cr supplementation on contractile properties, strength, fatigue and recovery were examined in the elbow flexors. Following Cr supplementation (20g x 5 days) body weight increased significantly (~ 1.0kg) in the younger and older men. In both age groups MVC, and stimulated contractile properties at rest were not affected by supplementation. In addition, there were no alterations in time to fatigue, MVC, central activation, EMG, stimulated tensions and durations during fatigue and recovery in younger and older men. These results indicate that Cr supplementation did not affect muscle performance in either age group.

In summary results indicated that when the mechanical aspect (Study 1), or excitation component (Study 2), of the MU was altered, strength and/or fatigue were affected. However, when the MU did not change (Studies 3 & 4) neither strength nor fatigue were modified. It appears that contractile and excitation components of the MU can change independently, and in the upper limb the excitation component is more malleable than the mechanical component.

Keywords: isometric, ageing, creatine, Addison's Disease, women, men, motor unit firing rates, contractile properties, elbow flexors, elbow extensors, quadriceps

CO-AUTHORSHIP

The following thesis contains material from published (Chapters 4,5) and submitted (Chapter 2) manuscripts. As well, Chapter 3 is in preparation for submission. The first author on all manuscripts was J.M. Jakobi and Chapter 2 was co-authored by D.W. Killinger, B.M. Wolfe, J.L. Mahon and C.L. Rice, whereas Chapters 4 and 5 were co-authored by C.L. Rice, S.V. Curtin and G.D. Marsh.

All of the experimental data presented in this thesis were collected and interpreted by Jennifer M. Jakobi.

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GLOSSARY OF TERMS
For this Thesis

Accommodation Session: A familiarization session of the experimental protocol

Action Potential (AP): A brief (< 1.0 ms) electrical signal recorded by an intramuscular needle representing the transmembrane voltage changes that occur in response to excitable cell stimulation. The APs that occur in nerve and muscle cells may be in response to either normal voluntary nervous system activation, the application of sensory stimuli or electrical stimulation (Robinson 1995)

Addison's Disease (AD): A disease of the adrenal cortex which was first described by Thomas Addison in 1855. This disease is primarily caused by auto antibodies directed at adrenal cortical cells, although a secondary cause is tuberculosis.

Ageing: The process of senescence from birth to death

Agonist: A muscle or muscle group that makes the major contribution to movement at the joint (Clarkson and Gilewich 1989)

Antagonist: A muscle or muscle group that has an opposite action to the agonist (Clarkson and Gilewich 1989)

Binned: The process of group MU firing rates in 5.0 Hz increments in order to construct a frequency histogram.

Contraction Duration (CD): The sum of time to peak tension and half relaxation time of a twitch

Corticospinal Pathway: A descending pathway with neuron cell bodies located in the cerebral cortex, and axons of this pathway do not synapse before connecting with spinal neurons.

Corticosteroid: A C-21 steroid produced by the adrenal cortex

Creatine (Cr):(N-[aminoiminomethyl]-N-methyl glycine). This substance is synthesized in the liver, pancreas and kidney. Most people consume 1-2g of Cr per day from a regular diet, although some people also supplement their diet with this substance. Additionally it is a product of anaerobic resynthesis of ATP

$$PCr + ADP + H+ \xleftrightarrow{CPK} ATP + Cr$$

Creatine Phosphate (PCr): A high energy phosphate molecule important in synthesis of ATP

Dehydroepiandosterone (DHEA): A C-19 steroid produced by the adrenal cortex

Dependent Variable: The statistical variable being measured

Electromyography (EMG): A complicated and summated signal that represents the extracellular voltage-time measure of the level of the intrinsic excitation during voluntary contraction in a skeletal muscle (Enoka 1988). Such data are obtained by applying surface electrodes or by inserting a needle electrode into the muscle and observing electrical activity with an oscilloscope and listening with a loudspeaker

Excitation Contraction Coupling (EC coupling): Sequence of events by which an action potential in the plasma membrane of a muscle fibre leads to cross-bridge activity through the interaction of actin and myosin

Fatigue: an acute impairment in the ability to produce a pre-determined target force, as well as an increase in perceived effort to the extent that the target force cannot be, or will not be produced

Half Relaxation Time: The time required for the twitch to fall to half of its peak amplitude

Glucocorticoid: A C-21 steroid produced by the adrenal cortex

Isometric contraction: When tension is developed in the muscle but no movement occurs, and the joint angle or position does not change

Kinase: enzyme that transfers a phosphate (usually from ATP) to another molecule

Maximal Voluntary Contraction (MVC): The maximal torque produced in a muscle contraction in which a subject employs voluntary activation/effort

Motor Unit (MU): The motor neuron plus all the muscle fibres it innervates

Peak Torque (PT): The maximal force produced from an electrically evoked muscle contraction (amplitude of the twitch or tetanus)

Supine: The position of lying on the back

Tetanus: The mechanical response of a muscle to a 50 Hz series of 16 electrical pulses which last 320 ms.

Time to Peak Tension (TPT): The time required for the twitch to attain peak amplitude

Twitch: The mechanical response of a muscle to a single brief electrical pulse

Twitch Interpolation: A method of assessing central activation (maximal drive) of a muscle. Two superimposed pulses at 100 Hz are given to a muscle during and following a MVC. Twitch interpolation is quantified by expressing the superimposed twitch as a percentage (ratio) of the post twitch.

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CHAPTER 1:

INTRODUCTION AND BACKGROUND

1.0 GENERAL INTRODUCTION

The generation of voluntary movement is a complex process that requires complementary interaction between the nervous system and skeletal muscle. Movement patterns are organized in the central nervous system in a functional hierarchy. The somatotopically organized motor cortex (primary motor area) transmits information through major efferent pathways (corticospinal tract) to the spinal motor neurons and their related interneurons. Two other brain structures (cerebellum, basal ganglia) send parallel projections to the brain stem descending pathways to aid in the modulation of movement (Heimer, 1995). Ultimately the entire motor command to move is transmitted from the central nervous system (brain structures, corticospinal tract, descending pathways) to the spinal motor neuron pool of the peripheral nervous system. This motor neuron pool is composed of many motor units (MU), and each unit is defined as an alpha motor neuron and all the muscle fibres that it innervates.

Motor units can be categorized into excitation and mechanical elements (Enoka 1988). The mechanical component of the MU is the skeletal muscle (in this thesis assessed by measures of contractile properties), whereas in this thesis the excitation component refers to the firing rate of the alpha motor neuron (assessed by a population mean of MU firing rates). Motor units produce and modulate force

through the number of MUs which are active (recruitment), as well as the rate of activity (rate coding). Both the mechanical and excitation components of the motor unit are adaptable, and there are many inter-related factors which can alter the inherent characteristics of the MU. (Figure 1.1). Acute factors investigated in this thesis included *nutrition and fatigue*, whereas the chronic perturbations studied consisted of *physical activity/inactivity, endocrine factors, ageing and disease processes* (Figure 1.1). Regardless of whether the perturbation is acute or chronic, one of the components of the MU will be affected, and in turn the manner in which force is produced and regulated will be altered.

1.1 FACTORS WHICH AFFECT FORCE PRODUCTION

Endocrine Factors: The majority of research relating to muscle function and alterations in endocrine hormones has centred upon menopause (estrogen), andropause (testosterone) and somatopause (growth hormone). There are a limited number of studies on adrenopause (adrenal steroids), but recent reports suggest that lack of dehydroepiandosterone (DHEA) and its sulfate (DHEAS) may play a role in the development of muscle wasting, metabolic abnormalities and impaired physical functioning (Proctor et al. 1998). DHEA is a precursor for androgenic and estrogenic steroid formation in peripheral tissues (Herbert 1995). In males, most androgens are produced in the testes, whereas in females most androgen production occurs from progenitors such as the C-19 steroids (DHEA, DHEAS) (Lieberman,1995). Thus a deficit in these precursors will attenuate muscle

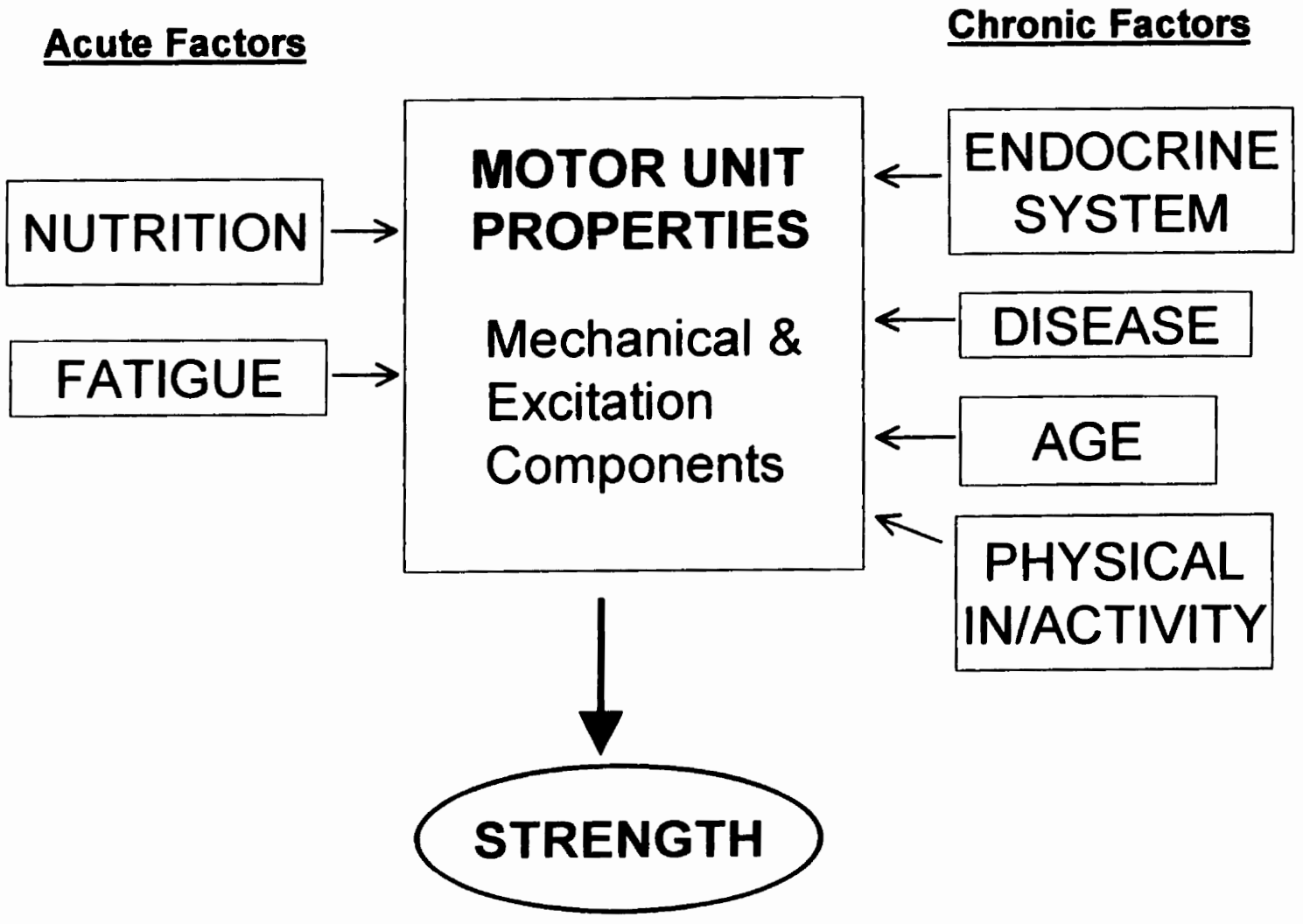

Figure 1.1: Factors which influence the motor unit as investigated in this thesis. Please note additional factors which are not indicated, such as afferent feedback also may have a direct influence upon the MU.

strength through a decrease in concentration of androgens. Glucocorticoids and mineralocorticoids (C-21 steroids) which also are produced in the adrenal cortex are involved in regulating carbohydrate metabolism and electrolyte balance, respectively. Consequently, C-21 steroids might influence muscle strength by altering ATP production, or ionic shifts across the membrane, and this may affect membrane properties and possibly excitability. The effect of a lack of these adrenal cortex steroids has not been assessed in relation to the excitation or mechanical components of the MU.

Disease:

Primary adrenal insufficiency (Addison's Disease) is a disease of the adrenal cortex that results in lack of production of C-21 and C-19 steroids. Patients with Addison's Disease (AD) frequently report muscle weakness and fatigue (Kong & Jeffcoate 1999), yet there has been no attempt to measure these symptoms and determine whether they are related to alterations in the mechanical or excitation component of the MU. Inactivity frequently results as a consequence of disease, and a decrease in activity has profound affects upon muscle, particularly in the lower limb (Bloomfield 1997). It is often difficult to discern whether MU alterations occur because of the disease, treatment of the disease, or disuse.

Age: A decrease in strength occurs with an increase in age, but this decline does not become apparent until approximately 60 years (Doherty et al. 1993), and it

seems that age-related weakness differs among muscles (Jakobi et al. 1999, Porter et al. 1995). There are inherent age-related changes that directly influence muscle strength and function (Porter et al. 1995) such as the loss of alpha motor neurons from the spinal cord, with the subsequent degeneration of their axons (Tomlinson & Irving 1977). This ultimately results in a decrease in the number of MUs, with a concomitant increase in size of the remaining MUs (reviewed in Porter et al. 1995). Furthermore, ageing is generally associated with slowed contractile properties and weakened muscle (Doherty et al. 1993), a shift in the MU recruitment threshold toward lower force levels (Erim et al. 1999), and an overall decrease in mean firing rate (Roos et al. 1997).

The literature is vast concerning age-related changes in human muscle. However, very little attention has been directed towards the upper limb, or functional muscle pairs. Only one study (Howard et al. 1988) has assessed the excitation component of the MU in the upper limb, and firing rates were measured only at submaximal levels. Since the corticospinal system exerts monosynaptic facilitation in the flexors, and inhibition in the extensors, with a greater influence over distal (hand) than proximal (arm) limb muscles (Palmer & Ashby 1992), it is important to determine whether MU properties of the elbow flexors and extensors are affected similarly with age, and to the same degree as other muscle groups. If differential alterations occur between the elbow flexors and extensors it might be related to the elbow flexors, compared to the extensors, receiving more direct corticospinal input. For example in hemiplegic patients the specific pattern of

weakness in the upper limb has been related to the distribution of corticospinal projections (Palmer & Ashby 1992).

Nutrition: Dietary depletion of protein results in a marked decrease in protein content (Felgines et al. 1999), and reductions in myosin heavy chains. Furthermore, low mitochondrial protein synthesis rates have been correlated with decrements in muscle strength and exercise tolerance (Proctor et al. 1998). Loss of muscle mass due to poor nutrition has a dual impact upon skeletal muscle function because it not only accounts for a decrease in the basal metabolic rate, but also reduced physical activity levels which lead to a decrease in the production of lean mass (Evans & Cyrcampbell 1997). Creatine (Cr) is a nonessential dietary protein that is found primarily in meat and fish, but also is synthesized in the liver, and eventually taken up by the muscle via a sodium dependent transporter on the membrane. Numerous studies have been conducted on Cr supplementation and most of these report a positive effect on exercise performance and strength, and many hypothesize that the effect of Cr occurs through the enhanced phosphocreatine stores, or rephosphorylation of ADP from PCr via the Cr kinase reaction (Terjung et al. 2000). Alternative suggestions have been proposed such as enhanced protein synthesis (Terjung et al. 2000), but there has been no attempt to ascertain whether the MU is altered following supplementation in younger, or older individuals.

Physical Activity & Inactivity: Muscle strength is a strong predictor of functional

independence (Brown et al. 1995), and yet in many populations (rheumatoid arthritis, post polio, older people) weakness is a primary deficit. Furthermore, the percentage of non-contractile tissue in muscle has been related to physical activity (Kent-Braun et al. 2000), and an increase in activity (training) will induce hypertophic adaptations in skeletal muscle and result in an increase in strength (Frontera et al. 1988). In many populations baseline activity levels have not been measured.

Fatigue: Fatigue is one of the most frequently studied acute perturbations to the neuromuscular system. Although the literature is expansive, interpretation is confounded by the variety of paradigms that have been employed and the numerous definitions of fatigue. For the purpose of this thesis, fatigue will be denoted as an acute impairment in the ability to produce a pre-determined target force, as well as an increase in perceived effort to the extent that the desired force cannot, or will not be produced (Bigland-Ritchie et al. 1995). The literature suggests that no single factor results in fatigue, rather it is a complex multi-factorial phenomenon that changes based upon the task *(task-dependency)*. Furthermore, it has also been reported that the greater the force exerted during a task, the more rapidly the muscle fatigues (*force-fatiguability*) (cf Enoka & Stuart 1992). Fatigue might also arise from an increase in the *perception of effort*. Typically fatigue is first 'felt' through the perception that the effort required to sustain the task increases, and this occurs well before task failure. Furthermore, in some patient populations

there are no peripheral impairments in the ability to exert force, but individuals report an effort-related fatigue (Lloyd et al. 1991) and task cessation occurs.

1.2 MODELS TO STUDY FACTORS WHICH AFFECT MU PROPERTIES:

The models chosen to assess the factors which might alter the MU, and inevitably influence muscle strength, were Addison's Disease, age, and dietary creatine supplementation. As well, fatigue was induced to aid in determining whether observed differences were restricted to rested muscle, or also evident during fatigued conditions. Four sets of experiments were conducted and are presented as manuscript chapters in this thesis.

Experiment 1:

Objective: To assess central and peripheral components of muscle strength and fatigue in women with Addison's Disease (AD), and to compare these results to age matched women with normal adrenal cortex function. Physical activity levels and general fitness in these two groups of middle-aged women (~ 53 years) also were compared.

Rationale: The destruction of adrenal cortex cells in AD results in the lack of production of glucocorticoids, mineralocorticoids (C-21 steroids), DHEA and its sulfate (C-19 steroids). Since conventional treatment of AD involves replacement of the glucocorticoids and mineralocorticoids which are catabolic, but not the C-19 steroids which are anabolic, AD provides a unique model to assess both artificial replacement of C-21 steroids, and insufficiency of C-19 steroids. This disease is

primarily autoimmune (65 - 75% of all cases), with a female to male predominance of 2:1 (Mason et al. 1968), and muscle weakness and fatigue are commonly reported with the most pronounced impairment associated with the proximal limb muscles (Kong & Jeffcoate 1999). Although weakness and fatigue are commonly cited as symptoms there has been little attempt to document objectively these characteristics. Since patients frequently report impairment in the lower limbs, the knee extensors were investigated. Inasmuch as disease affects activity levels, and this influences muscle strength, daily activity and fitness were measured. There are no reports in the literature regarding muscle strength, physical activity level and general fitness in AD patients.

Experiment 2:

Objective: The primary purpose of this study was to investigate whether age-related alterations in the excitation (MU firing rates), and mechanical (contractile properties) component of the MU are similar in an agonist - antagonist muscle pair in younger (24 - 25 years) and older (79 - 89 years) men.

Rationale: Although it is interesting to understand age-related change in the MU, very few studies (Vandervoort & McComas 1986, Howard et al. 1988) have investigated functionally related muscle pairs. Since complementary interactions between the elbow flexors (EF) and elbow extensors (EE) are necessary for effective upper limb movement, it is important to understand whether the MU is altered differentially with age in these muscle groups. It is known that the

corticospinal input is dissimilar for these muscles (Palmer and Ashby 1992), and the unique projections of this tract have been associated with differential alterations in muscle strength following stroke. Furthermore, it is important to assess an upper limb muscle pair since it has been suggested that differences exist in the control of movement between the upper and lower limbs (Day et al. 1984), yet the majority of MU and strength studies have been conducted in lower limb muscles. The few studies available on age-related alterations in MUs and contractile properties of the EF and EE indicate that these muscles are not affected by age as much as lower limb muscles. At low force levels (threshold, 10%, 30%) MU firing rates in the triceps and biceps brachii are not decreased with age (Howard et al. 1988), yet no study has considered high force levels. Furthermore, contractile slowing was not evident in the EF (McDonagh et al. 1984, Doherty et al. 1993), and in the EE contractile properties have not been compared between younger and older men. There is a need to determine whether age-related alterations are evident and similar in these muscles. Differential age-related alterations in agonist - antagonist muscles may result in changes in muscle strength, and ultimately the control of upper limb movement, while at the same time differential changes might provide indirect insight into the cortical control of these muscles.

Experiment 3:

Objective: To determine whether short-term Cr supplementation (5 days) alters the mechanical component of the MU in the EF of younger (19 - 28 years) men, and

induces a performance benefit in maximal voluntary force, time to fatigue, or rate of recovery in a submaximal isometric elbow flexion endurance task.

Rationale: Numerous studies have reported that short term creatine (Cr) supplementation in younger men enhances exercise performance (Terjung et al. 2000). The majority of reports suggest that Cr supplementation benefits performance through enhanced Cr content promoting rapid rephosphorylation of ADP from PCr via the Cr kinase reaction. Alternative mechanisms have been proposed such as the increase in PCr concentration, which occurs concomitantly with Cr retention in skeletal muscle, and optimizes sarcoplasmic reticulum Ca^{2+} ATPase activity, leading to a decrease in muscle relaxation time by promoting enhanced re-uptake of Ca^{2+}. A decrease in electrically stimulated muscle relaxation time has been reported in rat muscle (Wakatsuki et al. 1994), and a decrease in voluntary relaxation time in human EF (Van Leemputtee et al. 1999) following Cr supplementation. Since the chronic perturbation of age (Experiment 2) did not slow stimulated elbow flexor contractile properties, it was questioned whether acute Cr supplementation would alter these seemingly stable muscle properties, including muscle relaxation assessed from stimulated contractions. Furthermore, no studies have measured stimulated isometric contractile properties in younger muscle in non-fatigued and fatigued conditions in order to determine whether changes in the MU contribute to the performance effects which have been reported following acute Cr supplementation. As well, voluntary effort can be affected by central activation, and measured with the twitch interpolation technique, yet no study has ensured

that muscle activation is similar prior to and following supplementation.

Experiment 4

Objective: The purpose was to evaluate whether Cr supplementation alters the mechanical component of the MU and invariably induces a functional benefit in maximal voluntary strength, time to fatigue or recovery in older men (65 - 82 years).

Rationale: Although the mechanical component of the MU in the EF did not slow (Experiment 2, 3), it was hypothesised that Cr loading in older men would enhance muscle performance (contractile properties and recovery from fatigue) since phosphocreatine resynthesis is slower in aged muscle (Conley et al. 2000) and increases following supplementation (Greenhaff et al. 1994, Smith et al. 1998). There are a limited number of studies on Cr supplementation in older men, and the results are equivocal with respect to its effect on muscle performance. In older men Cr supplementation seems to increase endurance (Rawson et al. 1999, Rawson & Clarkson 2000, Bermon et al. 1998) independent of an increase in body weight (Rawson & Clarkson 2000, Bermon et al. 1998) and strength (Rawson & Clarkson 2000, Rawson & Clarkson 2000, Bermon et al. 1998). However, force and endurance time can be affected by central activation, and this has not been measured in previous Cr supplementation studies in older men. Central activation is determined by central nervous system factors related to practice, effort and neural drive (Gandevia et al. 1998, Bigland-Ritchie et al. 1986), thus the disparate findings of Cr supplementation in older adults might be attributed to variability in

performing maximal effort contractions. Central activation can be assessed objectively by twitch interpolation, and as well surface electromyography can be used as a co-measure of muscle activity. Combining these techniques could help resolve some of the controversy surrounding the Cr supplementation literature by ensuring that activation was similar for all test sessions. Furthermore, these techniques in combination with measuring stimulated contractile properties will aid in determining whether the mechanical component of the MU changes in older men following Cr supplementation.

1.4 SUMMARY

There are several acute and chronic factors which alter the MU and inevitably influence force. However, whether both the mechanical and excitation components of the MU must change in order to affect strength in humans is controversial (Connelly et al. 1999, Newton et al. 1988, Roos et al. 1999). Adrenal cortex steroids slow contractile properties in animal muscles (Gardiner et al. 1978). Thus, if similar alterations occur in the quadriceps muscle of humans following chronic steroidal replacement, then the role of the mechanical component in strength production may be assessed (Experiment 1). This thesis also attempted to provide insight into whether the mechanical and excitation aspects of the MU change en masse and in a similar manner (Experiment 2) in an agonist-antagonist upper limb pair. Finally, whether an acute perturbation (Cr supplementation) changes the mechanical component of the MU and affects elbow flexion strength was assessed

(Experiments 3, 4). A fatigue task was employed in each study to determine whether exercise effects MU properties with these perturbations.

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CHAPTER 2:

QUADRICEPS MUSCLE FUNCTION AND FATIGUE IN WOMEN WITH ADDISON'S DISEASE

2.0 INTRODUCTION

Addison's Disease (AD) develops following destruction of the cells of the adrenal cortex, most commonly by auto antibodies directed at specific steroidogenic enzymes, but may also be due to infection, or malignant disease (Oelkers 1996). The result is a lack of production of glucocorticoids, mineralocorticoids and C-19 steroids. The primary functions of glucocorticoids and mineralocorticoids are the regulation of carbohydrate metabolism and electrolyte balance, respectively. The C-19 steroids are precursors of testosterone and estrogen, and may play a role in regulating body composition. Initial studies suggested the prevalence of AD was 25-60 per million, with a female to male predominance of 2:1 (Mason et al. 1968). However, recent reports suggest that the prevalence is 2- to 3- fold higher than initially described, and there is no longer a female predominance (Kruse 1984).

Muscle weakness and fatigue are commonly reported by patients (Kong & Jeffcoate 1999, May et al. 1989), with the most pronounced impairment associated with the proximal limb muscles (Kong & Jeffcoate 1999). The quadriceps are one of the primary weight-bearing muscle group used for walking and balance, and loss of strength in this muscle group has been identified as a strong predictor of physical disability (Brown et al. 1995). Although weakness and fatigue are cited as symptoms (May et al. 1989) there has been little attempt to document objectively

these characteristics.

The physical demands of daily living which involve the quadriceps muscle can be reproduced in the laboratory by repetitive sub-maximal exercise (Lloyd et al. 1991). Muscle weakness and fatigue in AD patients during this type of activity may involve either a central or peripheral component. The peripheral component of force generation is generally associated with the neuromuscular junction and structures and processes distal to the neuromuscular junction. It is possible that the peripheral mechanisms related to the generation of force and the development of fatigue become impaired in AD since electrolyte balance is disturbed (Hiatt & Hiatt 1997). In part, these symptoms might be accounted for by altered sodium and potassium levels, since these are critical for the maintenance of membrane excitability, and muscle contraction. Furthermore, aspects of energy production, and utilization (Viru et al. 1994), protein synthesis and degradation (Robinzon & Cutolo 1999) are governed to a great degree by the adrenal cortical steroids. Electrical stimulation combined with voluntary efforts can be employed to assess peripheral aspects of strength and fatigue independent of central factors.

The central component of force generation involves motivation, and central motor drive which are often difficult to separate and measure objectively. It has been suggested that central fatigue, compared to peripheral fatigue is more common in patients with chronic disease, although the absolute contribution of each varies between conditions (Swain 2000). The modified method of twitch interpolation has been used as a measure of the ability of the central nervous

system to excite muscle during voluntary contractions (Allen et al. 1995, Merton 1954). A decrease in central activation will impair force production and this has been observed in other patient populations such as multiple sclerosis (Rice et al. 1992), post polio (Allen et al. 1994), amyotrophic lateral sclerosis (Kent-Braun & Miller 2000) and in some (Kent-Braun et al. 1993), but not all reports (Lloyd et al. 1991) of chronic fatigue syndrome. In addition, surface EMG has been used as a measure of central neural drive to a muscle during voluntary effort (Bigland-Ritchie et al. 1986). These techniques can be combined to assess CNS drive during voluntary isometric contractions, and they can be used to provide an index of subject effort during a fatigue task. In comparison to a control group, an elevated perception of effort, or muscle force can affect the ability of AD to generate maximal force and could limit muscle endurance (Lloyd et al. 1991).

Inactivity also may contribute to decreased strength and increased fatigue in AD. For example, in older adults it is known that inactivity results in generalized weakness and poor endurance which frequently develops into a state of frailty (Brown et al. 1995). Yet, numerous studies of older adults report that those individuals who exercise regularly, including those with chronic disease increase their strength and cardiorespiratory fitness with activity (Morey et al. 1998). General fitness and physical activity have not been evaluated in AD patients.

The primary objectives of this study were to assess the central and peripheral components of muscle strength and fatigue in women with AD, and since quadriceps data for these measures in women are not available, comparisons were

made with healthy women with normal adrenal cortex function. Central and peripheral components of strength and fatigue were measured in both groups in a non-fatigued condition and during a sub-maximal fatigue protocol. Physical activity levels and general cardiorespiratory fitness in these two groups of middle-aged women also were compared.

2.1 METHODS

2.1.2 Subjects

The study was conducted according to the guidelines established by The University of Western Ontario Review Board for Research Involving Human Subjects and performed according to the Helsinki Declaration. Informed written consent was obtained from all subjects prior to participation in the study. All Addison Disease (AD) patients were recruited through the offices of various endocrinologists in Southwestern Ontario, while the control (C) subjects were recruited from the Canadian Centre for Activity and Aging. Nine women with Addison's Disease (51 ± 2 years) and nine healthy age and menopausal matched women (56 ± 2 years) participated in the study (Table 1). The nine AD patients were a sub-sample of women from a larger clinical trial (n=63 men and women), which restricted data collection to one session. The nine control subjects were tested under identical conditions. Exclusion criteria for the AD patients and C subjects included myopathies, neuropathies, diabetes, alcoholism and hypertension. The criteria of age, absence of thyroid and pituitary dysfunction, and diabetes were utilized to select the nine AD patients from the clinical trial group.

The nine patients had primary adrenal failure (8= autoimmune, 1=tuberculosis) based on compatible history and laboratory examinations. Prior to neuromuscular testing the AD patients had normal serum Na^+ (139 ± 1.0 mmol/l; normal range 135 - 147 mmol/l) and K^+ (4.2 ± 0.1 mmol/l; normal range 3.5 - 5.2 mmol/l) levels. The average time since diagnosis of Addison's Disease was 15 ± 3 years, and all patients were taking standard doses of glucocorticoid and mineralocorticoid replacement.

Physical activity level was assessed in AD and C by questionnaire (Dipietro et al. 1993), and interview by the same investigator. Questionnaire results were used to estimate the amount of physical activity, and energy expenditure (per week). As well, the VO_2 max was calculated from the heart rate response to a sub-maximal step test (Petrella & Wright 2000). The subjects stepped up and down two steps (one cycle), and the time to complete 20 cycles was recorded. The predicted maximal energy cost was determined from the horizontal (steps/min x 0.35 mL/kg min per lifts/min) and vertical (step height x steps/min x 1.33 x 1.8 mL/kg min per m/min) components of stepping, such that the cost of stepping down is approximately 1/3 that of stepping up. Thus, for each complete cycle of up and down the energy cost is 1.33 times the energy cost of stepping along a flat surface.

2.1.2 Experimental Set-Up

To test knee extensor contractile properties and fatigue, subjects were seated in an isometric dynamometer with the knee joint at ~ 85° of flexion (Roos et

al. 1999). A seatbelt was secured over the hips to reduce extraneous movements during the isometric contractions. Voluntary and electrically stimulated isometric quadriceps forces were recorded from the dominant limb via a strap secured above the malleoli at the ankle, which was attached to the strain gauge (model 363-D3-300-20P3, Inter Technology Inc.) by a cable. The output from the strain gauge was sampled at 500Hz, amplified and filtered (60 Hz notch filter), and displayed in real-time on an oscilloscope within the visual field of the subject. After initial amplification and filtering, the force signal was converted from analog to digital format by a 12-bit A/D converter (model 1401 Plus, Cambridge Electronic Design Ltd.). The strain gauge was calibrated with known weights to confirm linearity and the response did not vary over several months.

2.1.3 Contractile Properties

Stimulation electrodes (5 X 20 cm) were constructed of aluminum foil, wrapped in gauze and paper towel, and soaked in saline. Stimulation was applied through the electrodes which were tightly taped transversely over the proximal (~ 30 cm from border of patella) and distal (~ 8 cm from border of patella) aspects of the quadriceps muscles. The pulse duration was 50μs and voltage levels for twitches (200 V - 400 V), were increased (model 3072-134, Digitimer Ltd.) to a level which activated as much of the muscle as possible, without interference from antagonists, or to the highest level tolerated by the subjects. This technique has been used previously in this laboratory (Roos et al. 1999). Two sets of ten twitches were applied at one Hz and twitch tension (PT), time to peak tension (TPT), and

half relaxation time (HRT) were determined from the average of the twitches. For the tetanic contractions 16 pulses at 50Hz (320 ms) were given, and voltage was increased in steps (80 V - 200 V). A plateau in tetanic force was obtained in the control group and in four AD patients, however, the remainder of the AD patients could not tolerate stimulation to achieve a plateau in force. Not all patients met the plateau criteria for tetanic tension (TT), thus off-line analysis of the non-fatigued 50Hz responses consisted of the tetanic half relaxation time ($T_{1/2}$). Since the 50 Hz TT in the AD was ~ 19% of MVC, contractile speed measures ($T_{1/2}$) provide similar results to those obtained from femoral nerve stimulation of the quadriceps (Hanchard et al. 1998).

Maximal voluntary contraction (MVC) and central activation were measured. To determine the MVC, the subjects were instructed to extend their knee as hard and as fast as possible, and hold this maximal effort for ~ five seconds. Subjects performed three to four MVCs with visual feedback and strong verbal encouragement, and approximately three minutes rest was given between attempts. A greater number of MVC attempts were not made because the AD patients were from a larger clinical trial (n=63 men and women), which restricted data collection to one session, and in pilot studies we observed that more than four attempts at an MVC in one session resulted in complaints of 'tiredness' and diminished motivation for the subsequent fatigue protocol. Thus, the control group were limited to an equivalent number of attempts as the AD group The modified (Allen et al. 1995) twitch interpolation technique (Merton 1954) was employed on two to three of these

MVCs to assess central activation. The twitch interpolation technique involves superimposing two supra-maximal pulses at 100 Hz to the muscle during the MVC, and following the contraction while the muscle was at rest. To gain an estimate of central activation the amplitude of the interpolated doublets (T_s) were compared to the amplitude of the post MVC doublets (T_r). A ratio of these two measures provides an index of how well the muscle was activated (% activation=[1-(T_s/T_r)]*100). The highest MVC was utilized in the analysis. The amplitude of the pre-MVC double twitch was compared with the post-MVC double twitch amplitude to provide an indication of twitch potentiation.

2.1.4 Surface Electromyography

A surface recording electrode was applied over the middle of the quadriceps, with an indifferent electrode on the patella. The raw electromyographic (EMG) data were sampled at 2500 Hz, wide band filtered (10Hz - 10KHz) and amplified (x1000) using a preamplifier (amplifier and filter model NL824, Neurolog). After initial amplification and filtering, the EMG signal was converted from analog to digital format by a 12-bit A/D converter (model 1401 Plus, Cambridge Electronic Design Ltd.). Off-line with computer software, the surface EMG was full wave rectified and integrated (IEMG) over 0.5s intervals over the duration of the force contraction. Force and EMG signals were monitored on the computer screen and collected on-line simultaneously to a VCR tape recorder.

2.1.5 Fatigue Task

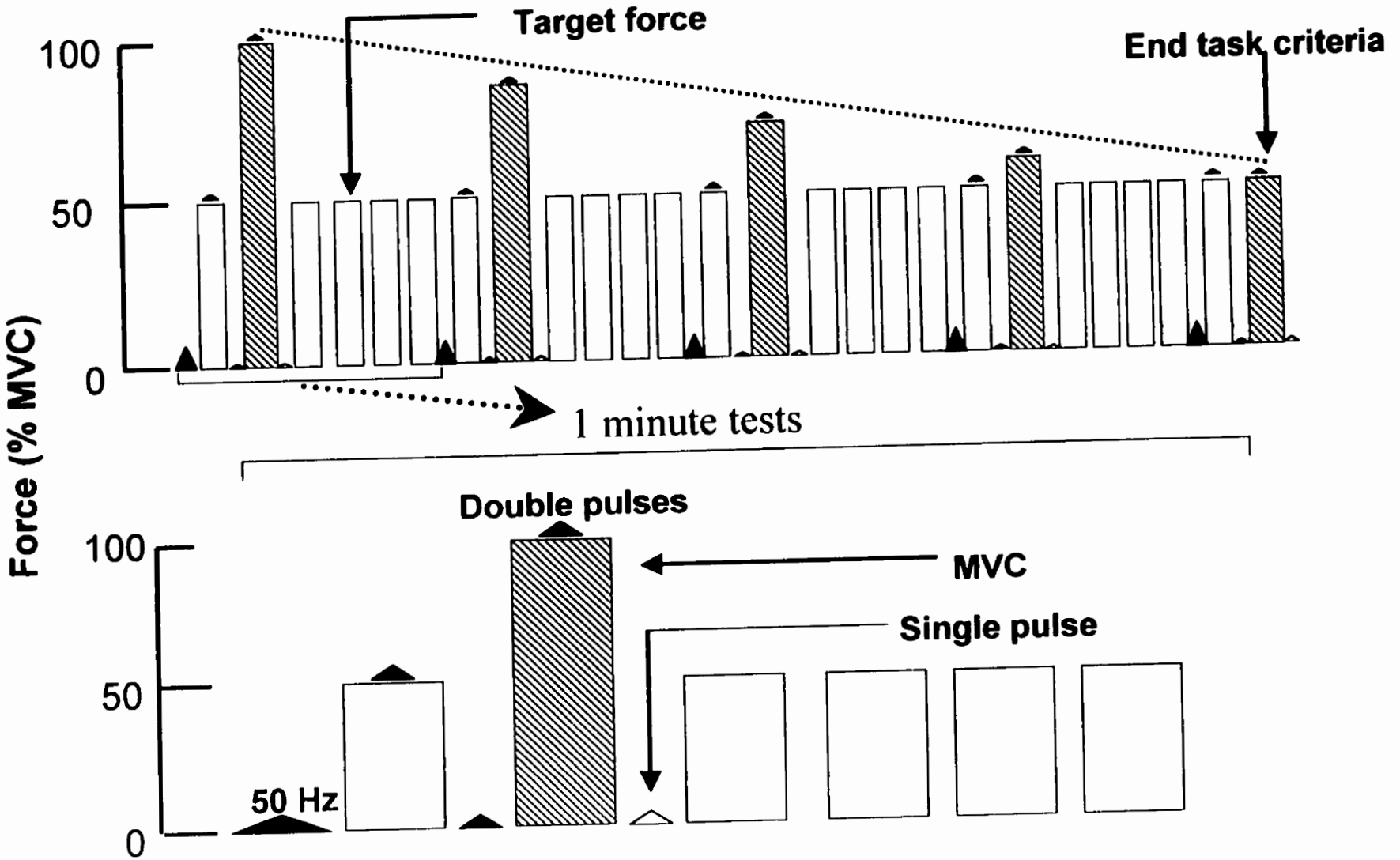
To assess fatigue an intermittent voluntary fatigue task was utilized (Bigland-

Ritchie et al. 1986). Subjects exerted target force contractions of 50% of their MVC for 6s followed by 4s of rest (60% work to rest ratio) and the degree of fatigue was tested by measuring the MVC every 60s during the fatigue protocol until the maximum force declined to the target force, or the subjects would not continue with the task (Figure 1). Visual feedback and strong verbal encouragement were given during the fatigue test. During the fatigue protocol sixteen pulses at 50 Hz stimulation were applied to the muscle prior to the 50% target contraction which preceded the MVC. Before and during the MVCs subjects were stimulated with double pulses to assess central activation, and a single pulse was applied after the MVC to assess twitch contractile properties. Off-line quantification of twitch and tetanic responses consisted of measures of amplitude (PT, TT) and half relaxation times (HRT, $T_{1/2}$). Tetanic tension (TT) is expressed as a percent change relative to each subjects baseline TT amplitude.

2.1.6 Statistical Methods

An unpaired t-test between the Addison's Disease (AD) patients and control (C) subjects was utilized to determine whether there were any differences between non-fatigue baseline measures of activity scores from the questionnaire, heart rate, stair time, predicted VO_2, contractile properties, MVC , central activation and twitch potentiation. As well, an unpaired t - test was used to determine the difference in time to fatigue between the two groups. Although the criteria to determine fatigue was a decrease in MVC force to a level whereby the maximal force became the target force (50%); the AD patients could not meet this criteria, and thus all

Figure 2.1: A schematic illustration of the experimental protocol. Upper panel depicts target force contractions repeated until the target could no longer be reached. Lower panel represents the test procedure which was performed every minute.


Target force
End task criteria
100
50
0
Force (% MVC)
1 minute tests
Double pulses
MVC
Single pulse
100
50
50 Hz
0

dependent variables in the fatigue protocol were compared for the first five minutes of the fatigue task. A two factor repeated measures analysis of variance using group; (AD, C) and time; (1,2,3,4,5 minutes) was employed to determine whether there were differences in force, central activation, contractile properties and IEMG during the fatigue protocol. Tukey Post Hoc tests were conducted to identify differences when statistical interactions occurred. The level of statistical significance was set at $p \leq 0.05$.

2.2 RESULTS

The amount of activity and energy expenditure tended to be lower in AD compared to C (Table 1). The resting heart rate was significantly higher in the AD patients ($p = 0.01$) than the C group, and it took the AD patients significantly longer to complete the step test than the C group (Table 1). As well, the predicted maximal VO_2 determined from the step test was significantly lower in the AD group ($p = 0.01$) (Table 1) compared to the C group.

Although the AD group showed a trend for a larger body mass (8kg, $p=0.08$), the MVC force was similar between AD (272 ± 14 N) and C (276 ± 14 N) (Table 2). The similarity in MVC is likely explained by a reduced central activation in AD (89 ± 3%) compared to C (96 ± 1%) ($p = 0.05$). The AD patients had a smaller twitch tension ($p = 0.01$), but unlike C, showed consistent and significant potentiation ($p=0.002$) (Table 2). Contractile speed, as assessed by TPT and T½, were both longer ($p=0.001$) in AD compared with C (Table 2).

There was a significant difference in the amount of time the AD patients (5

Table 2.1: Baseline characteristics for the Addison's Disease and control groups.

	Addison's Disease	Control	P Value
Age (years)	51 ± 2	56 ± 2	0.16
Height (cm)	162 ± 1	163 ± 2	0.97
Weight (kg)	73 ± 4	65 ± 2	0.08
Post menopause	n = 4	n = 3	---
Estrogen therapy	n = 1	n = 1	---
Time since diagnosis (years)	15 ± 3	---	---
Activity score (hours/week)	32 ± 6	43 ± 8	0.20
Energy expenditure (k cal/week)	6560 ± 1250	9752 ± 1957	0.18
Resting heart rate (bpm)	84 ± 2*	65 ± 2	0.01
Stair time (sec)	76 ± 3*	55 ± 3	0.0006
Post exercise heart rate (bpm)	122 ± 8	115 ± 5	0.1
Predicted maximal VO_2 (ml^{-1} kg min^{-1})	30 ± 2*	38 ± 2	0.01

Values are means ± standard errors of the mean. * represents significant difference ($p \leq 0.05$) between groups.

± 1 min) persisted with the fatigue task compared to the C group (10 ± 1 min) ($p = 0.006$) (Figure 2). All of the AD patients stopped the fatigue task prior to reaching the established end-point of the task (50% loss of MVC force), and thus at the end of fatigue the mean MVC of the AD group had decreased to 78 ± 4% of the pre-test force, whereas in the C group the force at fatigue was 57 ± 2% of the pre-test force (Figure 3). Seven of the control subjects met the criteria of a 50% decrease in force, but two of the control subjects stopped the fatigue task when MVC had fallen to only 65% MVC.

Since the time to fatigue (Figure 2) and force at the end of the task (Figure 3) were different, the AD and C groups were compared exclusively during the first five minutes of the fatigue task; the time corresponding to the mean time to fatigue of the AD group. The relative force loss was similar between groups, and the absolute MVC force at five minutes was less than the initial force for both AD and C (Figure 4a). Central activation for the first MVC was significantly less in AD compared with C, but it was similar to C for the remainder of the fatigue task (Figure 4b).

Twitch and tetanic tensions decreased similarly for both groups over the first five minutes of the fatigue task. Twitch tension decreased to ~59% in both groups (not shown), whereas tetanic tension decreased to ~ 80% in both groups (Figure 5). Half relaxation time of the single twitch and tetanic contraction during the fatigue task did not differ between the AD and C groups. At five minutes T½ slowed ~ 10% for both AD and C (Figure 5).

Table 2.2: Contractile properties for Addison's Disease and control subjects.

	Addison's Disease	Control	P Value
MVC (N)	272 ± 14	276 ± 14	0.64
Central activation (%)	89 ± 3*	96 ± 1	0.05
Peak tension (N)	35 ± 2*	48 ± 5	0.01
Time to peak tension (ms)	99 ± 2*	73 ± 2	0.0001
Half relaxation time (ms)	75 ± 5	69 ± 2	0.09
50Hz Half relaxation time (ms)	185 ± 10*	131 ± 3	0.0001
Fatigue time (min)	5 ± 1*	10 ± 1	0.006

Values are means ± standard errors of the mean. *, represents significant difference ($p \leq 0.05$) between groups.

Figure 2.2: The relationship between MVC force (N) and time to fatigue (minutes) for the Addison's Disease (solid line) and Control (dashed lines) groups during the fatigue protocol.

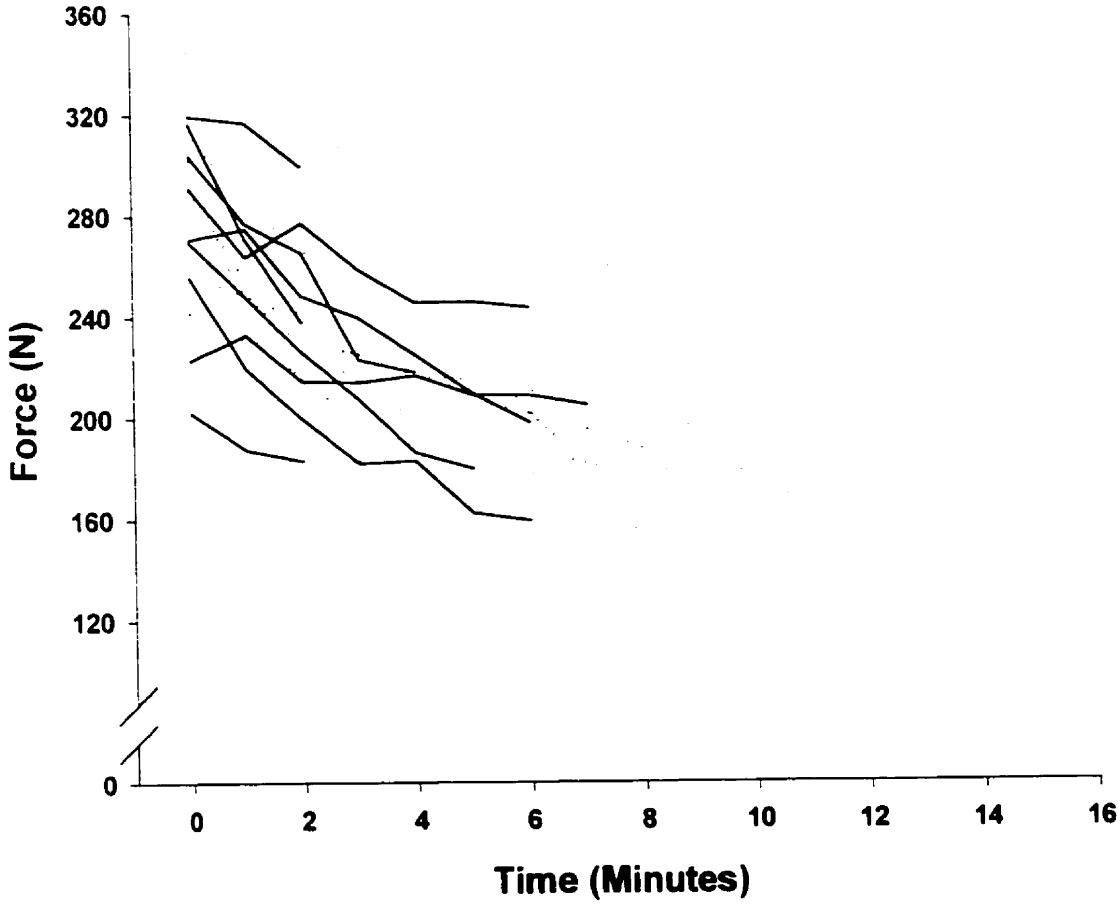

360
320
280
240
200
160
120
0
Force (N)
0
2
4
6
8
10
12
14
16
Time (Minutes)

Figure 2.3: Maximal force loss during the fatigue task for Addison's Disease (solid line, filled triangle) and control (dashed line, filled circle) expressed relative to the total endurance time. Force has been normalized to the pre fatigue value. There was a significant difference between AD and C between 33% to 100% of the total endurance time. Values are means, ± standard errors of the mean (se). * represents significant difference ($p \leq 0.05$) from pre fatigue. #, represents significant difference ($p \leq 0.05$) between Addison's Disease and control.

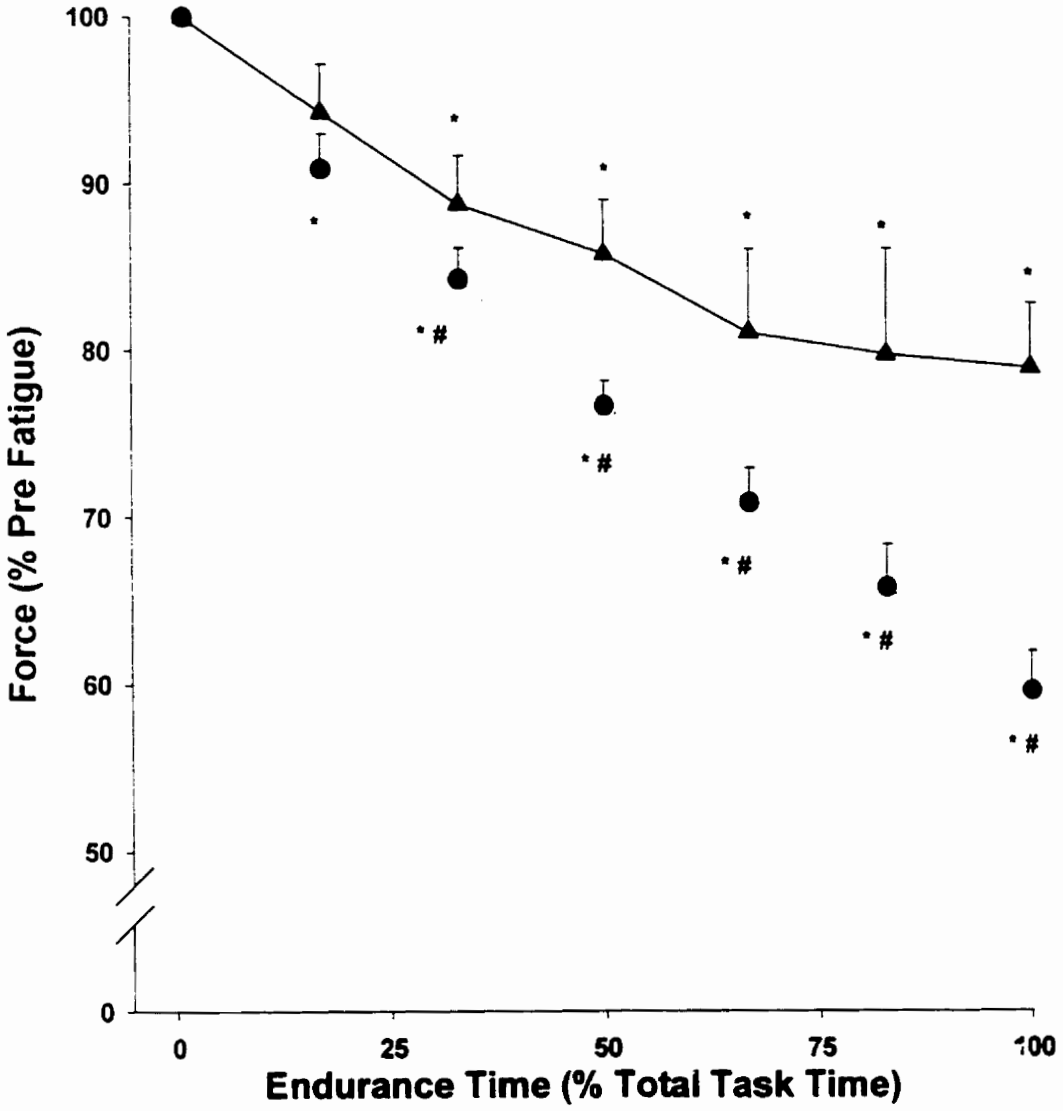

100
90
80
70
60
50
0
Force (% Pre Fatigue)
0
25
50
75
100
Endurance Time (% Total Task Time)

Figure 2.4: Maximal force loss (a) and central activation (b) during the first 5 minutes of the fatigue task (4a) for Addison's Disease (solid line, filled triangle) and control (dashed line, filled circle). The decrease in MVC was not different between groups for the first 5 minutes of the task, and at 2 minutes there was a significant decrease in force for both groups. Central Activation for the first 5 minutes of the fatigue task was significantly different between groups at the onset of the fatigue task, but there was no difference between minutes 1 - 5. Values are means, ± standard errors of the mean (se). * represents significant difference ($p \leq 0.05$) from pre fatigue.

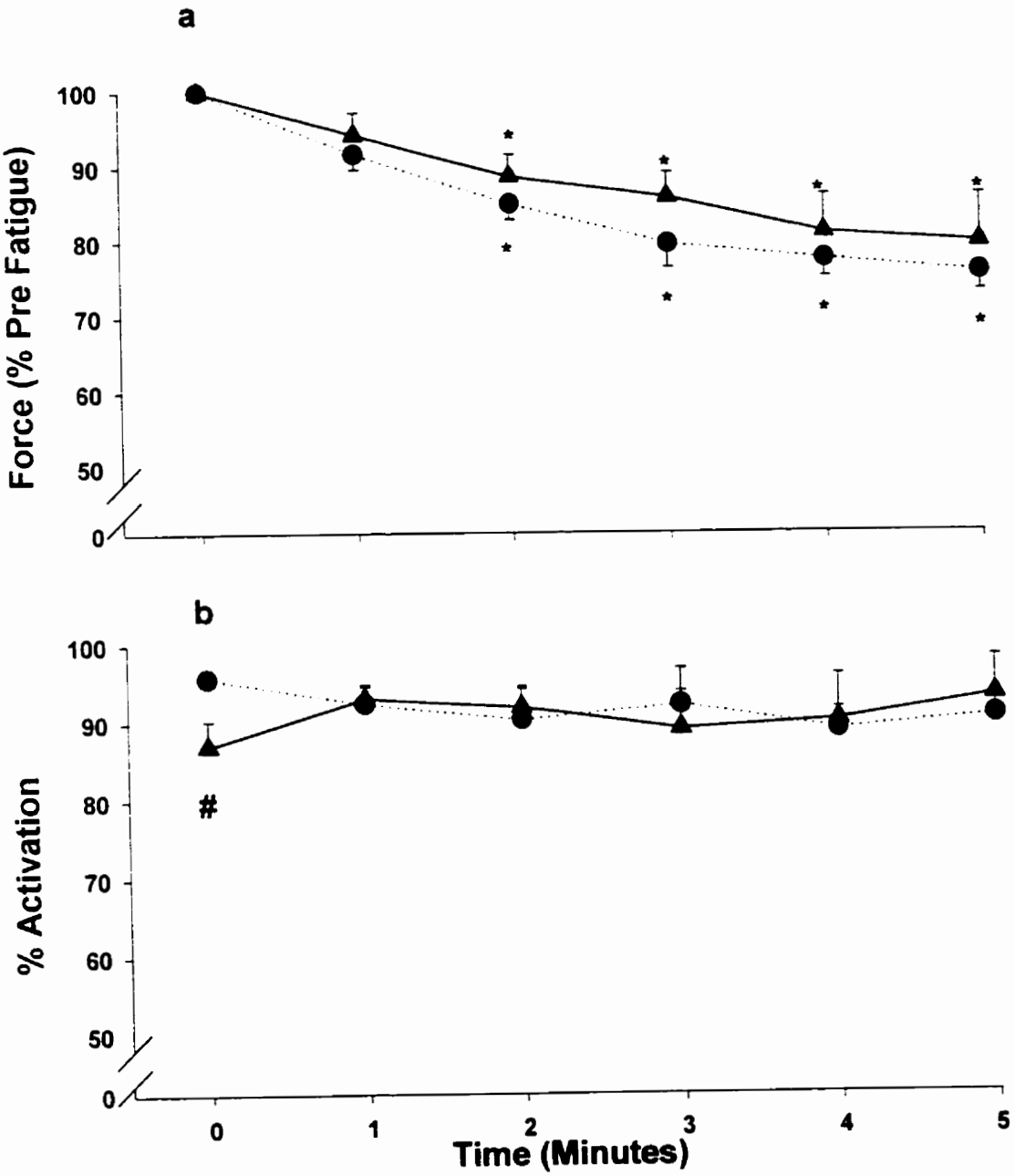

a
Force (% Pre Fatigue)
100
90
80
70
60
50
0
b
% Activation
100
90
80
70
60
50
0
#
0
1
2
3
4
5
Time (Minutes)

Figure 2.5: Tetanic tension (TT) and half relaxation time have been normalized to the pre fatigue value for the first 5 minutes of the fatigue task. There were no statistical differences between Addison's Disease and control groups for the rate of tetanic tension loss and muscle slowing during the initial five minutes of the fatigue task. The standard errors of the mean for Addison's Disease (solid line, filled triangle) and control (dashed line, filled circle) for HRT are upward bars, while the standard errors of the mean for Addison's Disease (solid line, open triangle) and control (dashed line, open circle) for TT are downward bars. * represents significant difference ($p \leq 0.05$) from pre fatigue.

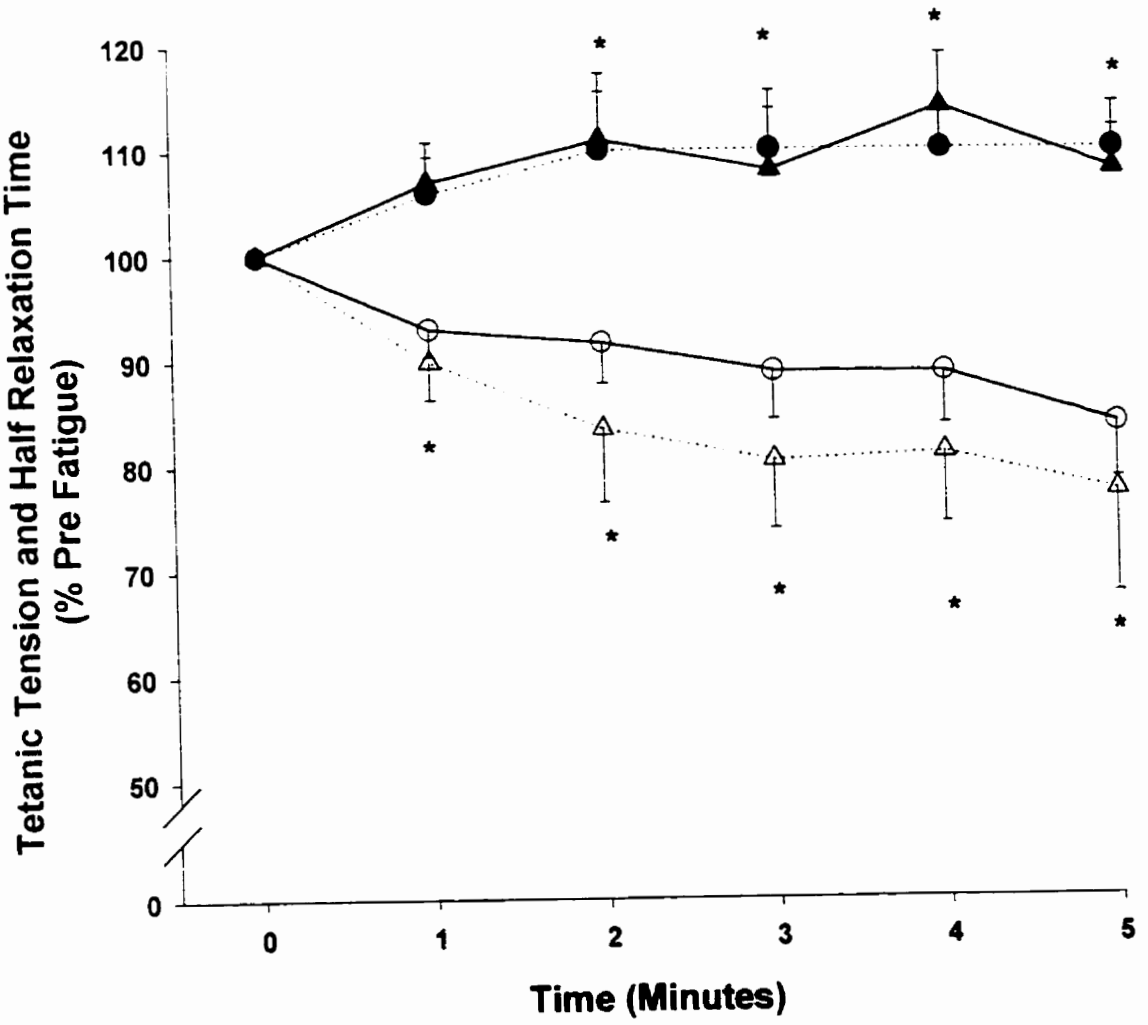
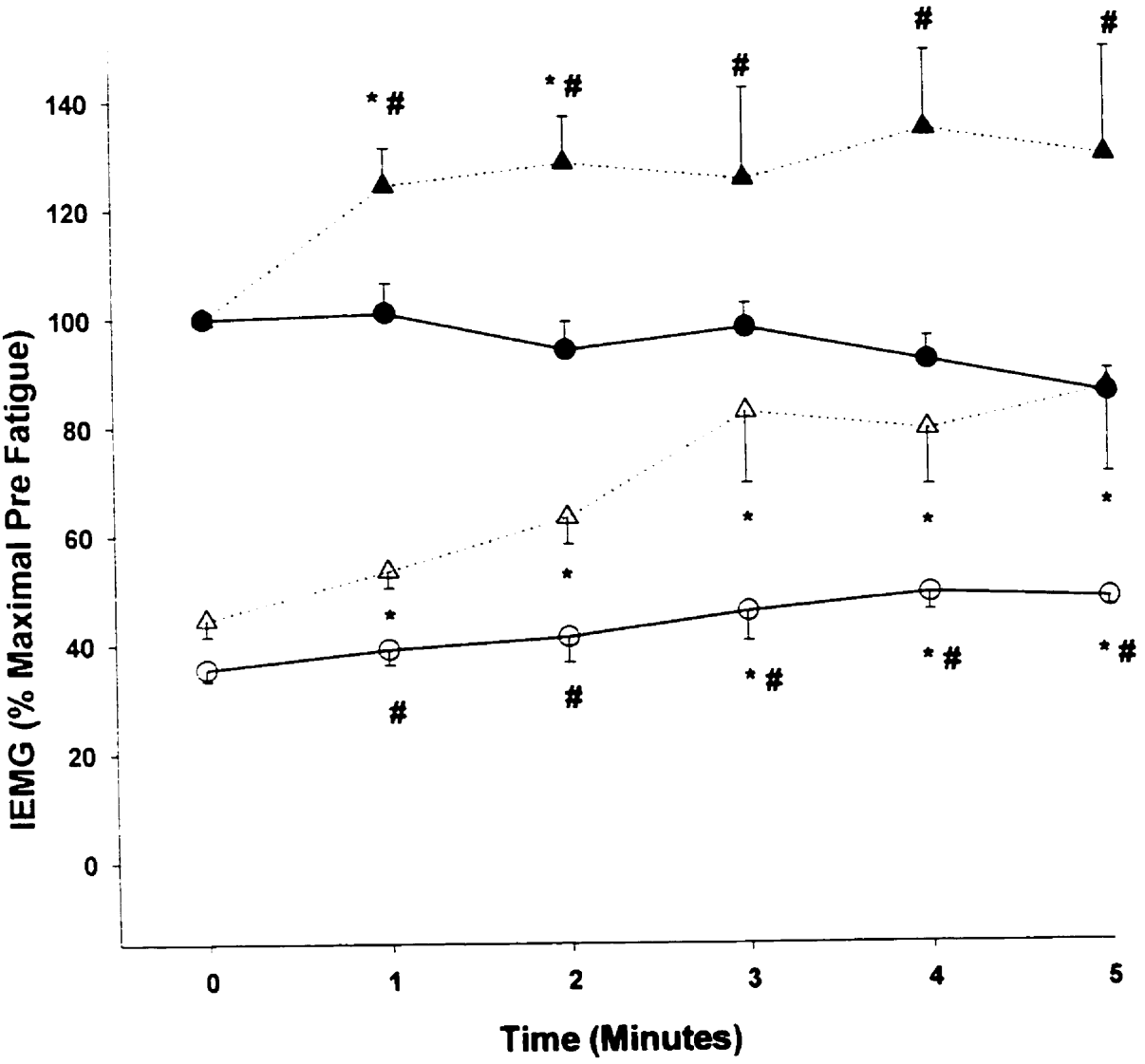

Tetanic Tension and Half Relaxation Time
(% Pre Fatigue)
120
110
100
90
80
70
60
50
0
0
1
2
3
4
5
Time (Minutes)

Figure 2.6: Submaximal and maximal IEMG for Addison's Disease and Control groups have been normalized to the maximal IEMG recorded for the pre fatigue MVC. Filled symbols and solid lines represent maximal IEMG, whereas open symbols and dashed lines represent sub maximal IEMG. Standard errors of the mean (se) for maximal IEMG for Addison's Disease (triangles), and control (circles) are upward bars, whereas se for submaximal IEMG standard errors of the mean are downward bars for Addison's Disease (triangle) and control (circle). There were significant differences between groups for both submaximal and maximal IEMG over the first 5 minutes of the fatigue task.* represents significant differences ($p \leq 0.05$) from pre fatigue. #, represents significant difference ($p \leq 0.05$) between AD and C.


IEMG (% Maximal Pre Fatigue)
140
120
100
80
60
40
20
0
0
1
2
3
4
5
Time (Minutes)

During the first five minutes of the fatigue task maximal IEMG in the C group did not differ from pre-fatigue (Figure 6), whereas sub-maximal IEMG in the C group increased significantly three minutes after the onset of the task. Both the sub-maximal and maximal relative IEMG in the AD group increased considerably one minute after the onset of the fatigue task (Figure 6), and at five minutes the sub-maximal IEMG in the AD group had increased 42% from its initial level, whereas the C group increased only 13%. The maximal IEMG in the AD group increased to 25% within the first minute and remained elevated for the next five minutes.

2.3 DISCUSSION

The physiological basis of the symptoms of muscle weakness and fatigue in primary adrenal failure have not been determined, nor have previous studies attempted to objectively quantify these symptoms and compare the results with age and gender matched controls. Based on a single laboratory session we observed that, despite a tendency for the AD group to be heavier, maximal voluntary isometric quadriceps force was similar between women with AD and a control group of women. However, central activation, as assessed by the twitch interpolation technique, was less in the AD group when an equivalent number of MVC attempts were given to patients and controls. The ~40% longer tetanic half relaxation time, and 26% longer TPT suggests that the quadriceps muscle of AD is slower than C, and the results from the fatigue task suggest AD patients will not endure a sub-maximal task for as long as the control group. This premature cessation of the fatigue task was not marked by a greater decline in force, or central activation in the

first five minutes as each of these variables were similar to those seen in the healthy, age-matched control groups. However, sub-maximal and maximal IEMG (relative to pre-fatigue) were considerably higher in AD compared to C.

In the middle aged healthy women central activation was similar to values previously reported for the quadriceps of healthy younger and older men (~ 95%) (Roos et al. 1999), however, central activation in the AD group was 7% lower. To our knowledge no study has reported central activation in quadriceps for women of this age group. Edwards et al. (1977) reported that the force of quadriceps MVC is related to body weight, and although the AD patients tended to be heavier (~ 8kg) than controls there was no difference in MVC force between groups. However, the inability to attain the expected MVC in the AD group, as predicted based on body weight (Edwards et al. 1977), could be related to the small but significant decrease in central activation which was observed in the patients. Impaired central activation has been reported in other patient populations (Allen et al. 1994, Kent-Braun & Miller 2000, Kent-Braun et al. 1993, Rice et al. 1992) and can be attributed to pain (Gandevia & McKenzie 1988), an alteration in the centrally generated motor command (Kent-Braun et al. 1993, Lloyd et al. 1991, Rice et al. 1992), decreased motivation (Kent-Braun et al. 1993), or lack of practise with the task (Gandevia et al. 1995). The AD women did not report pain for the pre-fatigue contractions, and for the three to four attempts given the AD group seemed to be as motivated as the C group. It is unknown whether AD could affect central motor commands, but it is conceivable that due to the reduced level of physical fitness and activity levels that

AD might require more practice to achieve similar activation levels as the C group. Some support for this speculation is provided from the results of the fatigue protocol in which during the first minute, central activation in AD improved to match the C group (see below).

Conventional treatment of Addison's Disease involves replacement of the glucocorticoids which are catabolic, but not the C-19 steroids (dehydroepiandosterone) which are anabolic (Robinzon & Cutolo 1999). It has been reported that following glucocorticoid and corticosteroid treatment the contractile properties of animal muscle are slowed because of a preferential atrophy of type II fibres (Gardiner et al. 1978, Wilcox et al. 1989). Since stimulated contractile properties provide a reasonable indication of both the fibre composition and intrinsic speed of the muscle (Hunter et al. 1999, Wilcox et al. 1989), it was important to measure these properties in AD and compare them to C. The increase in twitch TPT and tetanic half relaxation times observed in this study might indicate that slow twitch fibres occupy a greater percentage area of the quadriceps in AD patients compared to controls. Although there are no reports on muscle histochemistry for AD, these changes could suggest a change in fibre composition, similar to those observed in animal models following glucocortoid and mineralocorticoid treatment (Gardiner et al. 1978, Wilcox et al. 1989). However, it is also possible that the disease or the conventional drug treatment might slow contractile properties independent of an alteration in fibre type. This has been demonstrated in human muscle in which contractile speed was longer due to altered

Ca^{2+} activity because of mutations within the ryanodine receptors, and this occurred independent of a change in fibre type (Enzman et al. 1998). Twitch potentiation which was observed in the AD patients, but not in C, provides some support for altered Ca^{2+} activity. It is been shown that glucocorticoid treatment in cultured human muscle augments the number of dihydropyridine binding sites and acetylcholine receptors of the neuromuscular junction (Braun et al.1993, Braun et al. 1995). Thus, it is possible that during repeated excitations the efficacy of these processes would be enhanced which might explain the smaller twitch tensions observed at rest in the AD group, and their greater potentiation response following the MVC as compared with C. Furthermore, differences between twitch and tetanic force have been reported in other patient populations, and which have been associated with Ca^{2+} channelopathies (Greenberg et al. 1999).

During the first few minutes of the fatigue protocol, the AD patients frequently reported pain, or general 'soreness' which could not be localized to the knee joint or a particular area of the quadriceps muscle. This perception persisted throughout the fatigue task and likely caused the AD group to stop the test after only five minutes of exercise. Similar feelings were not reported in the C group until the end of fatigue when nearly 50% of MVC was lost. As suggested above, since the C and AD groups were equally practised with the experimental paradigm, but the AD patients had lower levels of physical fitness (Table 1), it is possible that AD require more time to learn how to produce MVCs and sustain voluntary effort during a sub-maximal fatigue paradigm. Thus, the inability of the AD patients to persist with the

fatigue task may be due to an altered perception of muscular effort (Miller et al. 1996) and related to their reduced level of physical fitness.

The resting heart rate of the C group was lower compared to AD patients, and the C group completed the step test considerably faster than the AD group, resulting in a higher predicted VO_2 for the C group. The predicted maximal VO_2 suggests that the cardiorespiratory fitness in the AD group is lower than the C group, and it is possible that the symptoms of muscular weakness and fatigue in AD patients relate to a decrease in physical activity. Individuals who are less fit, perform activities of daily living at a lower intensity, and Morey et al. (1998) suggested that low fitness is a risk factor for functional decline, independent of disease. Generally patients with primary adrenal failure who are receiving conventional glucocorticoids and mineralocorticoids function adequately, but they might tend to adopt a sedentary lifestyle to compensate for the symptoms of weakness and fatigue. Inactivity will affect muscle performance which in the long term can lead to an overall decline in physical function.

The improvement in activation observed in the AD group during the first minute of fatigue might be related to the patient group requiring more practise with the task, or also to the suggestion of altered Ca^{2+} processes which benefit from repetitive activation (discussed above). It has been reported that central activation does not decrease during a sub-maximal fatigue task (Gandevia et al. 1995), and the C group and AD group followed this pattern. Furthermore, during the first five minutes, the decline in both MVC and 50Hz forces were similar to each other and

not different between groups. Thus, these results suggest that fatigue in AD is not caused primarily by a limitation in the central component at least as assessed by the techniques used here, but by limitations distal in the system.

Within the first minute of the fatigue task, there were increases of 25% and 11%, respectively in maximal and sub-maximal relative IEMG for the AD group but not for the C group. It is likely that a portion of the increase in relative IEMG in the AD patients resulted from the increase in central activation which was seen during the first minute of the fatigue task. However, central activation per se may not fully account for the increase in relative IEMG during the fatigue task since it has been reported that an increase in surface IEMG during a fatiguing exercise can occur due to potentiation in the compound muscle action potential (M-wave) (Zijdewind et al. 1999). M-wave potentiation has been attributed to a Na^+ - K^+ pump induced increase in the muscle membrane potential (Cupido et al. 1992, Hicks & McComas 1989) and has been observed during fatigue (Cupido et al. 1992), and may be greater in people who are inactive (Cupido et al. 1996) such as AD patients. Since Na^+ and K^+ electrolyte balance is perturbed in AD (Fraser 1984, Hiatt & Hiatt 1997), the ionic shifts across the membrane, or Na^+ - K^+ pump activity may become altered affecting membrane properties. Although the plasma electrolytes at rest for the nine AD patients were in the acceptable physiological range, these numbers are not directly indicative of intramuscular levels (Juel et al. 2000). These preliminary findings suggest the need to assess the compound muscle action potential, and intramuscular electrolytes in future studies. Indeed, in a recent review, it has been

suggested that the first line clinical approach to any patient with symptoms of fatigue is to exclude any treatable cause, such as electrolyte imbalance (Swain 2000).

In summary, a variety of neuromuscular measures were utilized to assess central and peripheral factors related to the symptoms of muscle weakness and fatigue in female AD patients. In comparison to age matched female controls, conventionally treated Addison's Disease women did not have a lower MVC, but had a smaller stimulated twitch tension, greater twitch potentiation and slower contractile speeds. The changes in stimulated contractile properties suggest that the quadriceps muscle of AD patients might be occupied by a greater percentage area of slow twitch fibres compared to C, or that Ca^{2+} processes are altered in AD patients. Voluntary and stimulated force loss did not differ between AD and C in the first five minutes of the fatigue task, thus if the standard criteria of fatigue is the inability to sustain force, then AD patients do not fatigue more than controls. However, the time in which the AD patients can sustain the fatigue task was significantly less than the C group. In the AD patients an inability to sustain a relative effort compared to controls might be due to deconditioning which was evident from the higher resting heart rate and lower estimated VO_2 max. Since the AD group experienced a greater increase in both maximal and sub-maximal relative IEMG, compared to the control group, the sensation of muscular fatigue, which results in early task cessation in AD, may be attributable to a lower level of physical activity, or to altered Na^+ and K^+concentration, both of which could impair muscle

performance and alter the subjects perception of task effort.

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CHAPTER 3:

EFFECTS OF AGEING ON CONTRACTILE PROPERTIES AND MOTOR UNIT FIRING RATES IN UPPER LIMB MUSCLES

3.0 INTRODUCTION

The control of voluntary movement requires co-ordinated neuromuscular activity between agonist, antagonist and synergist muscles. In the upper limb the elbow flexors (EF) and elbow extensors (EE) represent a muscle pair which has been extensively researched in studies of motor control (Lemon 1999). It is known that corticospinal control of upper limb muscles exerts a greater excitatory influence over distal (hand) compared with proximal (arm) muscles (Palmer and Ashby 1992), and the biceps brachii (EF) receives short latency facilitation while the triceps brachii (EE) receives inhibition (Palmer and Ashby 1992). The anatomical projections of group 1 afferents to the motor neuron pool of upper limb muscles also have been studied and low threshold afferents from the wrist produce short latency facilitation in biceps, but inhibition in triceps motor neurons (Cavallari & Katz 1989). The distinctive neural connections to the biceps and triceps brachii have been related to the differential change in EF and EE strength following stroke (Colebatch et al. 1986). A further understanding of the interrelationship between the EF and EE of the arm might be gained by studying age-related alterations in these muscles. Substantial alterations occur in the neuromuscular system with normal ageing, as indicated by reductions in strength, contractile quality (Porter et al. 1995) and force

steadiness (Graves et al. 2000). However, neuromuscular factors related to force output in the EF and EE of older adults has not been described. Furthermore, studies in younger adults have focussed more on the EF compared with EE.

In younger subjects EF gross EMG activity has been described for isometric (Denier vander Gon et al. 1985, van Groeningen et al. 1999), dynamic (Kossev & Christova 1998) and eccentric (Kossev & Christova 1998) contractions, for a variety of muscle lengths (Christova et al. 1998). Overall it seems that the activation patterns of muscles are task dependent (Graves et al. 2000) in that the contribution of each individual elbow flexor depends upon whether the forearm is supinated or pronated (Naito et al. 1998), but the size principle of motor unit (MU) recruitment is maintained within each muscle of the group (Gielen and Denier vander Gon 1990). As well, in the biceps brachii of younger men the linear recruitment of MUs modulates force up to ~ 80% MVC (Kukulka & Clamann 1981). In the EF of older men contractile properties are not slower than in younger men, but voluntary and stimulated force is reduced (McDonagh et al. 1984, Doherty et al. 1993). There are no reports of EF motor unit firing rates at high force contractions in older men. However at low force levels (threshold, 10% and 30%) MU firing rates are less than those recorded in younger adults (Howard et al. 1988).

The EE have not been studied as extensively as the EF, but it seems that the central control mechanism of force production is similar between isometric and dynamic extension (Ivanova et al. 1997). Although during isometric movements the mono-articular heads of the EE (lateral and medial) primarily contribute to force

production, whereas in movement conditions there is a transfer of force to the bi-articular head (long) (van Groeningen and Erkelens 1994), and the order of MU recruitment is not altered whether the triceps functions as an agonist or antagonist (Garland et al. 1996). For submaximal contractions up to 30% of MVC, MU firing rates increased linearly, but did not differ between younger and older men (Howard et al. 1988). Similar to the EF motor unit firing rates for forces > 30% of MVC have not been compared in the EE of younger and older adults, and furthermore there is no information on age-related changes in contractile properties of this muscle group.

The extent to which contractile properties slow and MU firing rates decrease with age are dissimilar between muscles (Jakobi et al. 1999, Porter et al. 1995, Roos et al. 1997). For example, in older men contractile properties slow and motor unit (MU) firing rates are less in the first dorsal interosseous (Newton & Yemm 1986, Erim et al. 2000), and in the tibialis anterior (Connelly et al. 1999), whereas in the quadriceps MU firing rates do not change and contractile slowing is marginal (Roos et al. 1999). Differential age-related alterations might occur among muscle groups because of anatomic location, function, fibre type, or central neural control. Studying the EF and EE could provide further insight into the dissimilar age-related changes that occur between human limb muscles, since the EF are anti-gravity muscles whereas the EE are gravity aided, and neural connections (Palmer & Ashby 1992) and fibre composition (Elder et al. 1982, Johnson et al. 1973) have been identified to be different. However, each group consists of both mono- and bi-

articular muscles, are located proximally in the limb and are functionally inter-related for appropriate motor control tasks of the arm.

The purposes of this study were to describe and compare in younger men the contractile property and motor unit firing rate relationship in the elbow flexors and extensors, and to determine the effect of age on the agonist-antagonist relationship of these properties. Furthermore, it has been reported that force control decreases with age in the EF (Graves et al. 2000), thus studying the elbow flexors and extensors might aid in understanding the age-related reduction in motor control observed in the arm. It was hypothesized that the age-related weakness, and the decrease in MU firing rates would be greater in the EF than in the EE. Because excitability of the corticospinal tract decreases with age (Rossini et al. 1992) and this might affect corticospinal and group 1 afferent input more in the flexors because of the direct excitatory connections, whereas the extensors receive inhibitory input (Palmer & Ashby 1992). This disparate control to flexors and extensors has been associated with preferential weakness of the elbow flexors, compared to the extensors in hemiparesis (stroke) (Colebatch et al. 1986).

3.1 METHODS:

Exclusion criteria for participation in the study included diabetes, alcoholism, hypertension, angina, or known neurological, or upper limb orthopaedic pathologies, and extensive upper body training. To assess age-related alterations in contractile properties and MU firing rates both muscle groups were investigated in the same subjects. The six younger (24 ± 1 years) and six older men (83 ± 4

Table 3.1: Subject characteristics

	Younger	Older
Age (years)	24 ± 1 * (24 - 25)	83 ± 4 (79 - 89)
Height (cm)	173 ± 6 (167 - 180)	170 ± 9 (155 - 180)
Weight (kg)	77 ± 11 (64 - 89)	80 ± 5 (73 - 86)

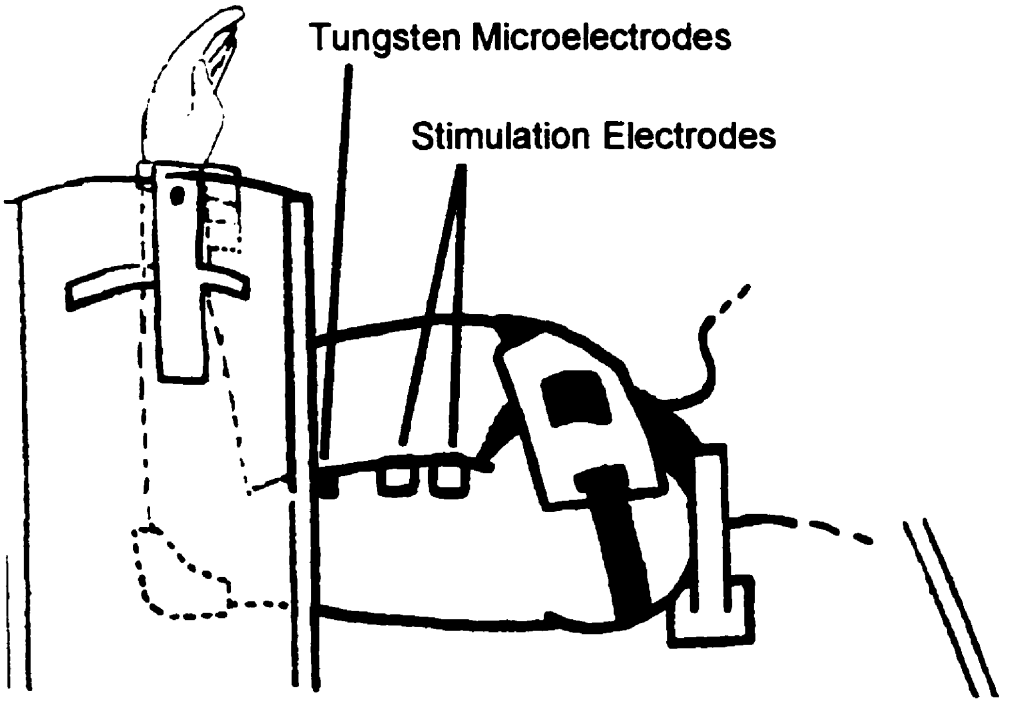
Values are means ± standard errors of the mean with the range indicated in brackets. * significant difference $p \leq 0.05$ between groups.

years) differed in age by ~ 60 years, but body weight and height were similar between groups (Table 3.1). All subjects signed written informed consent according to the guidelines established by the local university review board.

3.1.1 Experimental Set-Up

Elbow flexor and extensor contractile properties were assessed in a supine position, on a modified padded examination table with the subjects' legs elevated for comfort and as a means to prevent extraneous movements in the lower body which might influence upper body positioning or force generation. The chest and shoulders were secured to the table with stabilizing belts in order to minimize shoulder and trunk movement (Figure 3.1). The custom built dynamometer was fastened to a small platform which was attached to the side of the table. An opening (12.5 x 27.5 cm) in the platform allowed access to the extensor muscles on the posterior arm. The arm rested beside the body on the platform and the elbow joint was flexed to 90° for all EF and EE measures. The wrist was supinated for EF, but placed in a neutral position (semi-pronated) for EE. This was necessary because the supinated position for EE was uncomfortable and hindered the production of maximal voluntary force. In both positions the hand and wrist were wrapped with tensor bandages in order to prevent wrist flexion, extension, hand grip, or small movements of the passive hand during electrical stimulation of the elbow muscles. Detailed measures were made of all positions and straps in order to standardize the set up between sessions (Figure 3.1).

Elbow flexor and extensor force were measured by a strain gauge


Tungsten Microelectrodes
Stimulation Electrodes

(SST-700-100A, AS Technology) which was attached to the wrist plate of the dynamometer, and this plate rotated around the fixed central arm of the dynamometer. The strain gauge was calibrated with known weights to confirm linearity and to convert volts to Newtons of force (N). The output from the strain gauge used for EF and EE was sampled at 500Hz, amplified and filtered (60 Hz notch filter), and displayed in real-time on an oscilloscope in front of the subject. After initial amplification and filtering, the force signal was converted from analog to digital format by a 12-bit A/D converter (model 1401 Plus, Cambridge Electronic Design Ltd.) and stored on computer for off-line analysis.

3.1.2 Testing Sequence

Elbow flexor and extensor contractile properties were each measured on two sessions. To avoid an experimental order effect the muscle group tested first was randomized between sessions and subjects. Voluntary and electrically stimulated measures within each muscle group were tested in an identical sequence. For both muscle groups, twitches were elicited in the non-dominant arm and then subjects performed maximal voluntary contractions (MVCs) in which the modified twitch interpolation technique (Hales and Gandevia 1988) was employed.

3.1.3 Contractile Properties

Electrical stimulation of the EF and EE muscle groups was applied percutaneously through carbon rubber stimulation electrodes which were tightly bandaged over the muscle group under study. For the elbow flexors, the anode electrode (4 x 4.5 cm) was placed diagonally across the motor point of the biceps

brachii approximately 12 cm distal to the acromion process and the cathode electrode (4 x 4.5 cm) was located over the distal tendon approximately 2.0 cm proximal to the cubital fossa. Stimulation for the EE occurred similarly with the anode electrode (4 x 4.5 cm) situated diagonally across the proximal posterolateral portion of the long and lateral heads of the triceps brachii while the cathode electrode (4 x 4.5 cm) was placed over the triceps brachii tendon approximately 4.5 cm proximal to the olecranon process.

In order to elicit twitches at rest, 50 μs single pulses at a frequency of one Hz were applied to either the elbow flexors or extensors. On each visit, two series of 10 pulses were elicited and the series were separated by approximately two minutes of rest. To determine the intensity of the pulses, voltage was constant (400v) and the current was adjusted in incremental steps (DS7H, Digitimer Ltd.) until a level was attained which activated as much of the muscle as possible without interference from antagonists. Palpation, noticeable contraction of antagonists, or a decrement in force with an increase in current, were used to determine involvement of antagonist muscles. Maximal twitch tension for both muscle groups in the younger and older men was ~ 10% of MVC. Off-line quantification of the twitch responses consisted of measures of peak twitch tension (PT), time to peak tension (TPT), half relaxation time (HRT), and contraction duration (CD) which is the sum of TPT and HRT. Values reported for each subject were an average of approximately 16 twitches.

To measure the MVC, subjects were instructed to attempt to either flex, or

extend their elbow joint as hard and as fast as possible, and to sustain this effort for four to five seconds. Subjects performed three to four MVCs per muscle group on each session with three to five minutes rest given between each contraction. Visual feedback and strong verbal encouragement were given during the MVCs. The modified twitch interpolation technique was used to test the ability of the subjects to maximally activate their elbow flexors or extensors during a MVC. This technique consisted of applying a series of paired electrical pulses (two pulses at 100 Hz) to the muscle during and following the attempted MVC. Stimulation current for the double pulse was set at each subject's maximal tolerable level, but which did not demonstrate current spread to the antagonists and this intensity corresponded to approximately ½ - ⅔ of the current used for the single twitches. Muscle activation was estimated from a ratio of the amplitude of the interpolated paired response (T_s) to the amplitude of the post MVC paired pulse response (T_r) (% activation = [1 - (Ts/Tr)] x 100). If a subject was not exerting maximal effort a small twitch response would be superimposed on the voluntary force record.

3.1.4 Motor Unit Properties

Subjects visited the laboratory an additional four to seven times, over a period of 4 weeks, but not on consecutive days, in order to measure triceps brachii MU firing rates. The younger and older men who participated in the triceps MU experiments had previously (within 7 - 12 months) participated in similar sessions in which MU firing rates were recorded in the right arm of the biceps brachii (Jakobi et al. 1999). Although seated upright, the elbow angle (90°) for the biceps was the

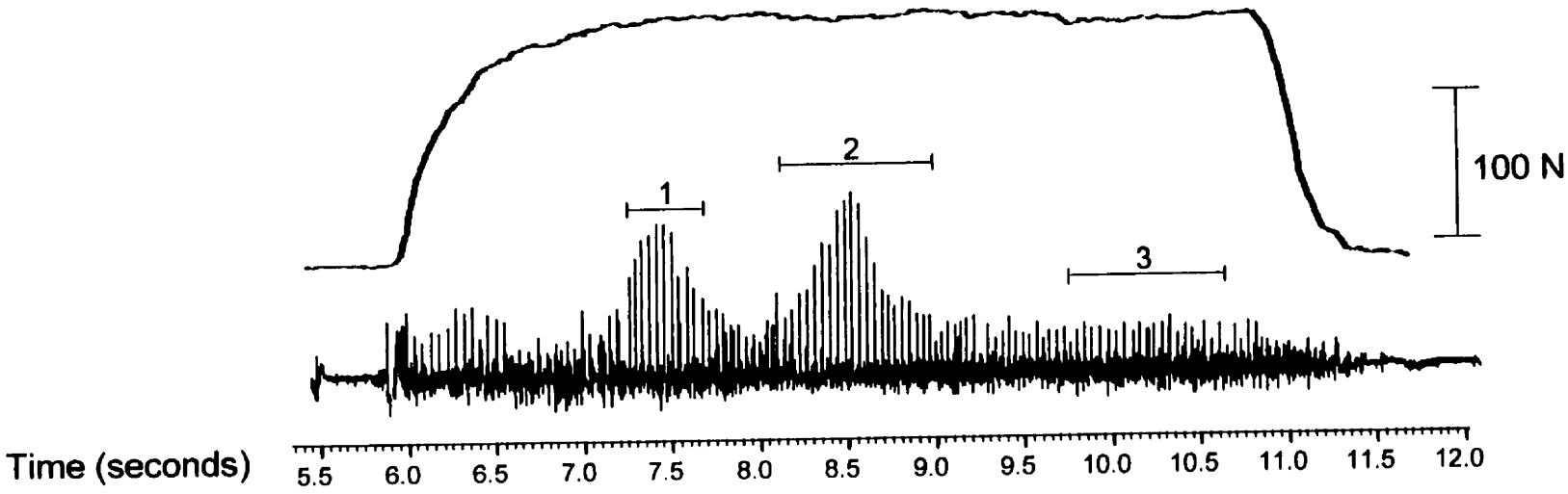
same as for the supine dynamometer. In order to record MU firing rates during steady state voluntary contractions custom-made tungsten microelectrodes (125 µm in diameter; 3 - 5 cm in length) (Bigland-Ritchie et al. 1992, Connelly et al. 1999, Roos et al. 1999) were inserted one into the long and one into the lateral heads of the triceps, or one each into the long and short heads of the biceps. When recording from biceps or triceps one common reference electrode was attached over the lateral epicondyle of the humerus. Before insertion of the microelectrodes, the skin area was cleansed thoroughly with 70% ethanol. During the brief (5 - 10 s) voluntary efforts at the five pre determined forces (10%, 25%, 50%, 75% and 100%), the microelectrodes were slowly advanced through the muscle to record from as many different MUs as possible. Recording from two separate heads in each muscle group and from many needle insertion angles, areas and depths enabled a comprehensive sample of average MU firing rates over the several sessions. The five target forces were randomly assigned during each test session to avoid the effects of fatigue on MU firing rates (Bigland-Ritchie et al. 1983). To further protect against fatigue, one to five minutes rest were given between contractions, and the 75% and 100% MVC contractions were limited to less than five seconds duration. During all contractions visual feedback and verbal encouragement were provided. Separate visual and auditory feedback were provided to the operators to help detect action potential trains.

The raw data from each of the microelectrode EMG channels in the triceps and biceps brachii were sampled at 12kHz, amplified (x100-5000) and wide band

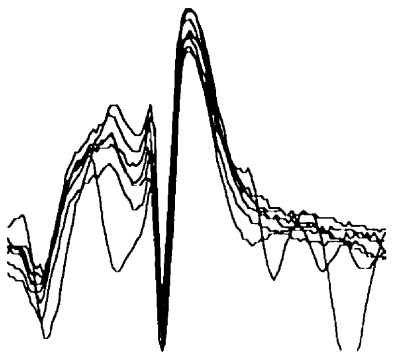
filtered (between 10 Hz and 10 kHz) using a Neurolog NL824 (Welwyn Garden City, Hertfershire, UK) preamplifier, amplifier, and filter. After amplification and filtering the signal was converted from analog to digital format by a 12-bit A/D converter (CED Model 1401 Plus; Science Park, Cambridge, UK). Data were collected concurrently to a videocassette recorder, online to computer disk, and displayed in real time with the force channel on a computer monitor. Subjects targeted the five force levels with force feedback from an oscilloscope positioned directly in their line of sight.

After data acquisition, off line analysis was conducted with a customized software package (Spike 2; CED, Science Park, Cambridge, UK). The raw electromyogram (EMG) from each microelectrode was analysed by comparing and overlaying individual sequential action potentials with respect to their shapes and amplitudes (Figure 3.2). Although a computer is required to overlay action potentials and calculate interspike interval statistics, the comparison of the shape and amplitude of the action potential was done manually. This technique is labour intensive but has been referred to as the 'gold standard' for MU frequency analysis (Stashuk et al. 1998). Beyond the criteria of shape and amplitude a minimum of six contiguous MU spikes were required for analysis, and the accepted MU discharge variability was $\leq 30\%$ (Fuglevand et al. 1993). Discharge variability was calculated by software as the coefficient of variation (CV), such that the standard deviation of the MU firing rate was divided by the firing rate to obtain a measure of variability, ($CV\ [\%] = [Sd_{p/s} / \text{mean firing rate}_{p/s}] \times 100$).

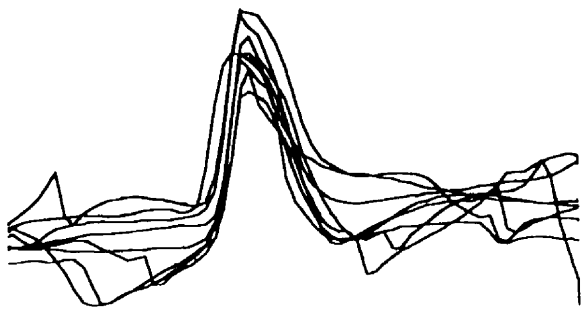
Figure 3.2: Example of a typical motor unit train of action potentials from the triceps of a 80 year old man. The top trace is the MVC force record (168 N), the middle trace is a recording from one of the tungsten electrodes and three MU trains are identified (1,2,3). The left section on the bottom of the figure shows the individual shape and overlay of the action potential from motor unit # 2, while the right panel on the bottom illustrates the shape and overlay of motor unit # 3.



Train # 2: Overlay of 23 APs
21 Hz; CoV 4.06



Train # 3: Overlay of 19 APs
25 Hz; CoV 17.1



In order to make statistical comparisons between age and muscle groups, the MU trains were grouped into five bins based on the force levels targeted. The 10% bin contained MU trains recorded at forces <12.5 % MVC; a 25% bin was 12.5 % - 37.5 %; a 50% bin was 37.5 % - 62.5 %; a 75% bin was 62.5 % - 87.5 %; and a 100% bin contained forces > 87.5 %.

3.1.5 Statistics

The four dependent variables of twitches recorded at rest (PT,TPT,HRT,CD), MVC and central activation were compared with a 2 x 2 (age group x muscle group) analysis of variance. Motor unit firing rate histograms were constructed for the triceps and biceps for each age group. Mean and median values of the histograms were calculated to determine central tendency and to compare the shape of the distributions. Regression equations were calculated for the MU firing rate - force relationships for each age and muscle group by plotting the mean frequency if each identified MU train. Age related comparisons in mean MU firing rates at the five target levels were assessed with a 2 x 2 x 5 (age group x muscle group x force level) analysis of variance. The critical value for statistical significance was set at $p \leq 0.05$ for all tests. Tukey post hoc tests were used to identify differences when statistical interactions were found. All data are presented as means and standard errors of the mean.

3.2 RESULTS

3.2.1 Age-Related Contractile Comparisons

Results for EF and EE MVC, central activation and contractile properties are

presented in Table 3.2. The age-related decrease in voluntary force was similar between muscle groups. In the older men compared to the younger EF MVC was ~ 42% less (207± 17 N; older, 357 ± 12 N; younger) whereas EE was ~ 46% less (173 ± 8 N; older, 321 ± 21 N; younger)(Table 3.2). The large age-related reduction in elbow extension and flexion MVC did not seem to be accounted for by a decrease in central activation, since twitch interpolation results were similar between age and muscle groups (94 - 98%) (Table 3.2). An age-related reduction in PT was also observed in the EF (~64%) and EE (~62%) (Table 3.2). However, since twitch TPT, HRT and CD were similar between age groups for the elbow flexors and extensors contractile slowing for either muscle group was not evident (Table 3.2).

3.2.2 Agonist-Antagonist Contractile Comparisons

In younger and older men voluntary force and stimulated twitch force were similar between the EF and EE muscles (Table 3.2). In younger men TPT and HRT tended to be faster in the EE compared to EF, which resulted in a significantly shorter CD in the EE. For the older men HRT was similar between muscles, TPT was significantly faster in the EE, thus CD was ~13 ms shorter in EE compared to EF muscles. Since PT was similar between the EF and EE for the younger and older men, and because the EE were slightly faster (~9%) than the EF for both age groups the contractile property relationship between these muscles was maintained with age.

Table 3.2: Voluntary force and stimulated contractile properties in the elbow flexors and elbow extensors.

	EE Younger	EE Older	EF Younger	EF Older
MVC (N)	321 ± 21	173 ± 8*	357 ± 12	207 ± 17*
Activation (%)	97 ± 1	98 ± 1	97 ± 1	94 ± 4
Peak tension (N)	26 ± 4	10 ± 1*	28 ± 3	10 ± 2*
Time to peak tensions (ms)	65 ± 3	63 ± 3†	73 ± 2	75 ± 3
Half relaxation time (ms)	63 ± 4	63 ± 3	67 ± 3	62 ± 4
Contraction duration (ms)	128 ± 4†	126 ± 2	140 ± 2	138 ± 5

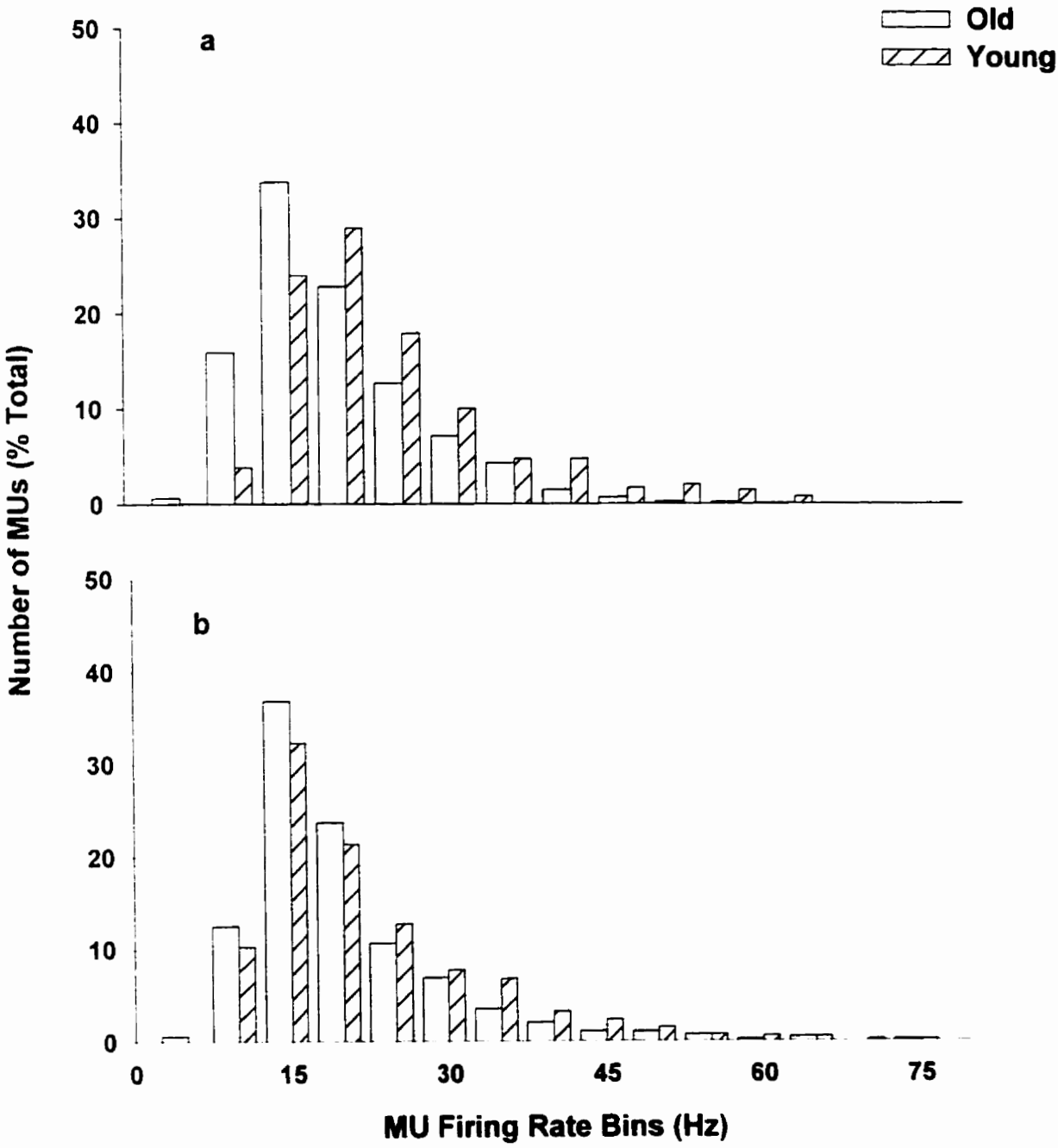
Values are means ± standard errors of the mean. Probability of signifcant differences set at $p \leq 0.05$. * represents significant difference between younger and older men within a muscle group. † represents significant difference between elbow flexors and extensors within an age group. EE, elbow extensors; EF, elbow flexors; MVC, maximal voluntary contraction; N, Newtons; ms, milliseconds.

3.2.3 Age-Related MU Firing Rate Comparisons

There were 1269 and 1189 MU spike trains recorded from the biceps of the older and younger men, respectively, and in the triceps of older and younger men 1071 and 1081 MU spike trains were recorded, respectively. In both muscles and age groups the greatest number of MUs were recorded at 25% MVC (~ 400) while the fewest were recorded at 10% MVC (~100), and approximately 265, 215 and 125 MU trains were recorded at 50, 75 and 100% MVC, respectively. There was a wide range of interspike intervals per MU train (5 -89), but the average number of inter spike intervals at each force level was similar for the two muscles and age groups.

Motor unit firing rate histograms in the younger and older men for the two muscles investigated are presented in Figure 3.3 a, b. The MU firing rate histogram across all force levels indicates the range of frequencies which occur in the motor neuron pool (Bigland-Ritchie et al. 1983). The range of MU firing rates across all force levels tested (obtained by subtracting the minimal from the maximal MU firing rate value) was 72 Hz in the younger and older triceps, whereas in the biceps the range was less for both the younger (54 Hz), and the older (47 Hz) men. For the younger and older the histograms for both muscles were positively skewed, however, the upper limit of MU firing rates was slightly higher for the triceps compared to the biceps. Since the histograms for both muscles are positively skewed the mean does not provide an accurate representation of the distribution, thus the median should also be considered as a marker of central tendency of the data. In the biceps of younger men the mean and median were 24 Hz and 21 Hz,

Figure 3.3a,b: Histograms representing the distribution of pooled MU firing rates for biceps brachii (a) and triceps brachii (b) for the young (hatched bars) and old (open bars) for all target forces. Percentage of total firing rates for each group are plotted in 5 Hz bins.


Old
Young
a
b
50
40
30
20
10
0
0
15
30
45
60
75
Number of MUs (% Total)
MU Firing Rate Bins (Hz)

respectively whereas in older men the mean was 17Hz and the median was 15Hz. Thus, these two descriptive statistics of central tendency indicate that the biceps histogram was shifted leftward with age. However, since the mean (20 Hz) and median (16 Hz) for the triceps of the younger and older men (17 Hz; mean, 15 Hz; median) were similar the leftward shift was marginal and less than the biceps.

The steady state force (% MVC) firing rate (Hz) relationship for all 2458 separately identified biceps MU trains is depicted in Figure 3.4a, whereas Figure 3.4b illustrates the relationship for the 2152 triceps MU trains. The greatest range in MU firing rates occurred at 100% for both age and muscle groups studied, and the smallest was at 10% of MVC (Figure 3.4a,b). The raw data set for the younger and older men was fit with a linear regression line in order to compare the MU firing rate and voluntary force relationship between age groups. The linear regressions were different between younger and older men for both muscle groups. The linear relations given for the biceps brachii muscle were:

$$\text{MU firing rate}_{(Hz)}\ (\text{younger}) = 0.31 \times \text{Force}_{(\%MVC)} + 9.34\ (r=0.78)$$

$$\text{MU firing rate}_{(Hz)}\ (\text{older}) = 0.20 \times \text{Force}_{(\%MVC)} + 7.89\ (r=0.66)$$

and the linear relations for the triceps brachii were:

$$\text{MU firing rate}_{(Hz)}\ (\text{younger}) = 0.26 \times \text{Force}_{(\%MVC)} + 9.22\ (r=0.70)$$

$$\text{MU firing rate}_{(Hz)}\ (\text{older}) = 0.19 \times \text{Force}_{(\%MVC)} + 8.81\ (r=0.58).$$

The slope was ~ 1.6 times steeper in the younger men compared to the older men for the biceps (0.31, 0.20), and the triceps slope was ~ 1.4 times steeper in the younger men compared to the older men (0.26, 0.19). The slopes indicate that for

every 10% increase in force the MU firing rate increases ~ 3.1 Hz and 2.6 HZ in the biceps and triceps of younger men, respectively, whereas in older men MU firing rates increase 2.0 Hz and 1.9 Hz for the biceps and triceps, respectively. Thus, the older men utilize 2.6% of the total range of MU firing rates to increase elbow extension force 10% (1.9 Hz (slope)/ 72 Hz (total range)), whereas the younger men utilize 3.6% of the total range (2.6 Hz/72 Hz) to achieve a similar relative increase in force.

In order to statistically compare the mean MU firing rate - force relationship between age groups, MUs were grouped around the five target levels (see Methods). In the biceps (Figure 3.5a) and triceps (Figure 3.5b) MU firing rates in the younger men were greater than the older men at force levels of 25%, 50%, 75% and 100%, but only in the biceps were the rates significantly greater at 10% MVC. When the change in firing rates was compared between consecutive target forces from 10% to 75% the relative differences were similar between younger and older men. However, between 75% and 100% of MVC the relative change in biceps mean MU firing rates were not similar between younger and older men. In the younger men the MU firing rates changed ~ 12Hz, whereas in the older men the increase was only 5 Hz (Figure 3.5a). The triceps followed a similar pattern as the biceps, the relative change in firing rates was similar between younger and older men at submaximal force levels, but between 75% and 100% the relative difference between age groups was considerably greater in younger (10 Hz) compared with older (4 Hz).

Figure 3.4a,b: Scatter plot of individual motor unit firing rates related to the normalized force levels for the biceps (a) and triceps (b). Open grey circles represent old, whereas open black squares symbolize young men. The grey solid line is the linear regression equation for old, whereas the black solid line is young men.

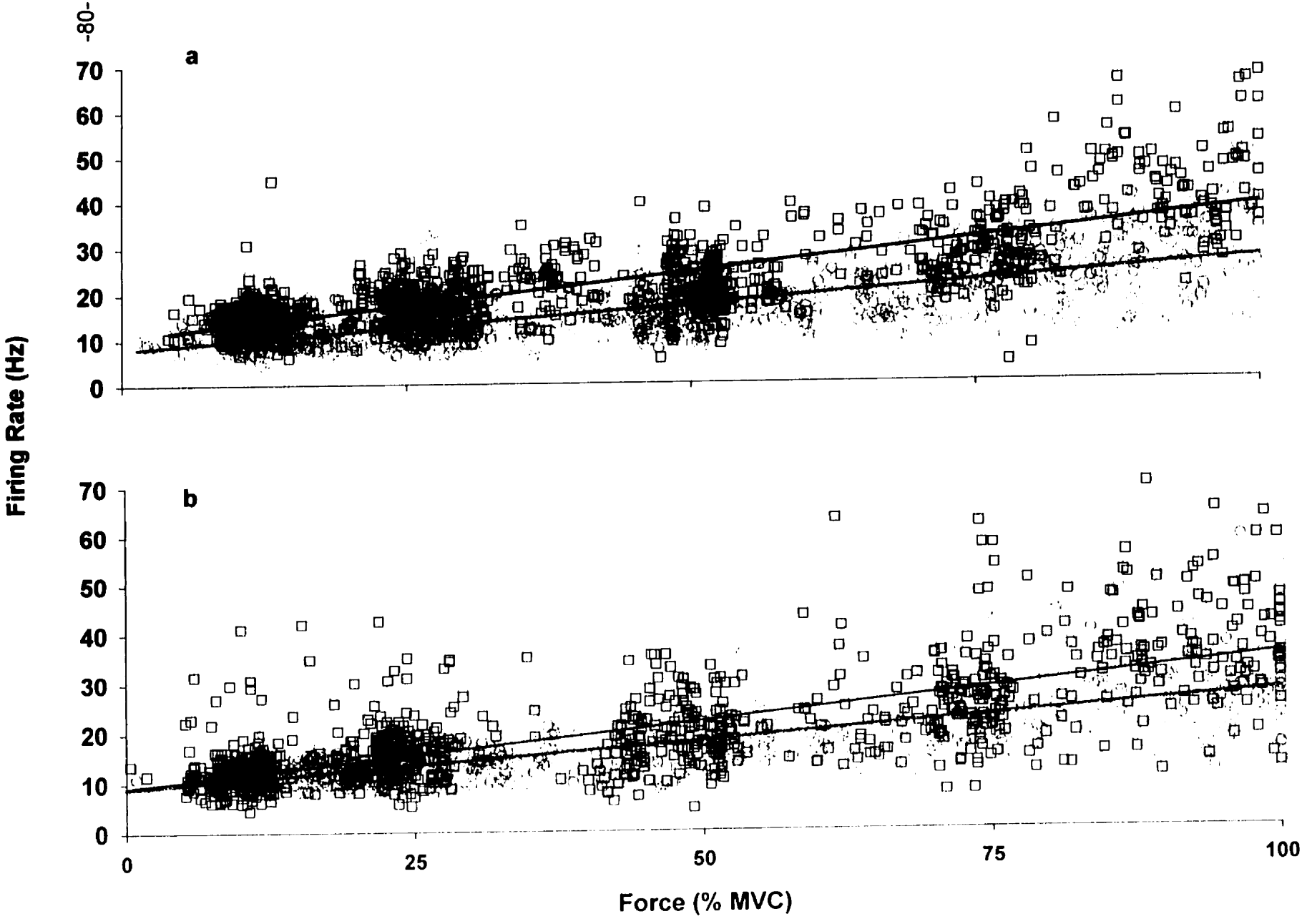

a
b
70
60
50
40
30
20
10
0
0
25
50
75
100
Firing Rate (Hz)
Force (% MVC)

Figure 3.5a,b: Means and standard errors of the mean for motor unit firing rates recorded at each of the five target force levels for the biceps brachii (a), and triceps brachii (b) for old ● and young □ men. *, represents significant difference ($p \leq 0.05$) between young and old men.

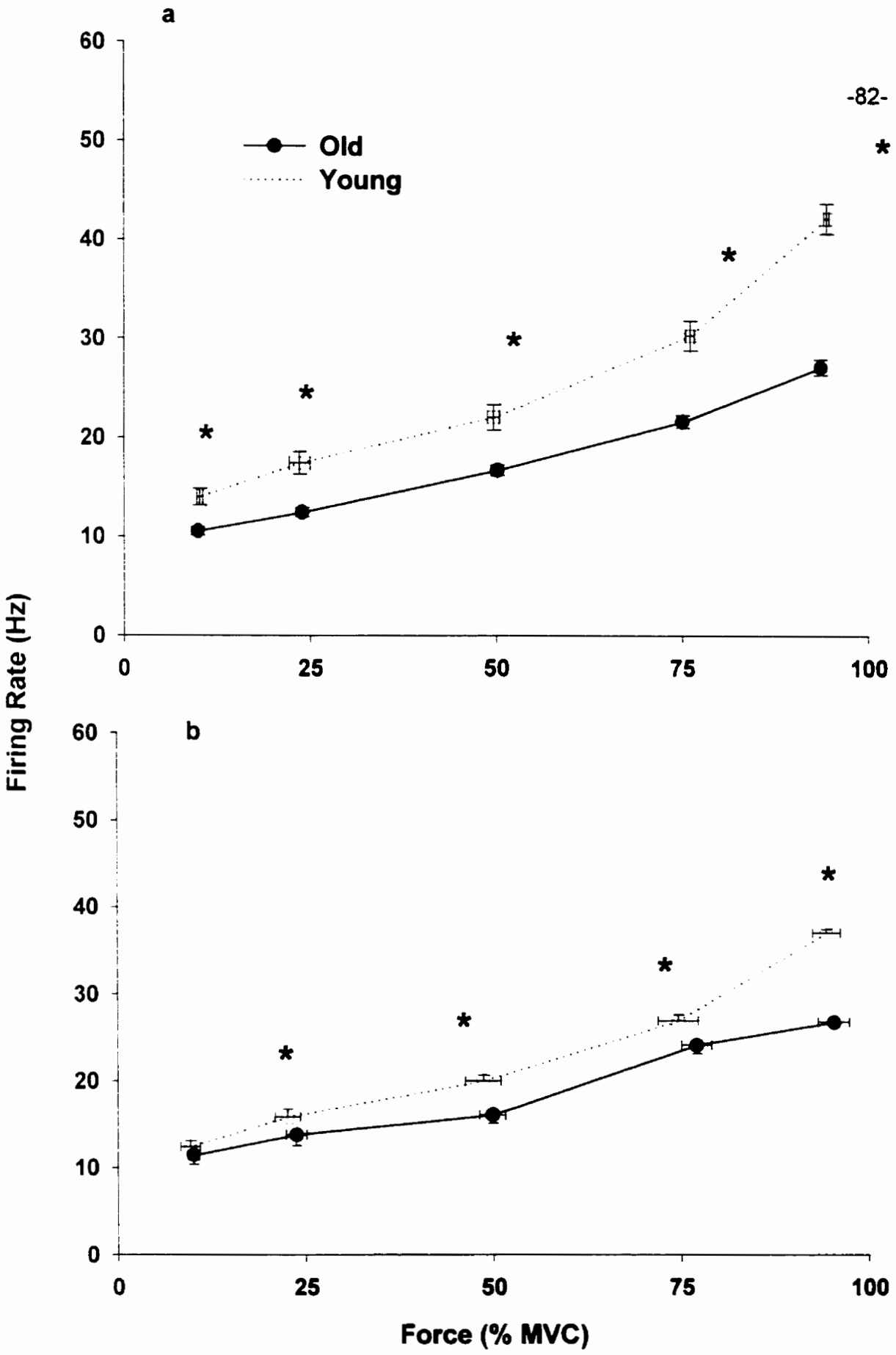
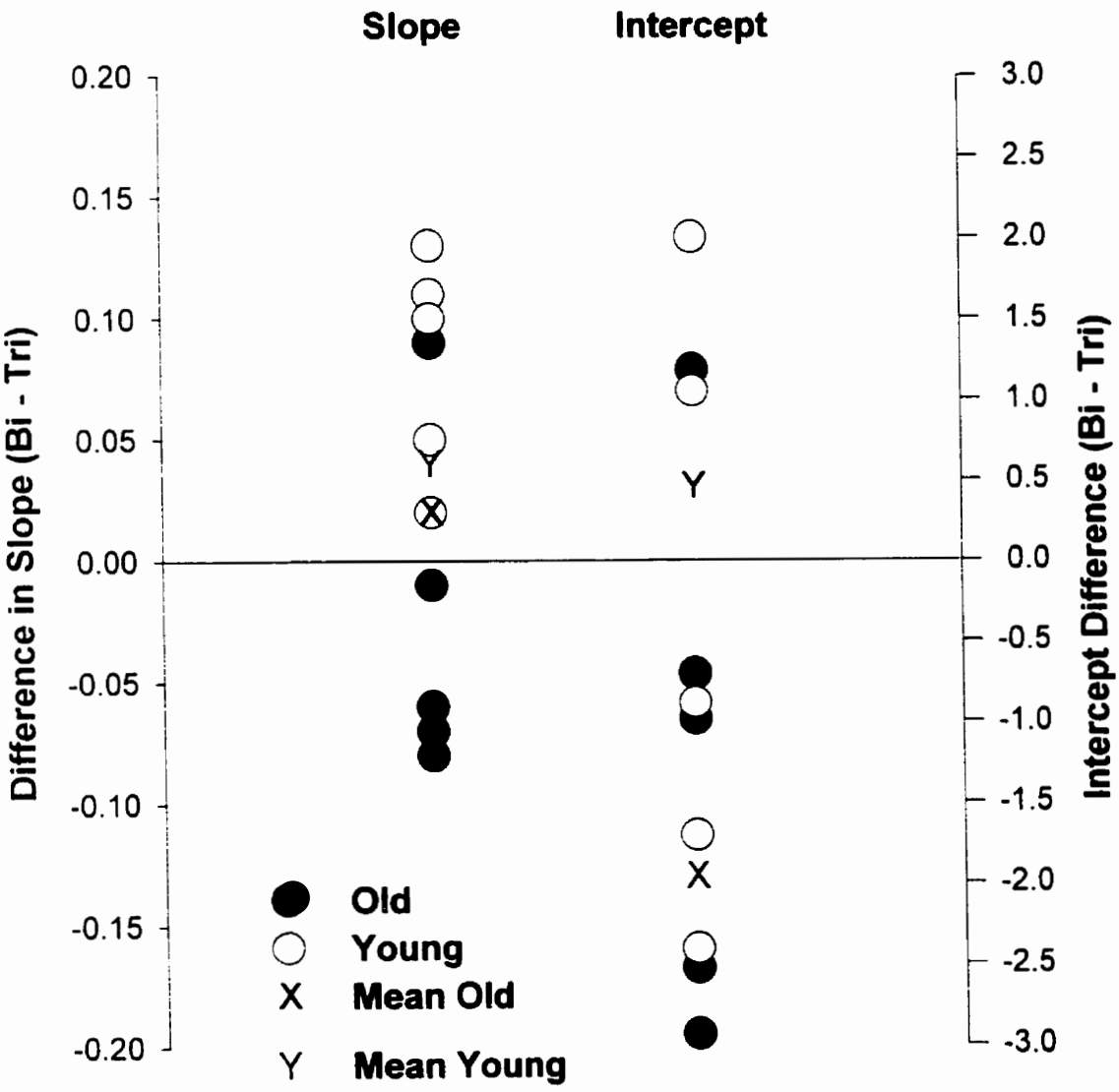

a
b
Old
Young
Firing Rate (Hz)
Force (% MVC)
0
10
20
30
40
50
60
0
25
50
75
100
*

Figure 3.6: Difference between biceps brachii and triceps brachii slope and intercept for old ● and young ○ men. Each result was obtained by subtraction of the triceps value from the biceps value. Group means for old and young men are X and Y, respectively.


Slope
Intercept
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
Difference in Slope (Bi - Tri)
Intercept Difference (Bi - Tri)
Old
Young
Mean Old
Mean Young

3.2.4 Agonist-Antagonist MU Firing Rate Comparisons

The MU firing rate - force relationship was assessed not only for differences between age groups, but between muscles within an age group. Overall, the mean difference in slope (biceps - triceps) was 0.05 for the younger and 0.01 for the older. However, when the slopes were compared (biceps - triceps) for each individual subject all of the younger men had a greater slope in the biceps compared to the triceps, whereas in the older men the triceps slope was usually greater than the biceps slope (Figure 3.6). Since the slope of the MU firing rate - force relationship was tightly grouped around zero (-0.08 - + 0.12) for all subjects, yet steeper in the biceps of the younger men, compared with the older men, these data indicate that the MU firing rate relationship in this agonist-antagonist pair is altered with age as characterized by the slope. The difference in intercepts were widely distributed across zero and more variable (-3.0 - + 1.5) between subjects than the difference in slopes. Thus, the intercept is not as a reliable descriptor of the MU firing rate - force relationship.

3.3 DISCUSSION

The purpose of this study was to compare EF and EE contractile properties and MU firing rates between younger and older men in order to determine whether the agonist-antagonist relationship was altered with age in these upper limb muscles. The age-related decrease in MVC and PT was similar in the EF and EE, and contractile properties did not slow in either muscle. Results from greater than

1000 MUs recorded over a wide force spectrum in each age and muscle group suggest that the change in mean MU firing rates is greater in the biceps than the triceps of older men. Thus, the contractile property relationship is maintained with age in these agonist-antagonist muscles, but the mean MU firing rate relationship is dissimilar between younger and older men.

3.3.1 Age-Related Contractile Properties

This is the first study to compare EE contractile properties between younger and older men and to make comparisons with EF properties in the same subjects. In these muscles PT was lower with age, but contractile slowing did not occur. The EF results from this study confirm prior reports in this muscle (Doherty et al.1993, McDonagh et al.1984), even though in previous studies the age of the subjects was approximately 70 years (Doherty et al.1993, McDonagh et al.1984) and in this study the older men were approximately 83 years of age.

Reports in the literature suggest that generally contractile properties slow with age (Porter et al. 1995), although results from the present study in combination with previous studies (Doherty et al.1993, McDonagh et al.1984) indicate that not all muscles slow, and not to the same extent (Jakobi et al. 1998). Differential age-related alterations might occur between limb muscles because the loss of type II fibres may be dissimilar between muscles (Grimby et al. 1982, Aniansson et al. 1986), possibly due to differences in the age-related remodelling of motor units (Lexell et al. 1988, Lexell & Downham 1992). Long axons which innervate distal muscles may be more susceptible to damage than short axons of proximal muscles

(Lexell 1997). For example it seems that contractile properties slow in distal lower limbs (Vandervoort & McComas 1986, plantar flexors; Connelly et al. 1999, dorsi-flexors) and in distal upper limb muscles (Newton & Yemm 1986, first dorsal interosseous) but not in proximal upper limb muscles (present study, EF and EE) and only modestly in proximal lower limb muscles (Roos et al. 1999; quadriceps). Furthermore, variable changes in contractile properties might exist because motor unit remodelling only accounts in part for the contractile changes observed with age (Rice 2000).

Age-related loss of MVC force in EE has not been documented previously. Voluntary force decreased substantially in the EF (42%) and the EE (46%). The decrease in EF MVC in this study was greater than values previously reported (Doherty et al. 1993, McDonagh et al. 1984) and this might be accounted for by the men in this present study being older (8 - 9th decade) compared to the men in prior studies (7- 8th decade) (Doherty et al. 1993, McDonagh et al. 1984). The substantial decline in EF and EE MVC with age did not seem to be related to an inability to activate skeletal muscle. The inclusion of the twitch interpolation technique indicated that the younger and older men were activating their EF and EE to the same extent. This supports the premise that weakness in older age results from myogenic alterations, but which may be related to neurogenic changes.

3.3.2 Agonist-Antagonist Contractile Comparisons

When comparisons were made within an age group, but between muscles (EF vs EE), voluntary force and stimulated force were similar between these

muscles. However, contraction duration was faster in the EE than the EF (~9%) in both age groups. It is conceivable that fibre type differences contribute to these results since the percentage of type II fibre area is higher in the EE (64%) (Elder et al. 1982, Johnson et al. 1973) compared to the EF(55%) (Elder et al. 1982, Johnson et al. 1973) in younger men. However, fibre composition has not been assessed in the EE of older men, whereas in the EF of older men the type II fibre area is similar (~ 49%) (Aniansson et al. 1986) to the value reported for younger men. Since the voluntary force relationship, and the contractile property relationship between the EF and EE is similar in younger and older men, results suggest that a comparable 'contractile' change occurs between these upper limb muscles with age.

3.3.3 Age-Related MU Firing Rate Comparisons

In younger men the slope of the MU firing rate - force relationship for the biceps muscle in this study was greater (0.31) than the slope reported by Seki & Narusawa (1996) (0.13), and at comparable high level target forces the mean MU firing rates in this study were higher than previously reported (Kukulka & Clamann 1981, Seki and Narusawa 1998). Differences likely exist between studies because the mean MU firing rate in the present study was calculated from a larger sample of MU trains (~ 3x greater) obtained at higher relative forces (> 80%). The relative increase in MU firing rates between high forces of 75% - 100% of MVC (12 Hz) is greater than between the low forces of 10% - 25% of MVC (3 Hz) resulting in an overall steeper slope compared to Seki & Narusawa (1998). In this study the slope

(0.20) of the biceps MU firing rate - force relationship for the older men was similar to the slope reported by Seki and Narusawa (1996) for younger men. The similarity between our older men and the younger men from an earlier study (Seki & Narusawa 1998) likely occurs because the change in MU firing rates between 75% and 100% was only 5Hz in the older men. Elbow extensor MU firing rates have not been compared between younger and older men over MVC forces greater than 30% (Howard et al. 1988). Similar to the EF, the slope of the EE MU firing rate - force relationship was not as steep in the older men compared to the younger.

It has been reported that the slope of the regression line for the MU firing rate - force relationship provides an indication of the rate coding strategy employed by the central nervous system to control force (Seki & Narusawa 1996). Seki and Narusawa (1996) speculated that the rate coding strategy differs between the first dorsal interosseous and the biceps brachii because the hand muscle has fewer MUs. Older people have fewer MUs (Doherty et al. 1993), yet results from this study of the EF and EE, and other studies which report a decrease in MU firing rates with age (Erim et al. 1999, reviewed in Roos et al. 1997, Connelly et al. 1999), indicate that the number of MUs is not likely a strong predictor in determining the rate coding strategy (Seki & Narusawa 1996). The rate coding strategy might differ between younger and older men because the relationship between recruitment and firing rate might be altered with age. During the process of re-innervation the faster fibres may be innervated by smaller motor neurons and in turn these fibres may be recruited earlier in older subjects (Merletti et al. 1992). Thus a mis-match between

the type of neuron and orphan fibre may cause a disturbance in the recruitment threshold and firing rate relationship (Erim et al. 1999).

The minimum and maximum MU firing rates recorded in each muscle did not differ between age groups. These data indicate that the range of MUs recorded was similar between younger and older men. However, with age the mean MU firing rate at each target force decreased in both muscles. Thus, older men retain some fast - firing MUs (similar range), but there are fewer available to contribute to the overall mean. Furthermore, it is unlikely that MU firing rates decrease with abe because force decreases. Roos et al. (1999) reported a substantial decline in quadriceps force but no age-related change in MU firing rates. Perhaps MU firing rates decrease because of physical inactivity. An increase in maximal MU firing rates has been observed in older men subsequent to training (Knight et al. 2000, Patten & Kamen 2000) and in younger men maximal firing rates decrease with disuse (Duchateau & Hainaut 1990).

3.3.4 Agonist-Antagonist MU Firing Rate Comparisons

In younger men when the slope of the MU firing rate - force relationship was compared between muscles for each subject, the biceps slope was greater than the triceps for all subjects, whereas in the older men the overall slopes were greater in the triceps compared to the biceps (Figure 3.6). The intercept data for both the younger and older men were variable between subjects and widely distributed across zero (-3.0 to +1.5). Hence, differences between muscle groups and age groups might be predominantly characterized by the slope, rather than the intercept.

The age-related reversal in the relationship between the triceps and biceps slopes indicates that the strategy employed by the central nervous system to increase force is differentially altered with age in these upper limb muscles. Since an age-related alteration has been reported in the recruitment - decruitment relationship (Erim et al. 1999, Patten & Kamen 2000) and this study indicates that the rate-coding strategy is differentially altered in these agonist - antagonist muscles, it seems that the change in the motor neuron pool with age is not the same for all muscles.

Dissimilar age-related alterations might occur between the EF and EE because habitual function is an influential factor in determining the control strategy of MUs (Deluca et al. 1982). Although these muscles work as a pair, the biceps typically functions against the resistance of gravity whereas the triceps normally operates in gravity-aided circumstances. Therefore, although these muscles work together they are involved in unique movement conditions that might impart a training or disuse interaction with the effects of age and this may contribute to the differential alterations in MU firing rates. As well, it is possible that corticospinal short latency facilitation of the biceps and inhibition of the triceps (Palmer & Ashby 1992), or presynaptic inhibition of 1A afferent terminals (Butchart et al. 1993) undergo age-related changes that are specific to each muscle group. These changes in combination with the sprouting of motor neuron dendrites and the subsequent formation of new synapses between re-innervated fibres (Ramirez & Ulfhake 1992) could result in a disparate decrease in the ratio of shared versus

unshared inputs which are received by the MUs, and in turn the MU firing rates would decrease differentially between muscles. Clearly further studies are needed which investigate age-related alterations in the spinal control of movement in functional muscle pairs.

3.4 CONCLUSION

Contractile slowing was not evident in the EF and EE, but there was a reduction in voluntary and stimulated force which was similar between these muscles. However, the decrease in MU firing rates was greater in the biceps than the triceps, thus the agonist - antagonist MU firing rate relationship was altered with age. The functional implications of these non-parallel changes in MU firing rates require further study, but it can be speculated that the decrease in force control (Graves et al. 2000) which has been reported in the upper limb with age might be due to disparate changes in MU firing rates between these arm muscles. Moreover, it does not seem that the control aspect of the MU is modified with age in a manner that denotes a compensatory mechanism to preserve force production and voluntary movement. Rather the age-related alterations which were detected might merely denote modifications in upper motor neurons which influence peripheral control of muscle force.

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CHAPTER 4:

CONTRACTILE PROPERTIES, FATIGUE AND RECOVERY IN YOUNG MEN ARE NOT INFLUENCED BY SHORT TERM CREATINE SUPPLEMENTATION

4.0 INTRODUCTION

Since Harris et al. (1992) first observed an increase in skeletal muscle creatine (Cr) content following several days of oral supplementation, many investigators have studied the effect of short term Cr ingestion on exercise performance. Most reports suggest that Cr supplementation enhances exercise performance (Balsom et al. 1993, Birch et al. 1994, Earnest et al. 1995, Greenhaff et al. 1993, Greenhaff et al. 1994, Prevost et al. 1997, Maganaris & Maughan 1998), although a number of studies have shown no effect (Febbraio et al. 1995, Redondo et al. 1996, Vandenberghe et al. 1996). In part, it seems the disparity in the findings occurs because of differences in the exercise model employed, or the intensity, duration, and frequency of the task, and the level of fitness of the subjects. Most studies have adopted cycling, swimming, or dynamic isotonic contractions, but few studies have employed an isometric model (Van Leemputte et al. 1999).

Compared with a dynamic task, the isometric model offers greater ability to

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assess in a controlled situation, the effects of various neural and contractile factors which might be affected by Cr supplementation. Motivation and effort are also important determinants of voluntary muscular output and are difficult to assess, especially during intense dynamic exercise. Isometric contractions allow measurements of both voluntary and stimulated contractile properties, and the twitch interpolation technique (explained in methods) can be employed as a means of assessing indirectly subject effort, and to separate the influence of central and peripheral neuromuscular factors in the generation of muscle force at rest and exercise. Although twitch interpolation is a well known technique in many studies of neuromuscular function (Bigland-Ritchie et al. 1986, Hales and Gandevia 1988), no previous studies have utilized this technique with Cr supplementation.

It is known that phosphocreatine (PCr) content in skeletal muscle is increased following Cr supplementation (Greenhaff et al. 1994), but the mechanism by which Cr might influence exercise performance is not known. Most research has centered upon the creatine kinase reaction (Casey et al. 1996), whereby increased availability of PCr enhances ATP resynthesis during high intensity exercise. Also, it has been proposed that Cr supplementation alters muscle relaxation through changes in local PCr concentration. Increases in PCr may optimize sarcoplasmic reticulum (SR) Ca^{2+} ATPase activity, thereby facilitating muscle relaxation (Van Leemputtee et al. 1999) by promoting enhanced re-uptake of Ca^{2+}. Indeed, it has been shown that following Cr supplementation in rats, twitch and tetanic half relaxation times (HRT) are decreased (Wakatsuki et al. 1994). In

human elbow flexors relaxation time of voluntary contractions is also less (Van Leemputtee et al. 1999). No studies to date, however, have assessed isometric stimulated contractile properties in human muscle following Cr supplementation. Furthermore, since muscle relaxation accounts for an important fraction of energy consumption (Bergstrom & Hultman 1988) and PCr is not only reduced during fatigue (Dawson et al. 1980), but presumably plays a role in altering muscle relaxation, it is important to assess muscle relaxation prior to and during a fatigue task.

Other mechanisms also have been proposed, such as enhanced protein synthesis occurring as either a direct (Ingwall et al 1976), or indirect result of an increase in cellular hydration (Sipila et al. 1981, Haussinger et al. 1993). An increase in intracellular skeletal muscle water has been observed following five days of Cr supplementation (Ziegenfuss et al. 1998). Cellular hydration (swelling) acts as an anabolic proliferative signal (Haussinger et al. 1993) inducing increased protein synthesis. Although it is unlikely that there would be significant protein synthesis in five days, cell swelling might alter muscle stiffness, or architecture (pennation angle) which could affect contractile properties (Lieber 1992).

Although a few studies have examined isometric voluntary force changes following Cr supplementation (Harridge et al. 1994, Maganaris & Maughan 1998, Van Leemputtee et al. 1999), stimulated contractile properties and maximal voluntary force have not been assessed concurrently in non-fatigued and fatigued muscle. Thus, the purpose of this investigation was to determine whether short

term creatine supplementation (5 days) in younger men would alter electrically evoked contractile characteristics of muscle, and induce a performance benefit in maximal voluntary force, time to fatigue, or rate of recovery in a submaximal isometric elbow flexion endurance task.

4.1 METHODS

4.1.1 Subjects:

Informed written consent was obtained from 14 moderately active younger men (ages 19 - 28) prior to participation in the study. All volunteers were students from the university community and the study was conducted according to the guidelines established by The University of Western Ontario Review Board for Research Involving Human Subjects and the Helsinki Declaration. Changes in daily physical activity (Robinson et al. 1999) as well as caffeine consumption were controlled during the course of the study by urging participants to maintain their normal daily activities, dissolve the powdered substance in non-caffeinated beverages, and abstain from caffeine one hour prior to neuromuscular testing. It has been reported that caffeine alters contractile properties (Fryer & Neering 1989, Nehlig & Debry 1994) and may decrease Cr uptake (Vandenberghe et al. 1996). Exclusion criteria involved creatine supplementation within the prior 12 months, myopathies or neuropathies, diabetes, alcoholism, and physical activity patterns which were deemed to represent highly trained men.

4.1.2 Study Protocol

For this double blind control study, subjects visited the neuromuscular

laboratory on three separate occasions for an accommodation, pre and post testing sessions. An accommodation session was conducted (first session) because prior reports (Maganaris & Maughan 1998) indicated that differences in force may exist between the first and second test as a result of familiarization with the experimental protocol. The second session (pre) was conducted two to five days following the first session, and the third session (post) was conducted within 8 - 14 hours following the last day of supplementation. Accommodation involved positioning the subject in the isometric arm device to ensure comfort and familiarity with the movements performed. Measurements of arm, shoulder, head and hip placement were taken to ensure identical subject positioning for the subsequent tests. At the pre and post test sessions body weight (kg), height (cm) and arm anthropometry (Rice et al. 1990) were determined prior to assessment of strength, and performance of the fatigue protocol and recovery. The estimated measure of total arm area (cm^2) (TAA), muscle plus bone area (cm^2) (MBA), and skin plus subcutaneous tissue area (cm^2) (SST) have been correlated (0.87, 0.91, 0.97, respectively) with computerized tomography scans (Rice et al. 1990).

Subsequent to the pre session, participants were randomly assigned to a dietary creatine supplementation (Cr; n = 7) or placebo (P; n = 7) group. The Cr group received 5.0 g of powdered creatine monohydrate in combination with 5.0 g of maltodextrin taken orally four times a day for five days (Green et al. 1996), while the P group received 5.0 g of maltodextrin four times a day for five days. The Cr and P samples were pre weighed and separated into twenty individual vials and

were of near identical taste and appearance. Volunteers were instructed to begin five consecutive days of supplementation two days following the pre test session by dissolving the pre measured powder in warm water or a non-citric acid juice and to consume a carbohydrate with the dietary supplement (Green et al. 1996). An information sheet was provided reminding volunteers to maintain their usual activity pattern and to dissolve the powdered substance in non-caffeinated beverages (Vandenberghe et al. 1996).

4.1.3 Muscle Strength

On a padded examination table elbow flexor measures were conducted in a supine position with the subjects' legs elevated for comfort and as a means of preventing the use of the pelvic girdle and lower limbs during contractions. The left elbow was flexed to 90° and the shoulders were secured to prevent flexion of the trunk. The wrist was secured to a plate which was attached to a strain gauge (SST-700-100A, AS Technology) and the elbow was positioned and secured perpendicular to the wrist. The output from the strain gauge was sampled at 500Hz, amplified and filtered (60 Hz notch filter), and displayed in real-time on an oscilloscope in front of the subject. After initial amplification and filtering, the force signal was converted from analog to digital by a 12-bit A/D converter (model 1401 Plus, Cambridge Electronic Design Ltd.). The strain gauge was calibrated with known weights to confirm linearity and to convert force values to Newtons. Detailed measures were made of all positions and straps in order to standardize the set up between sessions.

Voluntary and electrically stimulated contractile properties of the elbow flexors were measured. Electrical stimulation was induced through carbon rubber stimulation electrodes (4 cm x 4.5 cm) which were tightly bandaged over the proximal and distal portions of the anterior arm (shoulder to cubital fossa). For twitch and tetanic stimulation the electrical pulse duration was 50μs, voltage was constant (400V) and the current was applied in incremental steps (model DS7H, Digitimer Ltd.). Stimulation intensity for single twitches was set at a level which activated as much of the muscle as possible, or to the highest tolerable level without interference from antagonists. Palpation, noticeable contraction of triceps brachii, or a decrement in force with an increase in current, were used to determine involvement of antagonist muscles. To induce twitches, two sets of ten single pulses were delivered at one pulse per second while the subjects were at rest. The two sets of pulses were separated by approximately two minutes. Tetanic stimulation (16 pulses at 50 Hz) intensity was set according to the criteria used for single twitches. Off-line analysis for the single twitches consisted of twitch tension (PT), time to peak tension, (TPT) half relaxation time (HRT) and contraction duration (CD); the sum of TPT and HRT. Similarly for the 50 Hz force response, tetanic tension (TT) and half relaxation time (T½) were measured off line. Identical stimulation settings were used for each test session.

Maximal voluntary contraction (MVC) with superimposed twitches to assess central activation was measured after twitch contractile properties were collected. For these measures subjects were instructed to flex their elbow joint as hard and

as fast as possible, and sustain this effort for four to five seconds. Subjects performed three to four MVCs at each session. Five minutes rest was given between MVCs, and the highest value for that day was recorded as the maximum. Visual feedback from the oscilloscope was provided and strong verbal encouragement was given. Central activation was assessed using the modified twitch interpolation technique (Hales & Gandevia 1988). This technique involves superimposing a series of paired electrical shocks (2 pulses separated by 10 ms) during and following an MVC. If the subject was not exerting maximal effort a small twitch response would be superimposed on the voluntary force record. To obtain an estimate of activation the amplitude of the interpolated double twitch (Ts) was compared with the amplitude of the post MVC twitch (Tr). A ratio of these two measures provided an index (percentage) of how well the muscle was activated (activation % = [1-(Ts/Tr)]*100). A benefit of employing this technique is that, not only can activation be assessed, but subject effort can be monitored across sessions. It is unlikely that Cr would alter muscle activation per se, but it is important to ensure that all tests are conducted with equal voluntary effort.

4.1.4 Surface Electromyography

A surface electrode was applied over the belly of the biceps brachii, with an indifferent electrode placed over the lateral epicondyle of the humerus. The electromyogram (EMG) data were sampled at 2500 Hz, wide band filtered (10Hz - 10KHz) and amplified (x500) using a preamplifier (amplifier and filter model NL824, Neurolog). After initial amplification and filtering, the EMG signal was converted

from analog to digital by a 12-bit A/D converter (model 1401 Plus, Cambridge Electronic Design Ltd.). The surface EMG signal was analyzed off-line. The waveform was full wave rectified and then integrated (IEMG) over a 0.5s interval. Force and EMG signals were monitored on the computer screen and collected on-line simultaneously to a VCR recorder.

4.1.5 Fatigue and Recovery Protocol

Following the fatigue protocol developed by Bigland-Ritchie et al. (1986), the degree of fatigue and recovery were tested by measuring maximal voluntary force repeatedly during an intermittent fatiguing exercise. Subjects made a continuous series of target force contractions at 50% MVC for 6s followed by 4s of rest (60% work to rest ratio) until the maximal force became the target force of 50% MVC. MVCs were made every 60s during the fatigue protocol, and before and during the MVC, subjects were stimulated with double pulses to assess changes in muscle activation. Sixteen pulses at 50 Hz stimulation were applied to the resting muscle 11 seconds before every MVC. Off-line quantification of the twitch responses consisted of measures of PT, TPT, HRT, and CD. Similarly, 50 Hz stimulation force response was quantified off-line via assessment of TT and T½. Throughout the fatigue protocol and recovery EMG was collected. All IEMG values were normalized to the IEMG from the pre fatigue MVC.

Recovery timing began immediately after the last MVC of the fatigue test. The subjects remained secured to the arm device and no adjustments in posture or position were made. MVC force and electrically evoked contractile properties were

tested at one, three, five and ten minutes of recovery (R1, R3, R5, R10).

4.1.6 Statistics

A two factor analysis of variance using condition; (Cr, P), and day; (accommodation, pre, post test) was employed to determine whether there were any differences in baseline measures of force, activation or contractile properties. Similarly, time to fatigue was analyzed with a two factor analysis of variance (condition x day). The remaining dependent variables for the fatigue protocol were analyzed with a three factor analysis of variance (condition x day x time points of fatigue/recovery). The time points used for all dependent variables in the fatigue protocol were start of fatigue, middle time point of fatigue and final MVC. Normalizing the fatigue time points was necessary because not all subjects fatigued to ~ 50% force in a similar time. Recovery measures were recorded at one, three, five and ten minutes after the last MVC (R1, R3, R5, R10). Tukey's post hoc tests were used to identify differences when statistically significant interactions were found. All fatigue data are presented as means and standard errors of the mean (se). The level of statistical significance was set at $p \leq 0.05$.

4.2 RESULTS

Although some side effects were reported, such as frequent urination (n = 9), mild abdominal discomfort (n = 1) and bloating (n =1) all subjects were uncertain about the supplement they had been assigned. The two way ANOVA indicated MVC, and pre-fatigue contractile properties were not significantly different between the accommodation test and pre test, thus all post test measures were compared

to pre test measures. Anthropometric measures of TAA, MBA and SST were not different between groups or following dietary supplementation. Prior to supplementation, the Cr and P groups did not differ in age, height or weight (Table 4.1). Following supplementation there was a significant change in body weight for the Cr group while the P group did not change (Table 4.1). The average weight gain of the Cr group was 1.0 kg, which was similar to previous values in the literature for Cr loaded subjects. Six of the Cr volunteers gained weight (0.4 kg - 2.7 kg) whereas one had no change in weight. The P group had a net weight loss of 0.4 kg, five had no weight change, one had a loss (0.9 kg) and one had a weight gain (0.4 kg).

4.2.1 Force Measures

Voluntary force and electrically stimulated contractile properties are presented in Table 4.2. Maximal voluntary force did not change following supplementation for Cr (388 $\pm$ 25 N, 379 $\pm$ 23 N) or P groups (393 $\pm$ 16 N, 390 $\pm$ 15 N). Across all three test sessions maximal force varied ~ 4% in the Cr group whereas the P group varied ~ 2%. There were no statistically significant differences between the Cr and P groups in voluntary force. Maximal voluntary activation, as assessed using the twitch interpolation technique, indicated that all subjects, irrespective of the treatment, were able to equally activate their elbow flexors and there was no difference after treatment (Table 4.2). Twitch tension (PT) and

Table 4.1: Subject characteristics for men on placebo or creatine supplementation.

	Creatine Pre (n=7)	Creatine Post (n=7)	Placebo Pre (n=7)	Placebo Post (n=7)
Age (years)	22 ± 3	------	21 ± 1	------
Height (cm)	177 ± 6	------	176 ± 8	------
Weight (kg)	77 ± 4*	78 ± 4*	78 ± 5	78 ± 6
Total arm area (cm^2)	73 ± 8	75 ± 9	74 ± 8	76 ± 8
Muscle plus bone area (cm^2)	61 ± 6	63 ± 7	63 ± 8	63 ± 9
Skin plus subcutaneous tissue (cm^2)	12 ± 2	12 ± 2	11 ± 2	12 ± 2

Values are means ± standard errors of the mean. * Significant difference post- test compared with pre-test.

Table 4.2: Contractile properties of elbow flexors with creatine or placebo supplementation.

Muscle Property	Creatine Pre (n=7)	Creatine Post (n=7)	Placebo Pre (n=7)	Placebo Post (n=7)
MVC N	388 ± 25	379 ± 23	393 ± 16	390 ± 15
% Activation	98 ± 1	99 ± 1	99 ± 1	99 ± 1
Peak twitch tension (N)	29 ± 15	31 ± 13	21 ± 9	23 ± 10
Time to peak tension (ms)	78 ± 3	78 ± 4	75 ± 4	76 ± 3
Half relaxation time (ms)	59 ± 5	56 ± 3	60 ± 4	63 ± 3
Contraction duration (ms)	137 ± 5	134 ± 3	136 ± 5	139 ± 3
50 Hz tension (N)	69 ± 5	60 ± 4	69 ± 9	62 ± 7
Tetanic half relaxation time (ms)	94 ± 2	99 ± 4	101 ± 1	101 ± 2

Values are means ± standard errors of the mean.

contraction times (TPT, HRT,CD) were similar between the Cr and P groups and did not change as a result of Cr supplementation. There were no differences in TT and 50Hz T½ (Table 4.2) between the Cr and P groups before or after supplementation.

4.2.2 Fatigue Protocol

A three way ANOVA (condition x day x fatigue time) on the dependent variable of force, determined that there were no differences between accommodation and pre test measurements; thus all remaining dependent variables were compared post test versus pre test. Prior to supplementation, time to fatigue averaged 10 $\pm$ 4 minutes and 14 $\pm$ 3 minutes for the Cr and P groups, respectively (Figure 4.1). Following supplementation there was no significant change in time to fatigue for the Cr and P groups (11 $\pm$ 4; Cr, 15 $\pm$ 3; P). By the end of the fatigue protocol there was a significant decrease in maximal voluntary force of approximately 44% of the initial force for each group at both sessions ($p \leq 0.05$), indicating there was no significant effect of creatine (Figure 4.2). Although there was a significant recovery from the last minute of fatigue to the first minute of recovery (~ 15%), the force at one minute recovery was significantly less than the initial MVC in both groups prior to and following supplementation. By 10 minutes force was ~24% greater than the last MVC of the fatigue protocol in both groups. This resulted in a recovery of MVC force to ~ 84% of its initial value, but this was still significantly less than the pre fatigue force.

Creatine supplementation had no effect on the ability of subjects to activate their elbow flexors (Figure 4.2). At the onset of the fatigue protocol voluntary

Figure 4.1: The relationship between MVC force and time during the fatigue protocol at the pre test. The Cr (solid lines) and P (dashed lines) subjects did not differ in time to fatigue on the pre or post test.

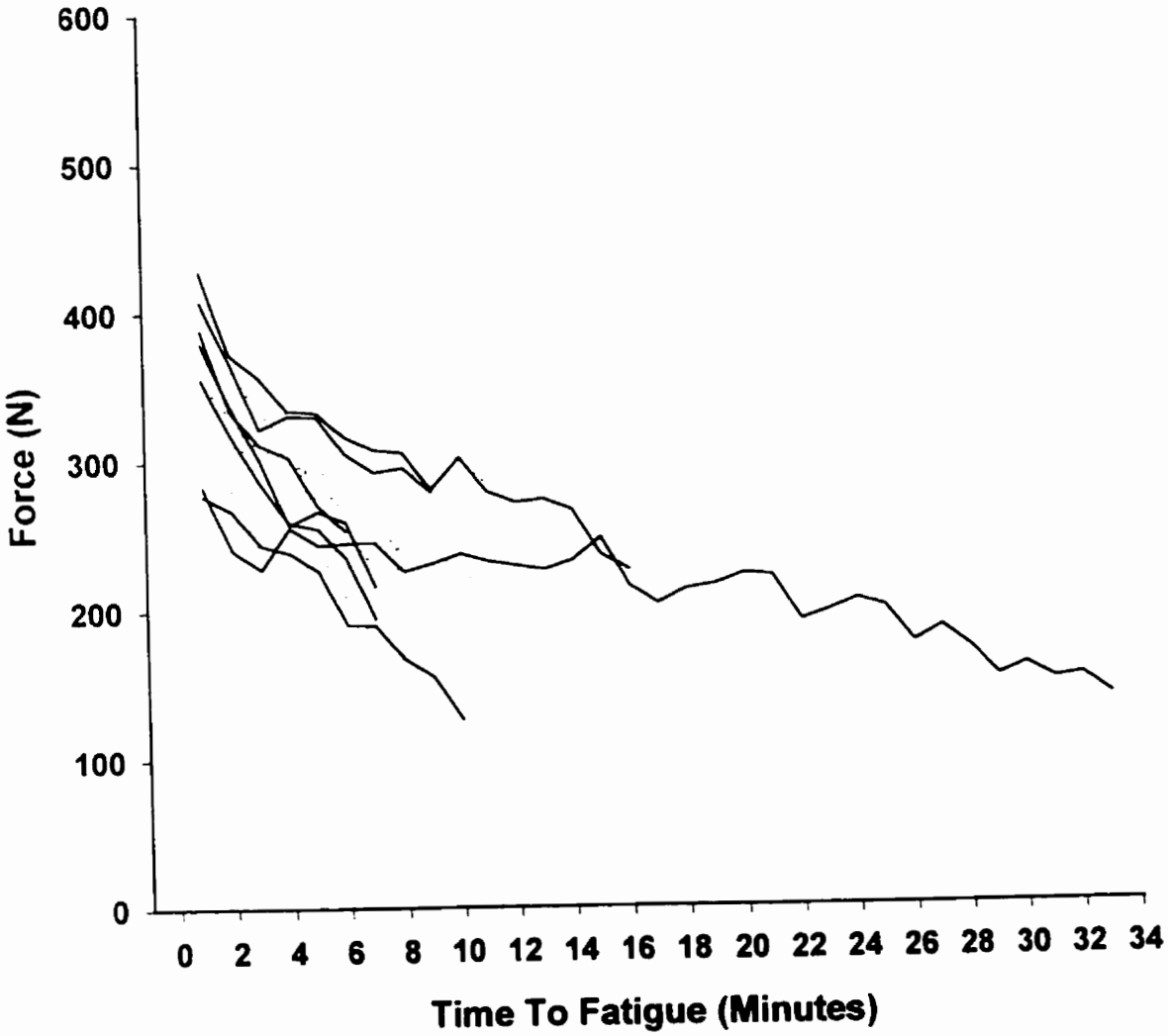
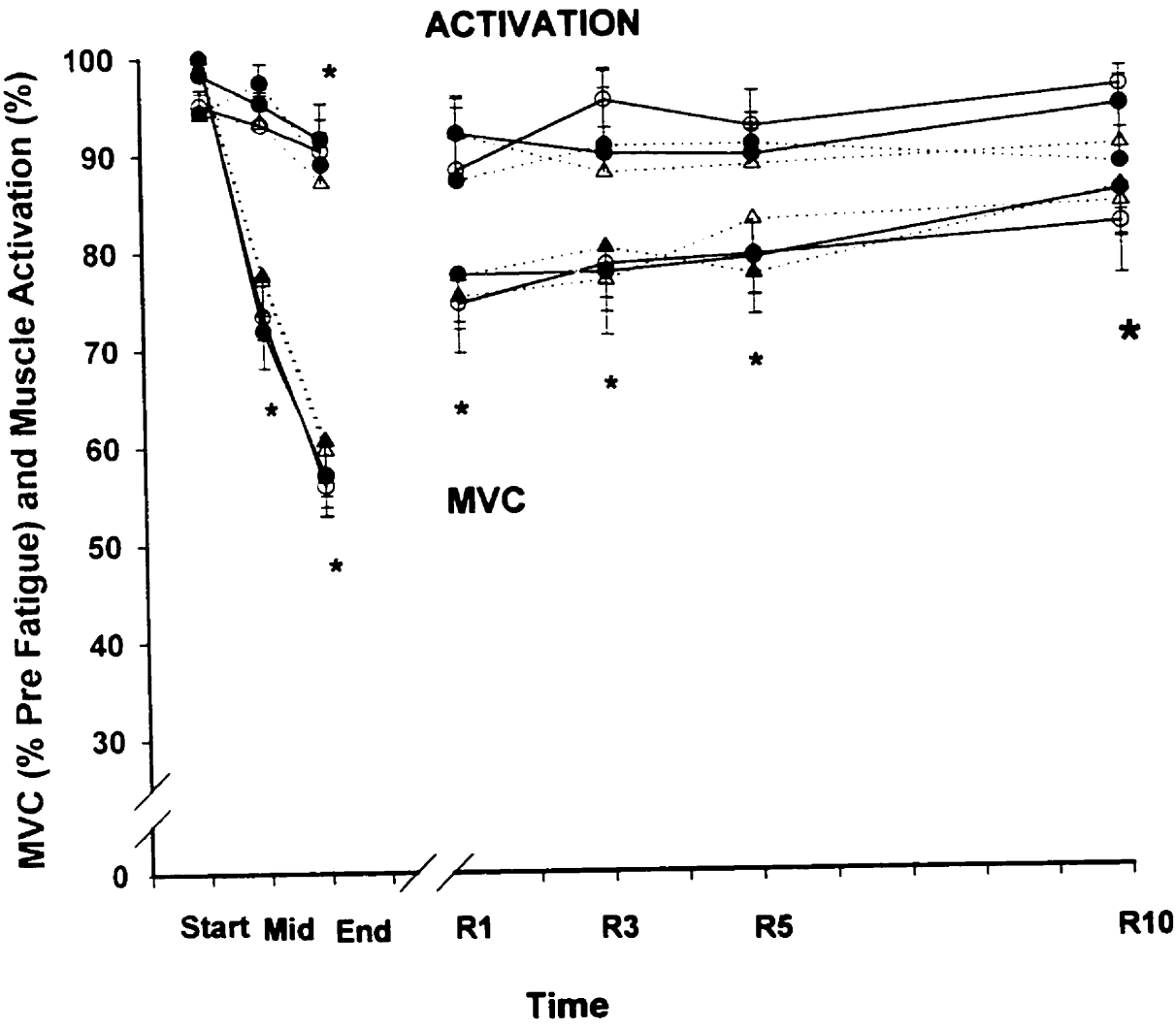

600
500
400
300
200
100
0
Force (N)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Time To Fatigue (Minutes)

Figure 4.2: Changes in MVC and activation during fatigue and recovery. MVC force (N) has been normalized to the pre fatigue force and plotted on a normalized time scale as start, middle and end. Recovery is shown in real time at one, three, five, and ten minutes (R1,R3,R5,R10, respectively). There are no statistical differences between the Cr and P groups during fatigue or recovery periods. Activation is given as a percentage (%activation = [1-(Ts/Tr)]*100), where Ts is the amplitude of the interpolated double twitch and Tr is the amplitude of the post-MVC twitch.) All pre data points are open, post are filled, continuous lines and ○/● are Cr, dotted lines and △/▲ are P. Downward bars represent standard errors of the mean (se) for force; upward bars represent standard errors of the mean for central activation. * significant differences ($p \leq 0.05$) from pre fatigue.


ACTIVATION
MVC
MVC (% Pre Fatigue) and Muscle Activation (%)
100
90
80
70
60
50
40
30
0
Start
Mid
End
R1
R3
R5
R10
Time

activation ranged 94 to 98% for each group prior to and following supplementation, whereas at the end of fatigue voluntary activation was significantly decreased ($p \leq 0.05$) in each group to ~ 87 - 90 %. Within the first minute of recovery voluntary activation had significantly recovered and was not different from pre fatigue values for either group.

Pre fatigue 50 Hz tetanic tension ranged 60 to 69 N for both groups, and the Cr group was not influenced by supplementation during fatigue. TT decreased in a similar manner (~ 64% decrease from initial TT) for each group prior to and following treatment (Figure 4.3) and neither the Cr nor P group had a significant increase in TT at R1,R3,R5, and R10. By R10 TT had recovered to ~ 36% of initial force. Single twitch tension followed a similar fatigue and recovery profile as TT. At the end of the fatigue protocol single twitch tension was ~ 13% of the initial force, and 10 minutes after the last MVC PT was ~ 38% of the pre fatigue MVC force in both the Cr and P groups.

The Cr and P group 50 Hz half relaxation time increased significantly ($p \leq 0.05$) over the duration of the fatigue protocol (Figure 4.4) for both the pre and post test. At the end of the fatigue protocol in each group, T½ increased to ~ 140% of its initial value and within the first minute of recovery HRT was significantly faster ($p \leq 0.05$) than the fatigue T½, but it was still statistically slower than pre fatigue. At five minutes of recovery, HRT was no longer statistically different from pre fatigue (11% slower than initial).

During the fatigue protocol and recovery there were no significant changes

Figure 4.3a,b: Tetanic tension (TT) (a) and single twitch tension (b) for Cr and P groups over the course of the fatigue and recovery protocol. The pre and post fatigue force for start (S), middle (M), end (E) and recovery (R1,R3,R5,R10) data have been normalized to the initial pre fatigue value. There were no statistical differences between Cr and P groups prior to, or following supplementation. All pre data points are open symbols, whereas post data points are filled symbols. ○/● and solid lines are Cr, and △ /▲ and dashed lines are P. Downward bars represent standard errors of the mean (se) * represents significant differences ($p \leq 0.05$) from pre fatigue.

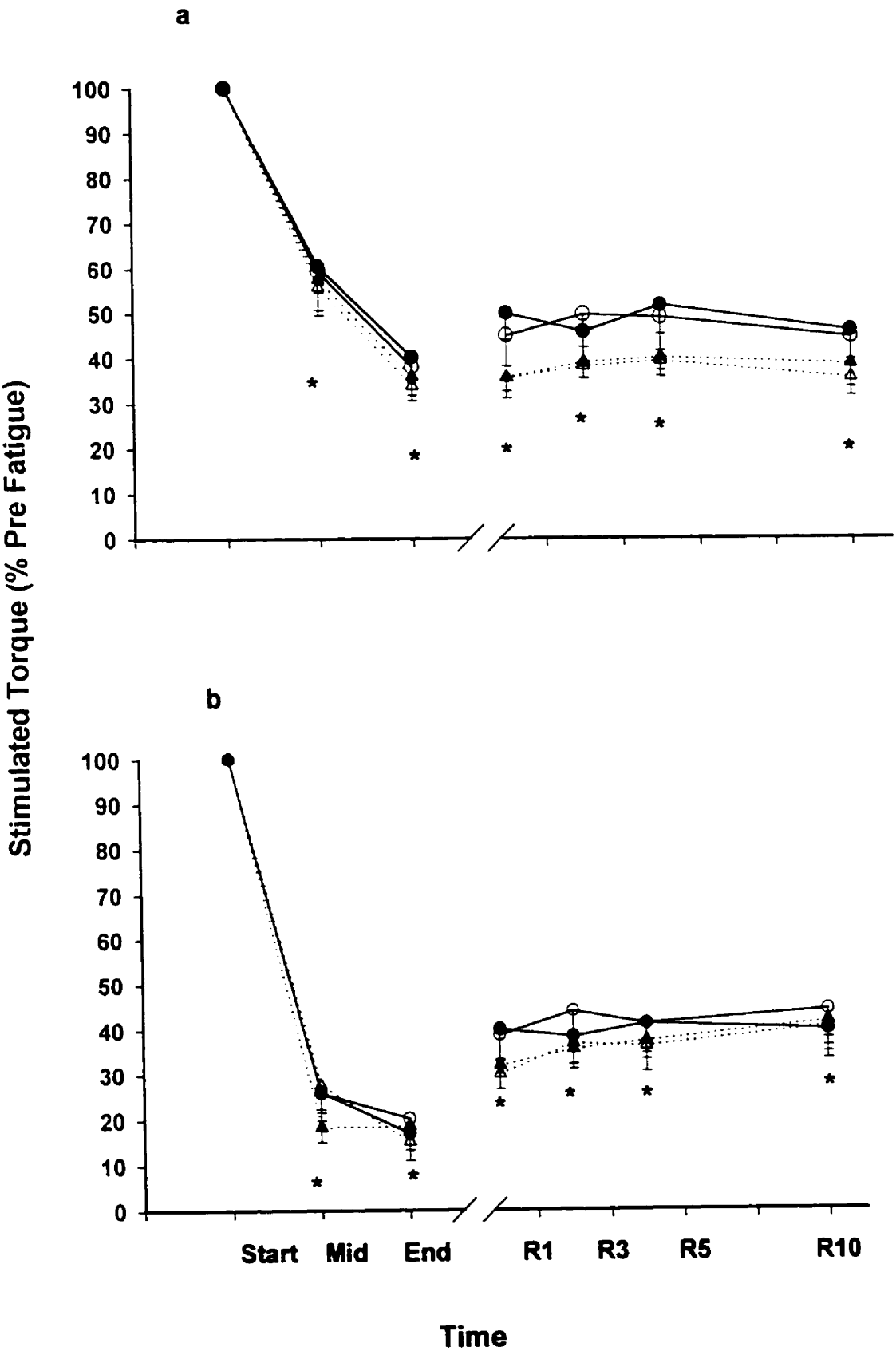

a
b
100
90
80
70
60
50
40
30
20
10
0
Stimulated Torque (% Pre Fatigue)
Start
Mid
End
R1
R3
R5
R10
Time

Figure 4.4: 50 Hz half relaxation time for the pre and post fatigue test. Data have been normalized to the initial pre fatigue value. There were no statistical differences between groups or following treatment. All pre supplementation data points are open, post supplementation are filled, solid lines and ○/● are Cr, dotted lines and △ /▲ are P. Downward bars represent standard errors of the mean (se). * represents significant differences ($p \leq 0.05$) from pre fatigue.

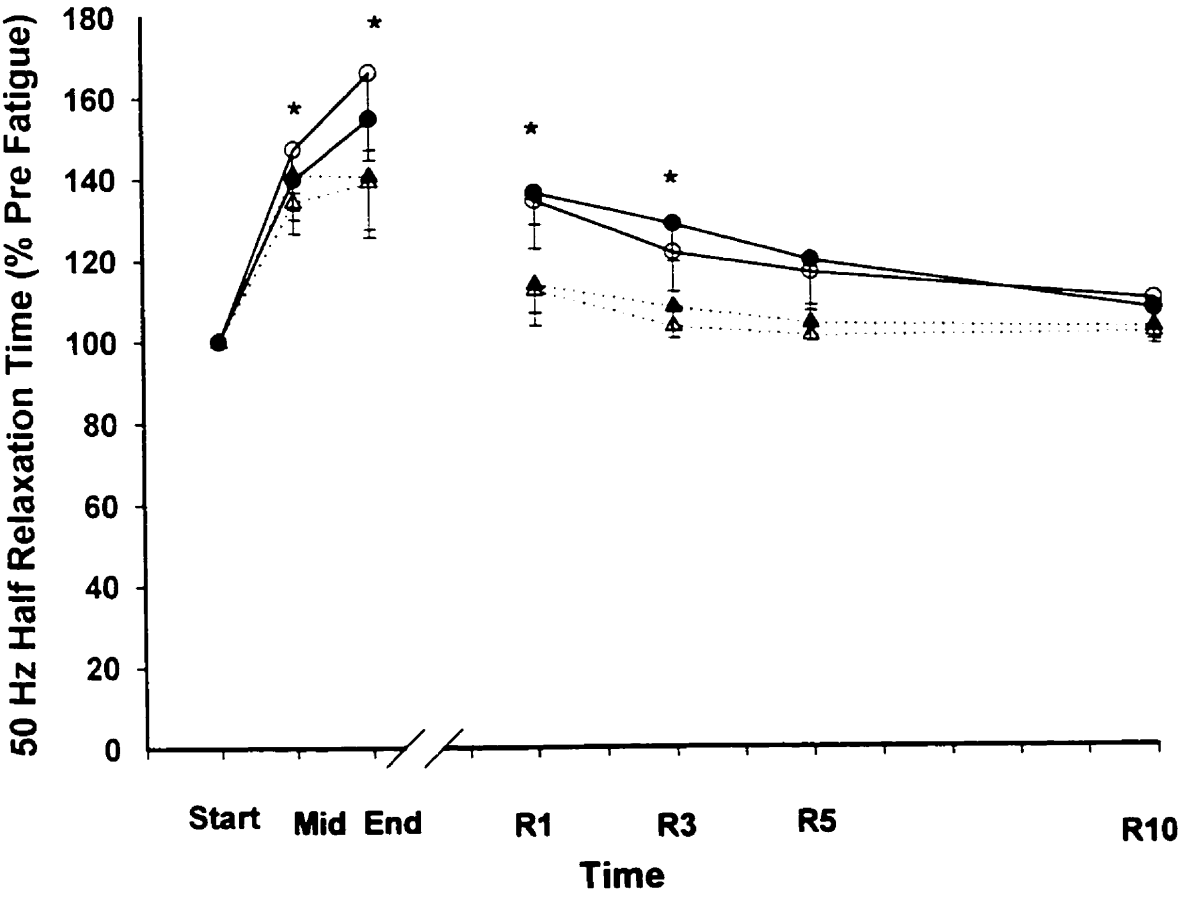
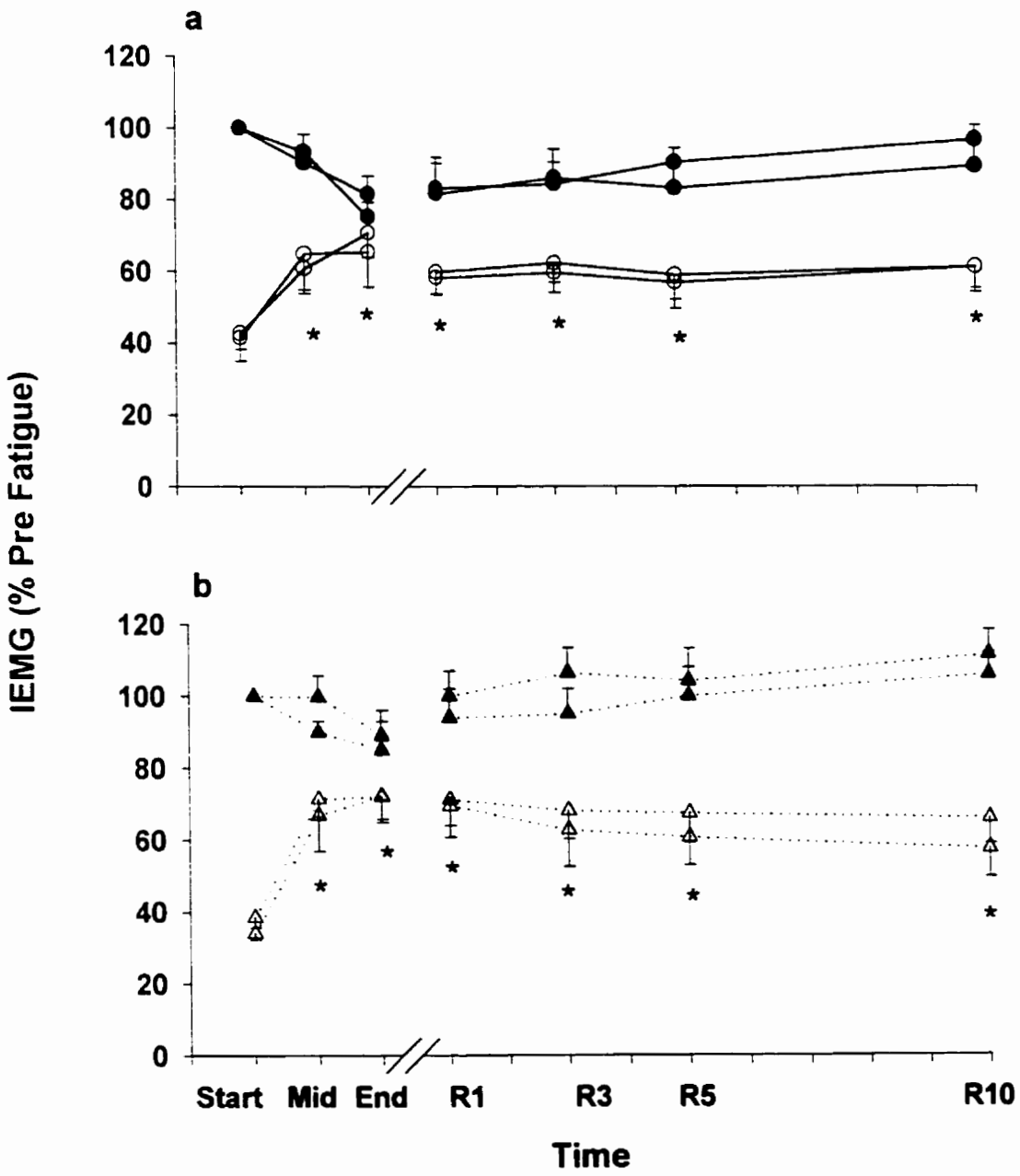

50 Hz Half Relaxation Time (% Pre Fatigue)
180
160
140
120
100
80
60
40
20
0
*
*
*
*
Start
Mid
End
R1
R3
R5
R10
Time

Figure 4.5a,b: Submaximal and maximal IEMG for pre and post test session for the Cr (a) and P group (b). All IEMG values have been normalized to the maximal IEMG recorded for the pre fatigue MVC. Filled symbols are maximal IEMG, open symbols are submaximal IEMG. For both groups there was a significant increase in submaximal IEMG during the fatigue protocol. ○/● are Cr, △ /▲ are P. Standard errors of the mean are shown as upward bars for maximal IEMG, and downward bars for submaximal IEMG. * represents significant differences ($p \leq 0.05$) from pre fatigue.


a
b
120
100
80
60
40
20
0
IEMG (% Pre Fatigue)
Start
Mid
End
R1
R3
R5
R10
Time

in maximal IEMG in either the Cr or P groups (Figure 4.5a,b), whereas submaximal IEMG increased significantly in both groups irrespective of the treatment. At the onset of all fatigue tests the submaximal IEMG was ~ 40% of the maximal IEMG and over the course of fatigue increased to ~ 63%. Prior to, and following supplementation, submaximal IEMG did not recover at one, three, five or ten minutes (Figure 4.5a,b).

4.3 DISCUSSION

The aim of this study was to investigate the influence of dietary Cr supplementation on elbow flexor strength, contractile properties, fatigue and recovery. The average weight gain in the Cr group of 1.0 kg was similar to previous values in the literature for short term Cr loading (Balsom et al. 1993), suggesting the supplementation protocol was effective in loading Cr. With no difference or changes in muscle activation and subject effort between groups or after supplementation, 20g of Cr per day, for five days, did not affect MVC, electrically stimulated contractile properties, and fatigue or recovery parameters of the elbow flexors in moderately active younger men.

4.3.1 Maximal Strength

The modified twitch interpolation technique acted as a subject motivator and as a means of discriminating central from peripheral factors involved with muscle force. Muscle activation in healthy moderately active younger men usually ranges between 95 and 98% (Hales & Gandevia 1988). Results from the present study illustrate that the 14 subjects produced near full activation (98 - 99%), with no

change following supplementation. Although the twitch interpolation technique has some limitations (Herbert and Gandevia 1999), especially in dynamic tasks in which many muscles are involved and their length and the joint angles change (Strojnik 1998), previous studies have not attempted to account for differences in subject effort and muscle activation which might contribute significantly towards the strength and performance improvements often found in other studies.

Most studies report an increase in isotonic dynamic strength following Cr supplementation (Earnest et al. 1995, Gordon et al. 1995, Vandenberghe et al. 1996), but the few studies of isometric strength are controversial. Results of isometric elbow flexion strength in this study are similar to those of Van Leemputte et al. (1999). Both studies suggest that there is no difference in absolute isometric elbow flexion strength following short term Cr supplementation in younger men. Conversely, Maganaris & Maughan (1998) and Lemon et al. (1995) reported an increase in isometric force of approximately 10% in the knee extensors and plantar flexors, respectively. Interestingly, Urbanski et al. (1999) observed increased knee extension strength, but no change in hand grip strength in the same subjects subsequent to five days of Cr supplementation. Although it is unclear how differences in muscle mass per se would affect Cr supplementation (Urbanski et al. 1999), it has been suggested that type II muscle fibres would benefit more by enhanced Cr availability, especially during exercise (Casey et al. 1996). Further studies are required to address the issue of muscle size and related fibre type differences with respect to the benefits of Cr. The addition of the twitch

interpolation technique in isometric studies might also help identify whether activation, or effort, are factors in strength changes between large and small muscle groups.

Previous short term Cr supplementation studies have utilized hydrostatic weighing techniques (Earnest et al. 1995), or skinfold thickness (Volek et al. 1997) in order to determine if the increase in body weight following Cr supplementation is due to a change in body composition. Rather than assess total body composition, measures of skinfold thickness and muscle girth were utilized to estimate muscle area (Rice et al. 1990) of the arm. The 1.0 kg increase in body weight observed in these younger men following short term Cr supplementation did not affect anthropometric estimates of muscle area (Table 4.1). The body weight increase is likely to be a result of increased water retention (Terjung et al. 2000, Volek et al. 1997, Zeigenfuss et al. 1998), which might increase muscle volume. Although measures of arm girth were made the anthropometric techniques which were employed (Rice et al. 1990, Thomas et al. 1998) may not have been sensitive enough to detect small changes in muscle area. Changes in muscle area within five days are not likely to occur as a consequence of an increase in contractile or structural proteins, but it is conceivable that an increase in intracellular fluid (Terjung et al. 2000) could affect muscle stiffness, or pennation angle of the muscle fibres which might affect overall muscle force output (Lieber et al. 1992). Other techniques, such as magnetic resonance imaging may help determine the extent of muscle volume changes following Cr supplementation and whether pennation

angles are altered.

4.3.2 Contractile Properties

Electrically evoked tensions and durations provide a reasonable indication of contractile processes (Hunter et al. 1998) which affect force production and endurance. In this study, electrically stimulated twitch and tetanic tensions and times in non fatigued muscle were not changed following short term creatine supplementation. Since stimulated contractile durations and tensions indirectly reflect calcium activity (Larsson & Salviati 1989) these results suggest that additional Cr does not alter excitation-contraction coupling in human muscle. No other human studies have measured stimulated contractile properties following short term Cr supplementation, and only one animal study has measured these. Creatine supplementation decreased stimulated relaxation time in the soleus muscle of rats (Wakatsuki et al. 1994), and it was suggested that this was due to a change in the high energy phosphate content. Besides possible differences between species, stimulated contractile changes in response to Cr might differ due to variations in fibre type (Casey et al. 1996, Terjung et al. 2000), or the role high energy phosphates play in human versus animal muscle. Clearly, further studies are needed to determine whether there is a relationship between Cr supplementation and contractile properties.

4.4.3 Fatigue

It has been reported that the amount of time isometric knee extension force (Maganaris & Maughan1998, Urbanski et al. 1999) and hand grip force (Urbanski

et al. 1999) can be sustained at a variety of submaximal levels increases following short term Cr supplementation. However, Van Lemputte et al. (1999) reported that Cr does not prolong elbow flexion fatigue when 12 consecutive maximal isometric contractions are performed. In this study, a submaximal isometric fatigue task was utilized, time to fatigue was determined, and maximal force was assessed intermittently in the elbow flexors, while monitoring subject effort, with the twitch interpolation technique. Prior to and following supplementation, maximal force decreased approximately 45% in a similar amount of time. Furthermore, in all sessions muscle activation decreased significantly by ~ 7%, by the end of the fatigue protocol, with the concurrent observation of no difference between groups in submaximal or maximal IEMG. This non-significant change in IEMG, muscle activation and maximal force following Cr supplementation suggests that elbow flexor neuromuscular characteristics over the course of an isometric fatigue task are not influenced by short term Cr loading.

Since slowing of muscle relaxation (Dawson et al. 1980) and a decrease in PCr (Westerblad and Allen 1993) have been reported in fatigue tests, stimulated contractile properties are important measures to consider during fatigue. Results from this intermittent voluntary fatigue protocol in which muscle stimulation was given at regular intervals suggest that there are no differences in stimulated force or contraction times as a result of Cr supplementation. The non significant change in stimulated force, in this study of the elbow flexors, is similar to a previous study of knee extensors and plantar flexors (Harridge et al. 1994) in which six days of Cr

supplementation had no effect on stimulated force at 40 Hz and 20 Hz, during a 2 minute fatigue test. Greenhaff et al. (1994) also employed electrical stimulation as a means of inducing fatigue, but force or contraction times were not reported, rather PCr and Cr content were measured from biopsy samples obtained from the vastus lateralis. During the second minute following intense electrical muscle contractions, Greenhaff et al. (1994) observed that there was an increase in PCr resynthesis, and it was suggested that this resulted from an increase in Cr content following five days of supplementation. If comparisons are made between studies, even though the protocols are different, it seems short term dietary supplementation of Cr may enhance total muscle Cr and PCr independently of any influence on contractile properties during fatigue.

The half relaxation time of 50Hz electrical stimulation did not change as a result of five days of Cr supplementation (Figure 4.4). This finding is inconsistent with that of Van Leemputte et al. (1999), in which a significant decrease in voluntary relaxation time was observed in twelve consecutive isometric elbow flexor contractions. Since the relaxation process accounts for an important fraction of total energy consumption (Bergstrom & Hultman 1988) during repeated muscle contraction there might be a performance benefit if relaxation time were decreased. Van Leemputtee et al. (1999) suggested that the mechanism facilitating relaxation was the rise in PCr concentration which enabled the sarcoplasmic Ca^{2+} ATPase to operate at a higher thermodynamic efficiency or at a higher rate of cross bridge detachment (Van Leemputte et al. 1999).

Changes in EMG in response to Cr supplementation have not been assessed extensively and comparisons between studies are difficult since different techniques have been employed. Following five days of Cr supplementation, Stout et al. (1999) observed a change in the EMG fatigue curves with cycle ergometry. This suggested that augmented PCr levels may influence anaerobic glycolysis and delay the onset of neuromuscular fatigue during incremental cycle ergometry (Stout et al. 1999). In the present study no changes were observed in maximal or submaximal IEMG in response to Cr supplementation in an isometric task. These IEMG findings support the maximal voluntary force data in the present study, indicating that there were no changes in either the neural drive or the contractile response which would delay time to fatigue.

4.3.4 Recovery

There is minimal information on neuromuscular recovery parameters following Cr supplementation, although there has been speculation in the literature that delayed depletion of PCr would enhance repeated bouts of exercise (Greenhaff et al. 1994) through facilitation of ATP resynthesis (Casey et al. 1996). In order to assess whether Cr supplementation enhances recovery voluntary force, IEMG and contractile properties were monitored for 10 minutes following the exercise. There were no changes in any measures of recover following Cr supplementation. This suggests that if changes in the phosphagen energy system occur (Casey et al. 1996) they are independent of alterations in muscle force or contractile properties. However, in order to conclusively assess this possible independence, both muscle

metabolism and contractile function should be measured in the same study.

In conclusion, short term Cr supplementation in younger men increased body weight, but did not influence cross sectional area of the arm, as assessed with anthropometric measures. Creatine did not alter isometric elbow flexion force, electrically stimulated contractile tension or times, fatigue or recovery in moderately active younger men. Thus, it appears that alterations in the strength or contractile properties of individual muscle groups do not contribute to the enhancement in whole body exercise performance frequently observed following short term Cr supplementation.

4.4 REFERENCES

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CHAPTER 5:

NEUROMUSCULAR PROPERTIES AND FATIGUE IN OLDER MEN FOLLOWING ACUTE CREATINE SUPPLEMENTATION

5.0 INTRODUCTION

Following short term (5 days) creatine (Cr) supplementation in younger men, several investigators have reported an elevation in Cr and phosphocreatine (PCr) content (Harris et al. 1992, Balsom et al. 1993), an increase in body mass (Balsom et al. 1993), strength (Earnest et al. 1995, Maganaris & Maughan 1998),and endurance (Balsom et al. 1993). There are a limited number of studies, and the results are equivocal with respect to the effects of Cr supplementation in older adults. It seems Cr supplementation in older adults results in an increase in endurance (Rawson & Clarkson 2000, Rawson et al. 1999) independent of any alteration in maximal strength (Rawson & Clarkson 2000, Rawson et al. 1999, Bermon et al. 1998) or body weight (Rawson et al. 1999, Rawson & Clarkson 2000). Further comparisons among the few studies (Rawson & Clarkson 2000, Rawson et al. 1999, Smith et al.1998) of short term Cr supplementation on older men are difficult because strength and endurance have not been measured in the same muscle groups and during similar tasks. The effect of acute Cr supplementation on endurance has been investigated during dynamic tasks in the lower limb (Rawson & Clarkson 2000), whereas strength has been investigated in isometric tasks in the

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upper limb (Rawson & Clarkson 2000). However, the effect of long term (8 weeks) Cr supplementation on strength and fatigue has been reported in the same muscle groups and tasks (Bermon et al. 1998).

It has been suggested that the performance benefit of Cr supplementation occurs through an elevation in Cr and PCr content (Harris et al. 1992, Balsom et al. 1993) which enhances PCr hydrolysis for ATP resynthesis (Terjung et al. 2000). Although controversial, it seems that the PCr content of skeletal muscle decreases with age (Kent-Braun et al. 2000, Moller et al. 1980, Smith et al. 1998). However, similar to younger muscle (Harris et al. 1992, Balsom et al. 1993) older muscle retains the ability to uptake Cr (Smith et al. 1998). Thus, supplementation may enhance performance during an endurance task. Furthermore, if ADP rephosphorylation is more rapid, and PCr resynthesis is accelerated (Greenhaff et al. 1994) following supplementation then it is likely that recovery from exercise will be improved. No study has assessed immediate recovery from fatigue in older men following Cr supplementation.

Force and endurance time can be affected by muscle activation, and this has not been measured in previous Cr supplementation studies in older men. Muscle activation is determined by central nervous system factors related to practice, effort (Gandevia et al. 1995) and neural drive (Bigland-Ritchie et al. 1986), and can be assessed objectively by using the techniques of twitch interpolation and surface electromyography. The application of these techniques could help determine whether changes in voluntary strength following Cr supplementation are due to

peripheral contractile muscle changes, or because of central neural factors affecting muscle activation.

It has also been suggested that an increase in PCr content would improve exercise performance by increasing the supply of ATP which would enhance Ca^{2+} kinetics at the level of the sarcoplasmic reticulum, thereby improving contractile speed (Van Leemputte et al. 1999). Furthermore, since muscle relaxation accounts for a significant proportion of the energy required for muscle activity (Bergstrom et al. 1988) endurance times also could be improved by faster contractile properties. Van Leemputtee et al. (1999) reported a decrease in relaxation time of a voluntary contraction in younger men, and in one animal study electrically induced twitch relaxation times were faster in adult rat soleus muscle after Cr supplementation (Wakatsuki et al. 1994). However, a change in electrically stimulated contractile properties was not found in younger men following Cr supplementation (Jakobi et al. 2000). Discrepancies may exist among these studies because of differences in measurement techniques, variations in fibre type between muscles and the role high energy phosphates play in human versus animal muscle (Casey et al. 1996, Terjung et al. 2000). Since contraction time of skeletal muscle in older men is longer (Porter et al. 1995) and PCr resynthesis slower following exercise (Conley et al. 2000) it is possible that Cr supplementation may enhance contractile function through increased PCr resynthesis (Greenhaff et al. 1994, Smith et al. 1998).

The purpose of this study was to combine neural and contractile measures to investigate the effect of short term Cr supplementation on muscle strength and

fatigue in the elbow flexors of older men.

5.1 METHODS

5.1.1 Subjects:

Volunteers were healthy, moderately active independent older men living in London, Ontario. The study was conducted according to the guidelines established by The University of Western Ontario Review Board for Research Involving Human Subjects and the Helsinki Declaration. Informed written consent was obtained from the 12 older men (ages 65 - 82 years) prior to participation in the study. Daily physical activity (Robinson et al. 1999), diet, and caffeine consumption (Fryer & Neering 1989, Vandenberghe et al. 1996), were controlled by urging participants to maintain their standard daily activities and energy intake, dissolve the powdered substance in non-caffeinated beverages. and abstain from caffeine one hour prior to visiting the neuromuscular laboratory. All subjects were meat eaters, and exclusion criteria consisted of creatine supplementation within the prior 12 months, myopathies or neuropathies, diabetes, alcoholism, and hypertension.

5.1.2 Study Protocol

Subjects visited the neuromuscular laboratory on three separate occasions (accomodation, pre and post test sessions) for this double blind control study. An accomodation session was conducted because prior reports in younger adults (Maganaris & Maughan 1998) indicate that differences in force may exist between the first and second test as a result of familiarization with the experimental situation as well as learning how to perform maximal voluntary contractions (MVCs).

Approximately one half of the older men in this study performed better on the second session (pre). The second session was conducted three to seven days following the first session, and the third session (post) was conducted within 8 - 14 hours following the last day of dietary supplementation. Accomodation involved positioning the subject in the arm device to ensure comfort and familiarity with the tests. Thorough measurements of arm, shoulder, head and hip placement were taken to ensure identical subject positioning on all test sessions. On the pre and post tests body weight (kg), height (cm) and arm anthropometry (Rice et al. 1990) were determined before assessment of strength, and performance of the fatigue protocol and recovery. Measures of total arm area cm^2 (TAA), muscle plus bone area cm^2 (MBA), and skin plus subcutaneous tissue area cm^2 (SST) were estimated from skin fold and arm girth measures (Rice et al. 1990).

Seven of the twelve participants were randomly assigned to the creatine supplementation group (Cr), while the remaining five subjects formed the placebo group (P). In addition to their normal diet subjects supplemented four times per day for five days, the Cr group with 5.0 g of powdered creatine monohydrate blended with 5.0 g of maltodextrin, while the P group received 5.0 g of maltodextrin. Volunteers were instructed to dissolve the pre measured substance in warm water or a non citric acid juice and to consume a carbohydrate with the dietary supplement (Green et al. 1996). The Cr and P samples were pre weighed and separated into twenty individual vials by someone not directly involved with the testing. Vials were given to each subject separately and subjects did not compare

samples, and the experimenters did not see the vials given to each subject.

5.1.3 Muscle Strength

Elbow flexor measures were conducted in a supine position on a padded examination table with the subjects' legs elevated for comfort and as a means to prevent extraneous movement in the lower body, which might influence upper body positioning or force generation. The left elbow was flexed to 90° and the shoulders were secured to prevent extraneous movements of the trunk. The wrist was secured to a plate which was attached to a strain gauge (SST-700-100A, AS Technology) and the elbow was positioned and secured perpendicular to the wrist. The strain gauge was calibrated with known weights to confirm linearity and to convert force values to Newtons. The output from the strain gauge was sampled at 500Hz, amplified and filtered (60 Hz notch filter), and displayed in real-time on an oscilloscope in front of the subject. After initial amplification and filtering, the force signal was converted from analog to digital by a 12-bit A/D converter (model 1401 Plus, Cambridge Electronic Design Ltd.).

To measure the MVC, subjects were instructed to flex their elbow joint as intensely and as quickly as possible, and sustain this effort for four to five seconds. Subjects performed three to four MVCs on each session. The importance of exerting maximal effort on all contractions was verbally encouraged and reinforced. As well visual feedback from the oscilloscope was provided. Five minutes rest was given between MVCs and the highest value for each session was recorded as maximum. Muscle activation was assessed with the modified twitch interpolation

technique (Hales & Gandevia 1988). This technique involved superimposing a series of paired electrical shocks (2 pulses separated by 10 ms) during and following a MVC. If maximal effort was impaired, a small twitch response would be superimposed on the voluntary force curve. To obtain an estimate of muscle activation the amplitude of the interpolated double twitch (Ts) was compared to the amplitude of the post MVC double twitch (Tr). A ratio of these two measures provided an index of how well the muscle was activated (%activation = [1-(Ts/Tr)]*100). A benefit of employing this technique was, not only to assess muscle activation, but also to monitor subject effort between sessions. It is unlikely that Cr would have altered activation but, it was important to ensure that all tests were conducted with consistent maximal efforts which could be objectively monitored.

Contractile properties of the elbow flexors were measured from twitch and tetanic recordings induced by electrical stimulation through carbon rubber stimulation electrodes (4 x 4.5 cm) which were tightly bandaged over the proximal and distal portions of the flexor area of the arm. The electrical pulse duration was 50μs, voltage was constant (400v) and the current level was adjusted in incremental steps (model DS7H, Digitimer Ltd.). Stimulation intensity was set to a level which activated as much of the muscle as possible, or to the highest tolerable level without interference from antagonists. Palpation, noticeable contraction of triceps brachii, or a decrement in force with an increase in current, were monitored to determine whether antagonist muscles were activated. Identical stimulation settings were used for each test session. To induce twitches, two sets of ten single

pulses were delivered at one pulse per second while the subjects were at rest. The two sets of pulses were separated by approximately one minute. Off line analysis for the twitches consisted of peak tension (PT), time to peak tension, (TPT) and half relaxation time (HRT). To assess the twitch contractile quality approximately 16 single twitches were averaged to determine PT, TPT and HRT, and the intra-class correlation coefficients between the accomodation and pre test sessions for these measures were 0.83, 0.86, and 0.86, respectively. For tetanic responses at rest, 16 pulses were delivered at 50Hz for 320 milliseconds duration. Stimulated tetanic contractions were elicited twice with 30 seconds rest given between each contraction. Peak tetanic tension (TT), and half relaxation time (HRT) were determined from the maximal response of these two contractions. The inter-day reliability for 50 Hz TT and HRT were each 0.95.

5.1.4 Surface Electromyography

A surface electrode was applied over the mid belly of the biceps brachii approximately five cm from the cubital fossa and a reference electrode was placed over the lateral epicondyle of the humerus. The electromyogram (EMG) signal was sampled at 2500 Hz, wide band filtered (10Hz - 10KHz) and amplified (x500) using a preamplifier (amplifier and filter model NL824, Neurolog). After initial amplification and filtering, the EMG signal was converted from analog to digital by a 12-bit A/D converter (model 1401 Plus, Cambridge Electronic Design Ltd.). Off-line the surface EMG was full wave rectified and subsequently integrated (IEMG) over the entire force record in 0.5s interval. Force and EMG signals were

monitored on the computer screen and collected on-line simultaneously to a VCR tape recorder.

5.1.5 Fatigue and Recovery Protocol

The fatigue protocol consisted of a series of target force contractions at 50% MVC for 6s followed by 4s of rest (60% work to rest ratio) until the maximum force became the target force of 50% MVC (Bigland-Ritchie et al. 1986), or subjects would no longer continue. Fatigue was monitored by measuring maximal voluntary force and electrically evoked contractile properties every 60s during the fatigue protocol. Sixteen pulses at 50 Hz stimulation were applied to the resting muscle prior to the 50% target force which preceded the MVC. Before and during the MVCs subjects were stimulated with double pulses to assess muscle activation and contractile properties, whereas following the MVC a single pulse was applied.

The recovery measures at one, three, five and ten minutes were the same as the tests applied every 60s during the fatigue protocol. Off-line quantification of the single twitch and 50 Hz stimulation response consisted of PT, TT and peak rate of fall ($N\,ms^{-1}$) (PRF). Peak rates of fall were measured, rather than half relaxation times, since the amplitude of the twitch and tetanic tensions decreased during the fatigue protocol. This value was calculated by software differentiation of the force signal. Throughout the fatigue protocol and recovery EMG data were collected. All EMG values were integrated and normalized to the pre fatigue MVC.

The time points used for all dependent variables in the fatigue protocol were start of fatigue (start), middle time point of fatigue (mid) and end of fatigue (end).

This normalization procedure was necessary because not all subjects stopped the fatigue task at the same time.

5.1.6 Statistics

A two factor repeated measures analysis of variance using condition; (Cr, P), and day; (pre, post) was employed to determine whether there were any differences in non-fatigued baseline measures of force, muscle activation or contractile properties. Time to fatigue was analyzed with a two way repeated measures analysis of variance (condition x day), and the remaining dependent variables for the fatigue protocol were analyzed with a repeated measures three factor analysis of variance (condition x day x time points of fatigue/recovery). All data are presented as means and standard errors of the mean (se). The level of statistical significance was set at $p \leq 0.05$.

5.2 RESULTS

None of the older subjects reported adverse side effects as a result of Cr or P supplementation, although all 12 subjects stated that urination was more frequent during the intervention period. Likely this was due to an increase in fluid consumption which was necessary to dissolve the powdered substances. The mean height (173 ± 4, 175 ± 4 cm) and body weight (83 ± 4, 81 ± 5 kg) of the Cr and P groups, respectively, were not different prior to supplementation. Following supplementation the body weight of the P group did not change (-0.7 - +0.4kg), but the Cr group had a significant ($p \leq 0.05$) increase of 1.0kg (0.3 - 2.8kg) (Table 5.1). Anthropometric measures of TAA, MBA and SST were not different between

Table 5.1: Subject characteristics for older men on placebo or creatine sypplementation.

	Creatine Pre (n=7)	Creatine Post (n=7)	Placebo Pre (n=5)	Placebo Post (n=5)
Age (years)	72 ± 2	-	73 ± 3	-
Height (cm)	173 ± 4	-	175 ± 4	-
Weight (kg)	83 ± 4	84 ± 4*	81 ± 5	81 ± 5
Total arm area (cm^2)	68 ± 2	68 ± 2	73 ± 5	72 ± 6
Muscle plus bone area (cm^2)	53 ± 2	54 ± 2	60 ± 5	58 ± 6
Skin plus subcutaneous tissue (cm^2)	15 ± 3	14 ± 2	13 ± 2	14 ± 2

Values are means ± standard errors of the mean. *, significant difference $p \leq 0.05$ post test compared to pre test.

Table 5.2: Contractile properties of elbow flexors with creatine or placebo supplementation.

Muscle Property	Creatine Pre (n=7)	Creatine Post (n=7)	Placebo Pre (n=5)	Placebo Post (n=5)
MVC (N)	282 ± 28	290 ± 22	277 ± 37	277 ± 26
% Activation	96 ± 2	97 ± 1	94 ± 1	95 ± 2
Peak twitch tension (N)	16 ± 2	17 ± 2	13 ± 2	14 ± 2
Time to peak tension (ms)	73 ± 4	69 ± 4	73 ± 4	72 ± 3
Half relaxation time (ms)	56 ± 4	59 ± 7	67 ± 6	68 ± 4
50 Hz tension (N)	36 ± 8	36 ± 6	22 ± 4	25 ± 4
Tetanic half relaxation time (ms)	99 ± 8	100 ± 7	105 ± 3	109 ± 3
Peak rate of fall (N ms^{-1})	-0.3 ± 0.06	-0.3 ± 0.09	-0.3 ± 0.06	-0.3 ± 0.08

Values are means ± standard errors of the mean.

groups either before, or following dietary supplementation.

Maximal voluntary contraction for the P group (277 ± 37N vs 277 ± 26N (se)) and Cr group (282 ± 28N vs 290 ± 22N (se)) did not change after supplementation (Table 5.2). Similarly, stimulated single twitch contractile properties (TPT,HRT, PT) did not differ between groups, prior to or following treatment (Table 5.2). As well, the 50Hz tension and HRT were not influenced by Cr supplementation, nor were they different between the Cr and P groups (Table 5.2).

The range in time to fatigue for the pre (5 - 38 minutes) and post tests (4 - 42 minutes) was similar between the groups irrespective of the treatment. Time to fatigue for the Cr and P groups on the pre test is presented in Figure 5.1. Following supplementation time to fatigue did not change in the Cr group (21 ± 5 min, 22 ± 5 min), or the P group (23 ± 4 min, 22 ± 4 min). During the fatigue task, there was a significant decline ($p \leq 0.05$) in voluntary force to 65% of the initial MVC for both groups prior to and following supplementation (Figure 5.2). In both groups the range in which force decreased was 49 - 73% of the initial MVC.

Recovery of one minute (R1) resulted in a 10% increase in voluntary force from the end of fatigue for both groups, but, at R1 voluntary force was still significantly lower than the pre fatigue MVC in both groups. At R10 MVC force was still significantly less (~ 22%) than the pre fatigue force (Figure 5.2). Although muscle activation varied ~ 10% over the fatigue protocol and recovery (Figure 5.2), it was never significantly less than pre fatigue in either group.

During fatigue, tetanic tension at 50Hz did not differ between groups or

Figure 5.1: The relationship between MVC force and time during the fatigue protocol at the pre test. The Cr (solid lines) (n=6) and P (dashed lines) (n=5) subjects did not differ in time to fatigue on the pre or post test.

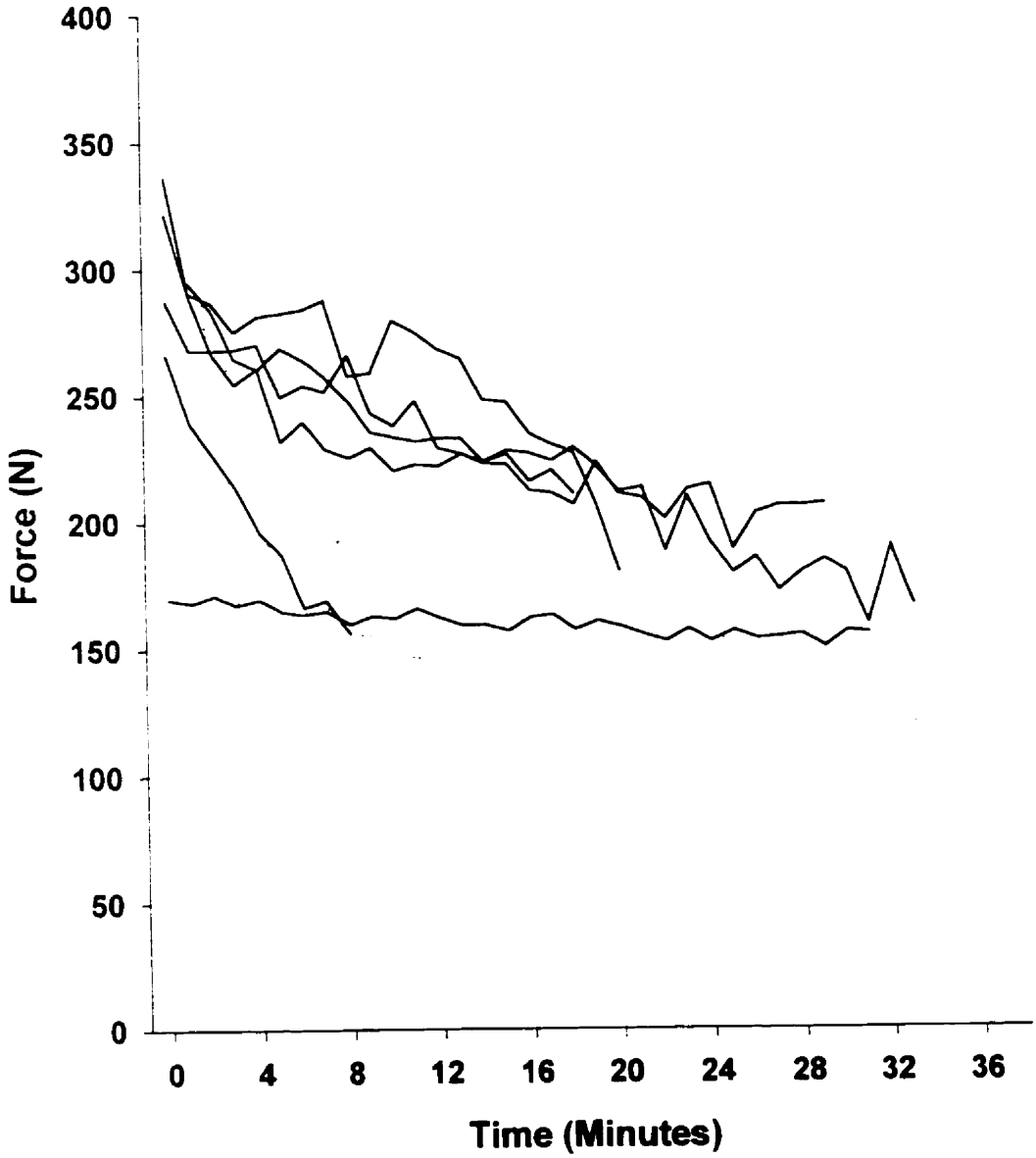

400
350
300
250
200
150
100
50
0
Force (N)
0
4
8
12
16
20
24
28
32
36
Time (Minutes)

Figure 5.2: Changes in MVC and activation during fatigue and recovery. MVC force and activation (% activation = [1-(ts/tr)]* 100) have been normalized to the pre fatigue value and presented on a normalized scale as start, middle and end. Recovery is shown in real time at one, three, five and ten minutes (R1,R3,R5,R10). There were no statistical differences between the Cr and P groups during fatigue or recovery. ○ represent Cr, △ represent P. All pre data points are open, post are filled, continuous lines are Cr, and dashed lines are P. Standard errors of the mean (se) for force are the downward bars, whereas se for muscle activation are the upward bars. * represents significant difference ($p \leq 0.05$) from pre fatigue.

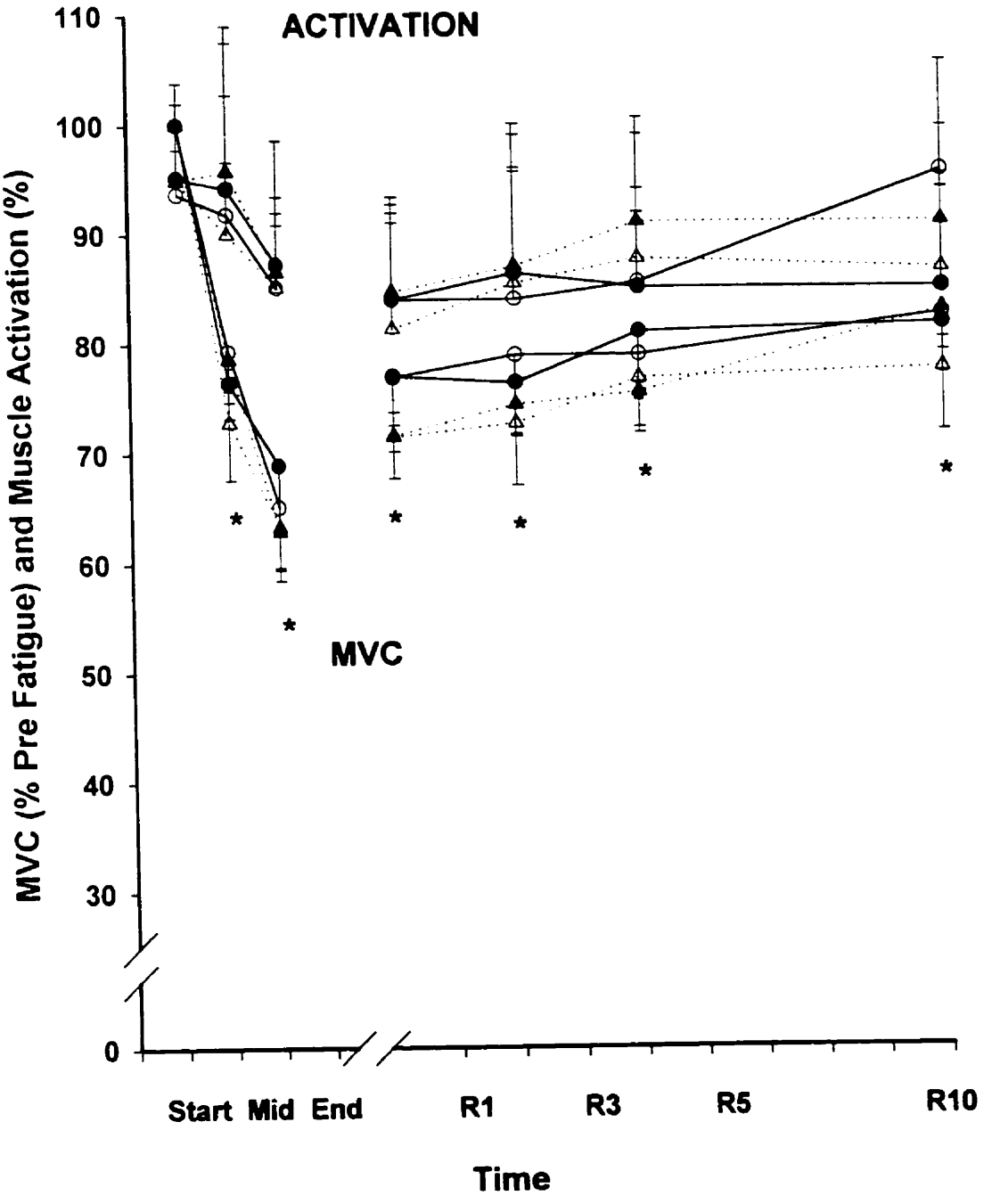

ACTIVATION
MVC
MVC (% Pre Fatigue) and Muscle Activation (%)
110
100
90
80
70
60
50
40
30
0
Start
Mid
End
R1
R3
R5
R10
Time

Figure 5.3: Tetanic Tension (TT) during fatigue (start, middle, end) and recovery (R1,R3,R5,R10) have been normalized to the pre fatigue value. There were no statistical differences between Cr and P groups prior to, or following supplementation. All pre data points are open, post are filled, solid lines are Cr, dashed lines are P,○ represent Cr, and △ represent P. * represents significant difference ($p \leq 0.05$) from pre fatigue.

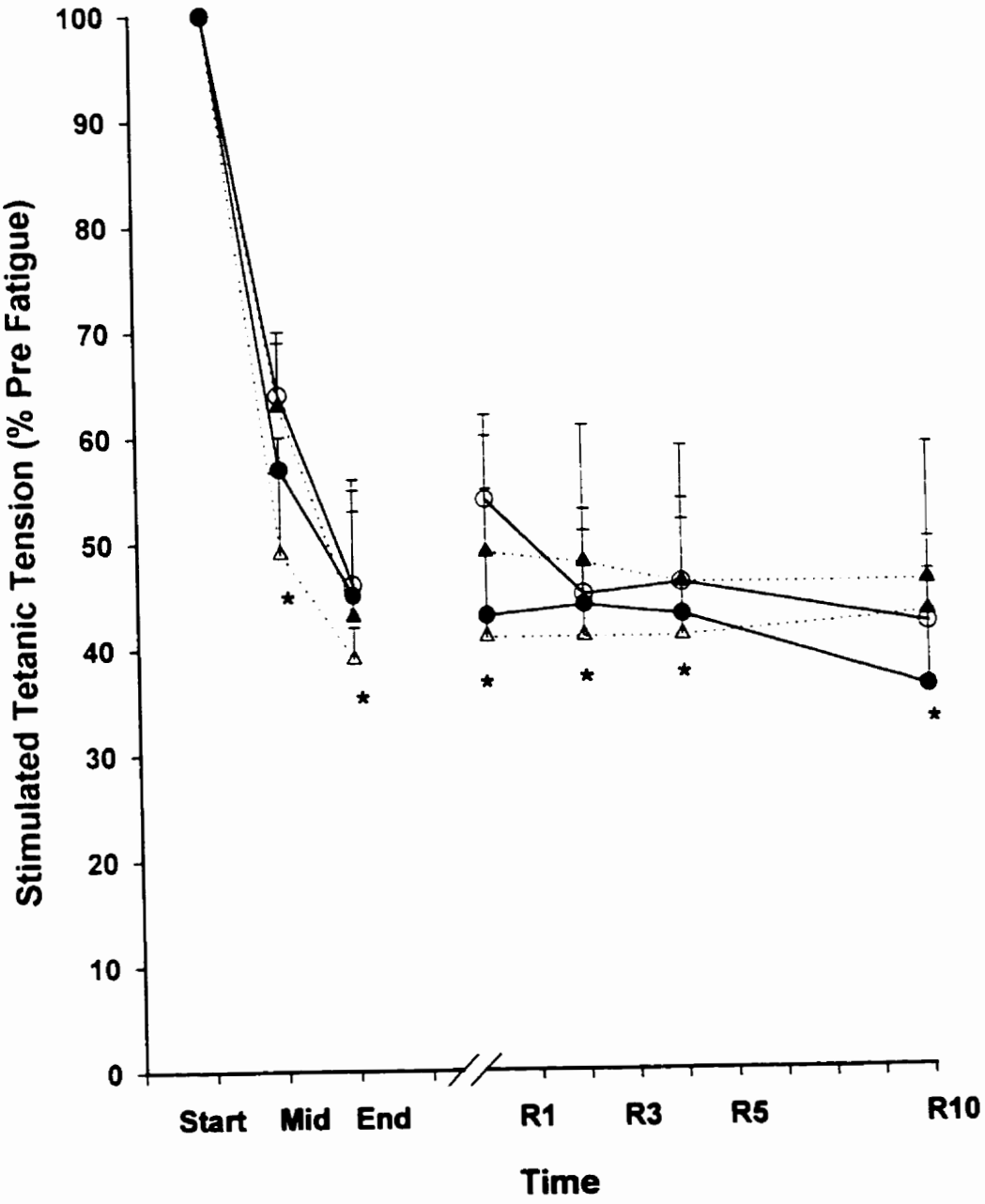
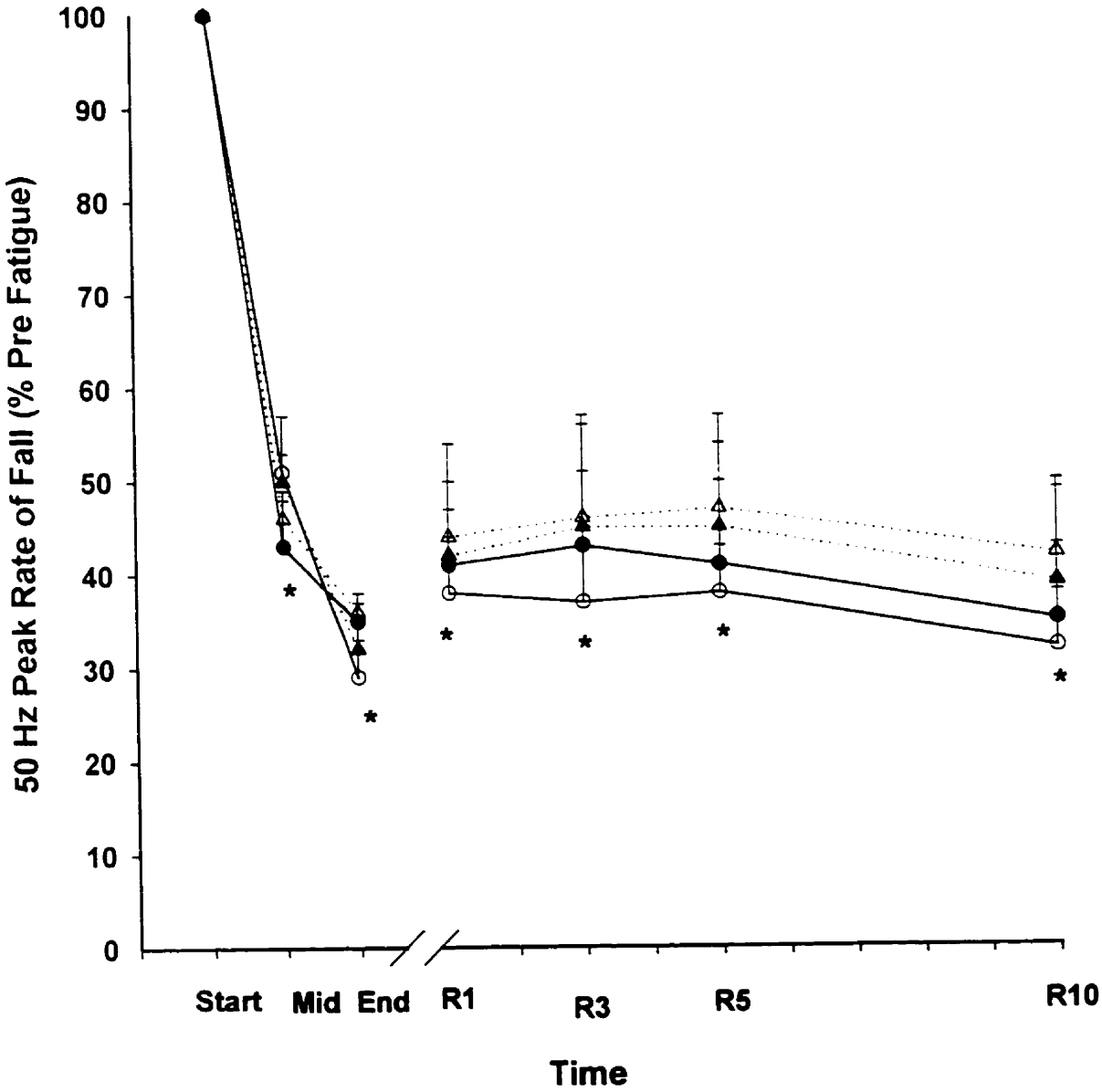

Stimulated Tetanic Tension (% Pre Fatigue)
100
90
80
70
60
50
40
30
20
10
0
Start
Mid
End
R1
R3
R5
R10
Time

Figure 5.4: 50 Hz Peak rate of fall for the pre and post fatigue tests have been normalized to the initial pre fatigue value. There were no statistical differences between groups or sessions. All pre data points are open, post are filled, solid lines are Cr, dashed are P,○ represent Cr, △ represent P.


100
90
80
70
60
50
40
30
20
10
0
50 Hz Peak Rate of Fall (% Pre Fatigue)
Start
Mid
End
R1
R3
R5
R10
Time

change as a result of dietary supplementation (Figure 5.3). The net decrease in tetanic force was ~ 57% of the initial pre fatigue tension in both groups. Tetanic tension did not recover, and by R10 it was still ~ 57% less than pre fatigue. Single twitch tension declined substantially and could not be measured reliably as fatigue developed. The point at which the twitch force could no longer be measured did not change as a result of Cr supplementation.

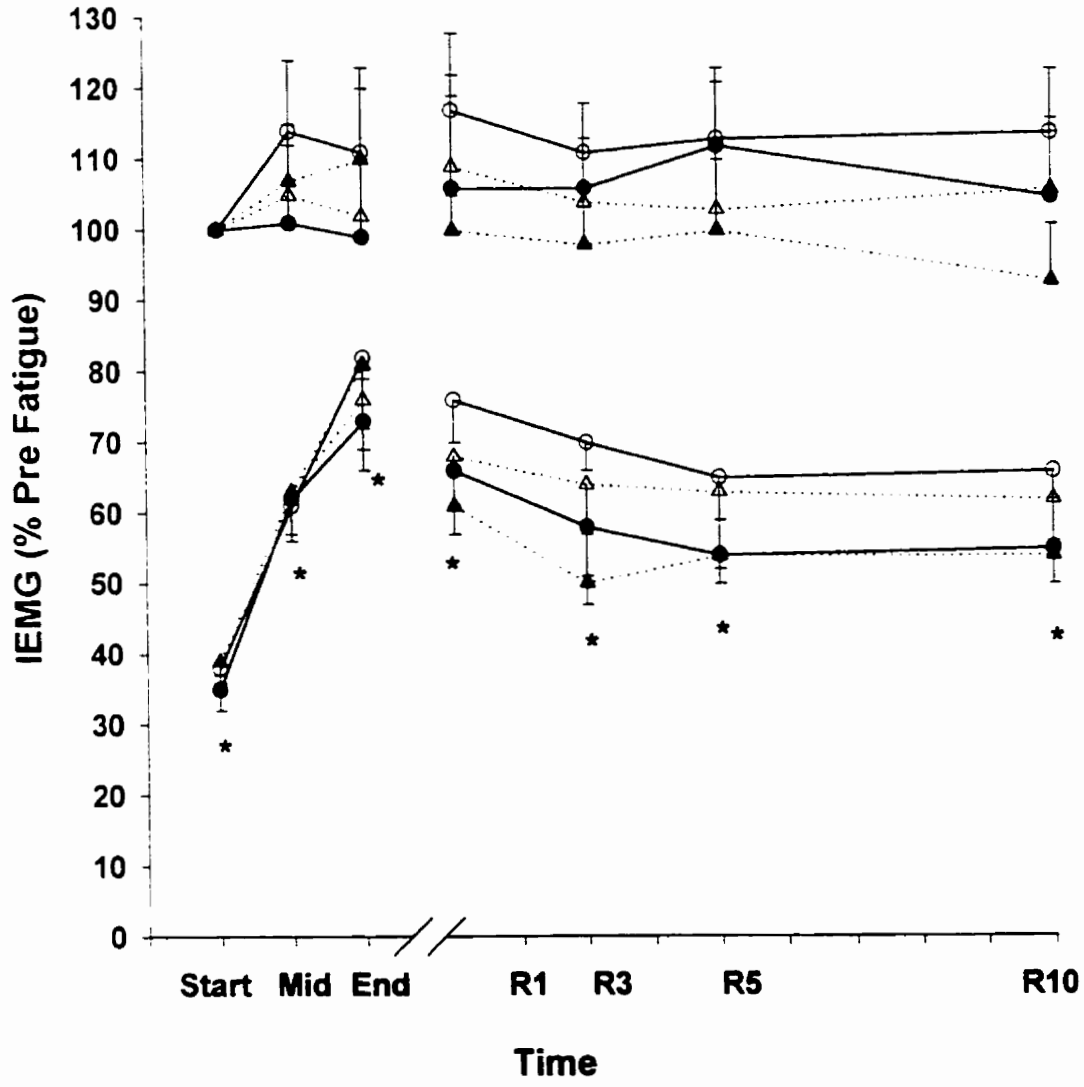
There was a significant decrease in the peak fall rate (N/ms) of tetanic tension during the fatigue task. The 50Hz peak fall slowed ~ 67% from the initial pre fatigue rate in each group and was not influenced by dietary supplementation (Figure 5.4). Peak fall rate was similar between groups, and at R10 the rate was still significantly slower (~ 63%) than pre fatigue (Figure 5.4).

During the fatigue protocol and recovery maximal IEMG did not change in either group prior to, or following supplementation (Figure 5.5). In each group the submaximal IEMG was initially 40% of maximal IEMG, increased to ~ 60% at the mid point of fatigue, and, at the end of the fatigue task it was 70% of the maximal IEMG (Figure 5.5). Submaximal IEMG did not recover in either group, it remained significantly greater (~28%) than the pre fatigue level prior to and following supplementation (Figure 5.5).

5.3 DISCUSSION

In this study a variety of neuromuscular parameters were measured to determine whether short term Cr supplementation influences isometric elbow flexor contractile properties of older men. Creatine supplementation had no effect on

Figure 5.5: Submaximal and maximal IEMG for the Cr and P groups have been normalized to the maximal IEMG recorded for the pre fatigue MVC. ○ represent Cr, △ represent P, open symbols are pre, filled symbols are post, Standard errors of the mean (se) for maximal IEMG are upward bars, whereas (se) for submaximal IEMG are downward bars. There were no significant differences between groups on the pre and post test sessions. Submaximal IEMG increased significantly while maximal IEMG did not change over the duration of the fatigue protocol. * represents significant differences ($p \leq 0.05$) from pre fatigue.


130
120
110
100
90
80
70
60
50
40
30
20
10
0
IEMG (% Pre Fatigue)
Start
Mid
End
R1
R3
R5
R10
Time

MVC, muscle activation, stimulated tensions and times at baseline, or during a submaximal voluntary fatigue task, and recovery. Although, the older men had a 1.0 kg increase in body weight, there was no change in arm size as measured by anthropometry.

In agreement with previous studies, in which no change in body composition (Rawson & Clarkson 2000, Rawson et al. 1999), or thigh muscle volume (Bermon et al. 1998) were reported, no change in the measures of total arm area, muscle plus bone area, or skin plus subcutaneous tissue were observed in this study. The 1.0 kg increase in weight is consistent with studies of younger men (Terjung et al. 2000), and one study in older men (Rawson & Clarkson 2000). A significant increase in body weight is not a consistent finding in the few studies in older men (Rawson et al. 1999, Rawson & Clarkson 2000, Bermon et al. 1998). It is possible that differences exist between studies and age groups because of non-responders. Uptake and transport of Cr is mediated by insulin activity (Steenge et al. 1998) and is inversely related to the initial Cr content (Terjung et al. 2000) and these factors differ among individuals (Dolan et al. 1995). However, the literature suggests (Terjung et al. 2000) that an increase in weight is indicative of water retention related to an osmotic load caused by Cr retention (Ziegenfuss et al. 1998).

To elicit an increase in maximal voluntary force either muscle activation, or contractile tissue content need to be increased. In this study an accomodation session was incorporated to ensure that subjects were familiar and comfortable with the experimental procedures and that the task under investigation (isometric elbow

flexion) had been practiced prior to the pre test. Approximately one half of the subjects performed better subsequent to accomodation. Furthermore, the twitch interpolation technique was used to ensure consistency in subject effort and found that there was no difference in muscle activation between the pre and post tests for either the P or Cr groups. There was no change in maximal voluntary force following supplementation. The results from older men in the present study are similar to those of Rawson & Clarkson (2000), in that maximal isometric elbow flexion force in older men is not altered following Cr supplementation.

Slowing of twitch contraction and relaxation times have been reported for many aged skeletal muscles (Roos et al. 1997), but to date there has been no attempt to measure contractile properties following Cr supplementation in older men. In this study electrically stimulated twitch tensions were small compared with MVC measures (~ 6%), and thus as suggested by others (Edwards et al. 1977, Hanchard et al. 1998) may not be representative of the whole muscle properties, unless stimulated tests generate forces of 10% of MVC. Furthermore, twitches are less reliable (in this study intra class correlation range 0.83 - 0.86) and much more affected by fatigue (Edwards et al. 1977) than higher frequency responses (in this study 50 Hz intra class correlation was 0.95), and thus the tetanic responses are often preferred to provide consistent measures of contractile relaxation times, or rates in whole human muscle (Cooper et al. 1988, Hanchard et al. 1998, Wiles et al. 1979). In non fatigued muscle of older men 50 Hz TT, T½ and PRF did not change following Cr supplementation. Since stimulated contractile property

relaxation measures reflect calcium activity (Hunter et al. 1999), these results suggest that at least in the short term Cr does not alter Ca^{2+} activity.

The mechanism by which Cr could prolong time to fatigue is believed to be related to increased potential for ATP resynthesis through the creatine kinase reaction (Casey et al. 1996, Terjung et al. 2000). If this reaction can be altered through enhanced PCr stores, conceivably older adults would be able to sustain everyday activities for longer periods of time. Furthermore, since muscle relaxation is an important component of the proportion of energy required for muscle work (Bergstrom & Hultman 1988), fatigue could be prolonged by factors related to contractile speed. Indeed, an increase in the supply of ATP which is known to alter calcium kinetics at the level of the sarcoplasmic reticulum (Duke & Steele 1999), may decrease relaxation time (Van Leemputtee et al. 1999). However, results showed that muscle relaxation was not faster in older men following Cr supplementation and isometric elbow flexion endurance time was not prolonged. In addition, when younger men performed an identical fatigue task Cr supplementation did not increase endurance time or decrease muscle relaxation time (Jakobi et al. 2000). Although previous studies have not evaluated changes in relaxation rate in older men after Cr supplementation, studies of dynamic knee extension have shown increases in time to exhaustion of ~4% in older men (Rawson and Clarkson, 2000) and 30% in men and women (Smith et al. 1998). Comparing the results of fatigue studies are difficult because of task specificity. Not only does the rate of fatigue differ between dynamic and isometric movements, but

between different muscle groups, between maximal and submaximal tasks (Bigland-Ritchie et al. 1995) and possibly with gender (Terjung et al. 2000). Thus, it is unclear from the results of these few studies whether, or to what extent, Cr supplementation affects fatigue in a single limb task.

Besides changes in contractile activity, changes in muscle activation would also alter isometric endurance time. In this study muscle activation was assessed by the twitch interpolation technique and EMG. Previous studies of Cr supplementation in older men have not used these techniques in combination with a well established voluntary fatigue protocol (Bigland-Ritchie et al. 1986). Muscle activation, and maximal EMG remained near 100%, and the submaximal EMG increased over the fatigue task, both prior to and following supplementation. These results are consistent with the literature for this task (Bigland-Ritchie et al. 1986), but more importantly indicate that acute Cr supplementation in older men does not increase muscle activation which could prolong fatigue. There are a limited number of studies available on recovery from fatigue in older adults. Data from these 12 older adults indicate that in the ten minutes following a 50% submaximal voluntary isometric elbow flexion task, force and submaximal EMG do not recover to pre-fatigue levels. This lack of recovery was not influenced by five days of Cr supplementation.

In conclusion, short term Cr supplementation increases body weight but does not alter maximal strength, contractile properties, time to fatigue or recovery in older men. The 1.0 kg weight gain which was observed in these healthy older men is

similar to the values reported for younger men, which suggests that aged human muscle can Cr load. Following five days of supplementation this weight gain did not result in a change in any of the measures made in this study of muscle strength and fatigue. Perhaps the benefit of Cr supplementation may only occur in older men subsequent to long term supplementation.

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CHAPTER 6:

GENERAL DISCUSSION AND CONCLUSIONS

6.0 GENERAL DISCUSSION

This thesis was an investigation into chronic and acute factors which might affect the motor unit (MU), and influence muscle strength. In particular this thesis examined an aspect of endocrine function, the factor of age and the use of a nutritional 'supplement' on contractile characteristics, central activation, strength and MU firing rates in rested and fatigued states.

6.1 Addison's Disease

Summary: This is the first study to measure contractile properties, voluntary strength, fatigue and recovery in AD patients. Results indicated that conventionally treated middle-aged women with AD, compared to healthy age-matched controls, have slower contractile properties and decreased endurance time in a submaximal fatigue task. The neuromuscular measures which were utilized to assess central and peripheral factors of strength and fatigue suggest that there is an altered sense of effort with this disease which contributes to early task cessation. The maximal and submaximal IEMG in the AD group were substantially, and quickly, elevated compared to controls. It is likely that a portion of this augmented relative IEMG in the AD patients resulted from the increase in central activation which was seen during the first minute of the fatigue task. However, central activation per se may not fully account for the increase in relative IEMG during the fatigue task since it has been reported that an increase in surface IEMG during a fatiguing exercise can

occur due to potentiation in the compound muscle action potential (M-wave). Thus, this measure indirectly suggests that the action potential during fatigue might be altered (potentiated) more in the disease condition compared to healthy controls. Because Na^+ and K^+ electrolyte balance is regulated by the adrenal cortex steroids, it is possible that intramuscular concentrations of these ions are altered in this disease, and this affects membrane excitability. Furthermore, it has been shown that potentiation is greater in individuals who were sedentary, and the AD patients in this study were more inactive than the group of control subjects. These results indicate that the mechanical (contractile) aspect of the MU is altered in AD.

Limitation: The sample size may have been a concern in this study. Only nine women were investigated in each group. Thus, it is important not to make generalizations of these data to all AD patients. Furthermore, it is difficult to ascertain whether the changes observed were due to the disease, inactivity, conventional hormone replacement or lack of DHEA and its sulfate. However, it would be unethical to assess AD patients following a lapse in steroid replacement since glucocorticoids and mineralocorticoids are necessary for survival.

6.2 Agonist-Antagonist Muscle Alterations with Age

Summary: To produce a goal directed upper limb contraction the pattern of neuromuscular activity must be co-ordinated between the elbow flexors and extensors. If age-related alterations are dissimilar between the MUs of these muscles, there might be a decrease in the ability to produce effective force and control movement. In agreement with earlier work no age related slowing was

observed, only weakening in these two muscle groups, and the decrease in strength was similar in the agonist and antagonist muscles. While these data suggest that the mechanical component of the MU was altered similarly with age, this study further suggested that the excitation component seems to be affected differentially in these muscles. The total range of MU firing rates recorded in the biceps brachii and triceps brachii were similar in younger and older men. However, the MU histogram was shifted leftward in only the biceps with age, and the age-related decrease in MU firing rates at five target forces were greater in the biceps than the triceps. These data provide indirect evidence to support the suggestion that the projections from the corticospinal tract might alter the excitation component of the biceps more than the triceps in older men, however, this change in activation does not negatively affect force production per-se since age-related force loss was similar between these muscles.

Limitations: Elbow flexion strength is not only a product of the long and short heads of the biceps brachii, but also the brachialis, however, MU firing rates were not measured in the brachialis, nor the medial head of the triceps (elbow extensors). Thus, although a great many MUs were sampled they are not a complete representation of the entire flexor and extensor muscle compartments. As well the MUs recorded in this study are an average population response (cross sectional sample) The subject sample was small for contractile properties, voluntary strength, and central activation (6 younger and 6 older men).

6.3 Cr Supplementation in Younger Men

Summary: Acute Cr supplementation in younger men did not alter the mechanical component of the MU. The average weight gain in the Cr group of 1.0 kg is consistent with previous reports in the literature and suggests that the supplementation period was effective. Following a standard loading period, electrically stimulated contractile properties, strength, fatigue and recovery were not altered in the elbow flexors of younger men.

Limitations: Since Cr did not change the mechanical component of the MU it was not possible to assess the independent effect of this component on strength. Although the weight gain which was observed is typical of a successful loading paradigm no quantitative measure of muscle Cr content was made. The assumption of Cr uptake is founded entirely on reports in the literature that have related changes in body weight to measures of Cr uptake.

6.4 Cr Supplementation in Older Men

Summary: Following Cr supplementation the older men in this study gained ~ 1.0 kg weight, which is consistent with the literature for younger men, and greater than previous studies in older men. However, electrically stimulated contractile properties, strength, fatigue and recovery were not altered following acute Cr supplementation. This was the first study in older men to ensure that effort, assessed with the twitch interpolation technique, was similar between groups and sessions. Similar to younger men, the mechanical component of the MU is not altered in older men following short term Cr supplementation.

Limitations: The older men in this study were healthy, active, community living men, about 72 years of age. Perhaps supplementation was not successful because Cr content was not significantly lower in these older men compared to younger men. In other conditions that have low Cr content and poor muscle strength there is an increase in strength and exercise performance following supplementation (Terjung et al. 2000). Perhaps older men should have been investigated in this study. Furthermore, similar to the study of younger men absolute measures of Cr content would have provided a quantitative measure of the amount of creatine stored in the muscle.

6.5 FUTURE STUDIES

1. A double blind placebo-control clinical control study in Addison's Disease patients should be undertaken (placebo and DHEA) to investigate a large number of men and women, which would enable assessment of the multiple factors which might influence the neuromuscular results (age, gender, activity level and years of affliction with the disease). Furthermore, the intervention of DHEA will enable determination of whether the change in contractile properties is due to lack of DHEA, or conventional hormonal treatment. These patients should not have diabetes mellitus, hypertension, thyroid or pituitary problems, but should be undergoing standard hormonal replacement, and have recorded normal serum NA^+ and K^+ levels in the prior year. Intramuscular measures of these electrolytes should be made, and neural activity assessed with either compound muscle action

potentials or MU firing rates. These measures should be taken prior to, during, and following a fatigue task.

2. If recruitment occurs at lower thresholds in the triceps of older men, MU firing rates will not have to increase as rapidly to attain a given relative force level. Perhaps the MU firing rates in the triceps in this study were altered less with age because recruitment occurred at lower thresholds in this muscle of older men. Furthermore, if antagonist coactivation of the biceps during EE decreases more with age than antagonist activity of the triceps during EF, the biceps MU firing rates can be lower in older men, and still produce a similar relative force level as the triceps. Recruitment, and coactivity need to be assessed in the triceps and biceps of older and younger men. Furthermore, the optimal joint angle for the EF and EE might change with age, and if the EF are closer to an optimal joint angle than the EE of older men, the MU firing rates can be less in the biceps and still attain a similar relative force as the triceps. Finally, to substantiate the non-significant change in contractile properties, measurements of fibre type need to be made in the EF and EE of younger and older men.

3. Contrary to the vast majority of Cr literature on dynamic performance, no effect was observed in this study on isometric elbow flexion strength, fatigue and recovery. In order to substantiate these findings Cr content needs to be measured to ensure loading occurred. Furthermore, other performance measures (cycling, dynamic contractions) should be assessed along with isometric muscle strength and contractile properties during a high intensity fatigue task. Possibly Cr may prolong

time to fatigue during a maximal short duration isometric task during which anaerobic energy supply predominates.

4. Acute Cr supplementation should be investigated in very old and frail men. Perhaps Cr supplementation will alter the mechanical aspect of the MU if it has undergone extreme change. If frail men are investigated, Cr content of muscle should be measured prior to and following supplementation. As well, an overall performance test should be conducted (eg. timed up and go). The benefit of Cr in a frail population might arise through enhanced energy levels which will increase the amount of daily activity and intensity, and these will ultimately promote strength gains. Furthermore, five days of Cr supplementation may not be a sufficient amount of time to induce an effect; long term supplementation needs to be considered.

6.6 CONCLUSIONS

The models utilized in this thesis were employed to investigate acute and chronic adapted states. Results from this thesis affirm that when the mechanical (Addison's disease), and excitation components (ageing) of the MU are altered, strength and/or fatigue are affected. However, when the MU is not altered following acute Cr supplementation, neither strength nor fatigue are modified. Results from the EF and EE study of younger and older men indicate that ageing does not result in slowing of contractile properties in the upper limb, and changes in the mechanical and excitation components of the MU do not necessarily occur concomitantly (ie,

no change in contractile properties, decreased MU firing rates). Since Cr uptake and storage occurs in skeletal muscle it was hypothesized that supplementation would exert an effect upon the mechanical component of the MU in the EF. The lack of change in contractile properties whether perturbed with acute (Cr) or chronic (age) perturbations suggests that the mechanical aspect of the EF is resistant to change. Thus, it seems that the excitation component is more malleable than the mechanical component in the upper limb. Earlier studies of older and younger men have indicated that the mechanical component of the quadriceps changes independent of an alteration in the excitation component (Roos et al. 1999). In Study One contractile properties were slower in AD compared to controls, and interestingly the contractile times in the quadriceps of AD women who were ~ 50 years old were analogous to the healthy 80 year old men studied previously (Roos et al. 1999). Perhaps, and in contrast to the upper limb, the mechanical component is more malleable than the excitation component in the lower limb. This might be due to functional differences because the quadriceps supports the body (strength) but does not participate in complex movements, whereas the arms are involved in highly co-ordinated movements. It should not be surprising that the excitation component seems to regulate strength more in the upper body than the lower body since the motor homunculus conveys the concept that different parts of the body are represented according to their functional importance, and the upper limb occupies more area than the lower limb. Furthermore it is known that the corticospinal control of upper and lower limbs differs. To date, no studies have clearly addressed

how changes in the excitation and mechanical components of the MU in humans are regulated, but these four studies affirm that agonist-antagonist muscles in the upper limb do not change in a like manner, and furthermore alterations between the upper and lower body are dissimilar.

6.7 REFERENCES

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Appendix A

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Appendix B

From: **"Emma Ward" <eward@physoc.org>**
To: **<jakobi@julian.uwo.ca>**
Subject: **Re: copyright MS2021**
Date sent: **Tue, 9 Jan 2001 08:47:16 -0000**

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From: **"Essenpreis, Alice" <Essenpreis@Springer.de>**
To: **"'jakobi@julian.uwo.ca'" <jakobi@julian.uwo.ca>**
Subject: **AW: EJAp copyright**
Date sent: **Thu, 18 Jan 2001 09:31:27 +0100**

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Appendix D

Addison's Disease Study

Control Characteristics

SUBJECT CODE	TREATMENT	AGE years	HT cm	WT kg
1	Control	59	162	63
2	Control	60	168	58
3	Control	62	163	69
4	Control	50	153	61
5	Control	54	162	64
6	Control	55	161	68
7	Control	49	165	64
8	Control	53	168	72
9	Control	69	159	69
MEAN		**56 ± 2**	**163 ± 2**	**65 ± 2**

HT = height, WT = weight

Addison's Disease Study

Patient Characteristics

SUBJECT CODE	TREATMENT	AGE years	HT cm	WT kg
1	AD	53	158	73
2	AD	60	165	64
3	AD	50	160	81
4	AD	57	160	66
5	AD	42	168	66
6	AD	46	158	73
7	AD	47	165	105
8	AD	55	160	71
9	AD	51	165	59
MEAN		**51 ± 2**	**162 ± 1**	**73 ± 4**

HT = height, WT = weight

Addison's Disease Study

Control Characteristics

SUBJECT CODE	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)	TIME TO FATIGUE (min)	FORCE AT FATIGUE (%)
1	345	98	41	76	65	141	9	67
2	210	97	38	80	76	155	17	65
3	242	91	31	72	65	137	10	62
4	290	93	56	69	70	140	7	62
5	241	94	40	81	75	156	7	50
6	302	98	55	67	67	134	3	56
7	285	96	36	75	68	143	12	56
8	314	96	66	70	63	133	9	53
9	258	97	71	66	77	143	13	57
MEAN	**276 ± 14**	**96 ± 1**	**48 ± 5**	**73 ± 2**	**69 ± 2**	**142 ± 14**	**10 ± 1**	**58 ± 2**

MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time,

CD= contraction duration

Addison's Disease Study

Patient Characteristics

SUBJECT CODE	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)	TIME TO FATIGUE (min)	FORCE AT FATIGUE (%)
1	270	94	38	89	60	149	5	67
2	256	93	29	100	76	176	6	60
3	223	76	40	98	61	159	7	91
4	271	96	30	100	77	177	6	73
5	304	98	31	94	56	150	4	72
6	203	86	47	100	92	191	2	90
7	320	85	36	101	61	161	2	93
8	291	95	35	97	87	183	6	84
9	317	98	26	112	86	199	2	75
MEAN	**272 ± 14**	**89 ± 3**	**35 ± 2**	**99 ± 2**	**75 ± 5**	**174 ± 7**	**5 ± 1**	**79 ± 10**

MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time,

CD= contraction duration

Appendix E

Younger and Older Men Motor Unit Firing Rate Contractile Property Study

Subject Characteristics

SUBJECT CODE	Group	AGE years	HT cm	WT kg
1	Y	24	167	73
2	Y	24	180	89
3	Y	24	170	83
4	Y	24	175	64
5	Y	25	178	75
6	Y	25	173	74
MEAN		**24 ± 1**	**173 ± 6**	**77 ± 11**
1	O	89	180	80
2	O	84	173	73
3	O	81	171	82
4	O	82	173	86
5	O	79	155	80
6	O	82	168	67
MEAN		**83 ± 4**	**170 ± 9**	**80 ± 5**

Y = younger, O = older, HT = height, WT = weight

Younger and Older Men Motor Unit Firing Rate Contractile Property Study

Elbow Flexor Contractile Characteristics

SUBJECT CODE	Group	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)
1	Y	367	96	36	74	57	131
2	Y	342	99	30	70	74	145
3	Y	319	95	24	67	73	141
4	Y	369	97	18	73	70	143
5	Y	391	98	30	81	61	142
6	Y	325	98	29	69	66	135
MEAN		**357 ± 12**	**97 ± 1**	**28 ± 3**	**73 ± 2**	**67 ± 3**	**140 ± 2**
1	O	161	88	10	83	51	134
2	O	248	100	16	75	61	136
3	O	193	97	12	80	77	156
4	O	244	90	6	68	63	131
5	O	180	87	7	84	45	129
6	O	216	97	9	81	56	136
MEAN		**207 ± 17**	**94 ± 4**	**10 ± 2**	**75 ± 3**	**62 ± 4**	**138 ± 5**

Y = younger, O = older, MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time, CD= contraction duration

Younger and Older Men Motor Unit Firing Rate Contractile Property Study

Elbow Extensor Contractile Characteristics

SUBJECT CODE	Group	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)
1	Y	362	98	20	62	57	119
2	Y	355	97	19	62	55	117
3	Y	315	97	39	75	61	136
4	Y	241	96	20	63	70	132
5	Y	335	96	30	61	75	136
6	Y	323	98	29	65		
MEAN		**321 ± 21**	**97 ± 1**	**26 ± 4**	**65 ± 3**	**63 ± 4**	**128 ± 4**
1	O	177	95	8	67	63	131
2	O	174	98	9	65	57	122
3	O	177	98	9	66	58	124
4	O	195	99	14	51	70	121
5	O	146	98	11	66	64	130
6	O	188	98	12	63	59	119
MEAN		**173 ± 8**	**98 ± 1**	**10 ± 1**	**63 ± 3**	**63 ± 3**	**126 ± 2**

Y = younger, O = older, MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time, CD= contraction duration

Appendix F

Younger Creatine Study

Subject Characteristics and Anthropometry

SUBJECT CODE	GROUP	AGE years	HT cm	WT kg PRE	WT (kg) POST	TAA cm^2 PRE	TAA cm^2 POST	MBA cm^2 PRE	MBA cm^2 POST	SST cm^2 PRE	SST cm^2 POST
1	C	23	180	71	72	72	71	64.03	64	7	7
2	C	19	180	76	78	72	67	61	57	11	10
3	C	28	188	87	90	76	79	67	71	10	8
4	C	22	178	75	75	69	69	57	61	12	8
5	C	23	173	98	97	115	124	93	100	22	24
6	C	22	173	68	67	52	52	41	41	11	11
7	C	20	170	66	65	58	62	45	50	13	12
MEAN	**n = 7**	**22 ± 3**	**177 ± 6**	**77 ± 4**	**78 ± 4**	**73 ± 8**	**75 ± 9**	**61 ± 6**	**63 ± 6**	**12 ± 2**	**12 ± 2**

C = creatine, P = placebo, HT = height, WT = weight, TAA = total arm area, MBA = muscle plus bone area, SST = skin plus subcutaneous tissue area

Younger Creatine Study

Subjects Characteristics and Anthropometry

SUBJECT CODE	GROUP	AGE years	HT cm	WT kg PRE	WT (kg) POST	TAA cm^2 PRE	TAA cm^2 POST	MBA cm^2 PRE	MBA cm^2 POST	SST cm^2 PRE	SST cm^2 POST
8	P	23	183	74	74	67	65	58	56	9	8
9	P	23	163	64	64	72	72	57	57	14	14
10	P	22	173	101	100	118	121	112	113	6	8
11	P	20	183	88	89	76	81	59	58	17	24
12	P	22	178	72	72	58	58	52	52	6	6
13	P	21	170	69	69	60	60	46	47	15	13
14	P	22	185	74	75	65	67	55	56	9	10
MEAN	**n = 7**	**21 ± 6**	**176 ± 8**	**78 ± 5**	**78 ± 8**	**74 ± 8**	**76 ± 8**	**63 ± 8**	**63 ± 9**	**11 ± 2**	**12 ± 2**

C = creatine, P = placebo, HT = height, WT = weight, TAA = total arm area, MBA = muscle plus bone area, SST = skin plus subcutaneous tissue area

Younger Creatine

PRE Contractile Characteristics for Creatine and Placebo Subjects

SUBJECT CODE	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)	FATIGUE Time (min)	FORCE AT FATIGUE (%)
1	351	99	25	86	61	147	6	55
2	433	98	43	81	52	133	8	69
3	391	99	33	66	54	120	6	57
4	421	100	49	71	61	132	5	65
5	491	99	30	79	61	140	4	49
6	325	95	8	89	40	129	32	49
7	301	100	13	78	82	160	9	48
MEAN	**388 ± 25**	**98 ± 1**	**29 ± 15**	**78 ± 3**	**59 ± 5**	**137 ± 5**	**10 ± 4**	**56 ± 3**
8	414	100	14	64	76	140	8	67
9	336	100	23	76	60	136	7	53
10	449	98	35	64	56	126	12	53
11	474	98	24	71	63	135	15	53
12	360	100	23	74	68	148	28	82
13	387	98	20	85	45	116	10	46
14	335	98	6	83	54	153	18	64
MEAN	**393 ± 16**	**99 ± 1**	**21 ± 9**	**74 ± 4**	**60 ± 4**	**136 ± 5**	**14 ± 3**	**58 ± 5**

MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time, CD= contraction duration

Younger Creatine

POST Contractile Characteristics for Creatine and Placebo

SUBJECT CODE	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)	FATIGUE Time (min)	FORCE AT FATIGUE (%)
1	347	97	31	86	60	146	9	57
2	427	100	43	81	48	129	6	48
3	398	98	36	62	63	125	6	55
4	400	100	44	74	61	135	5	51
5	474	99	17	80	54	134	6	58
6	318	100	14	86	50	137	37	81
7	294	100	12	79	80	159	11	51
MEAN	**379 ± 23**	**99 ± 1**	**31 ± 13**	**78 ± 4**	**56 ± 3**	**134 ± 2**	**11 ± 4**	**57 ± 4**
8	445	100	17	68	68	137	7	50
9	357	98	15	71	63	134	5	42
10	445	99	38	72	52	124	13	56
11	465	99	29	74	66	141	17	59
12	349	100	31	80	72	152	32	99
13	428	100	24	79	65	144	10	56
14	352	100	9	89	57	146	23	62
MEAN	**390 ± 5**	**99 ± 1**	**23 ± 10**	**76 ± 3**	**63 ± 3**	**140 ± 3**	**15 ± 3**	**59 ± 7**

MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time, CD= contraction duration

Appendix G

Older Creatine

Characteristics and Anthropometry

SUBJECT CODE	GROUP	AGE (years)	HT (cm)	WT (kg) PRE	WT (kg) POST	TAA PRE	TAA Post	MBA PRE	MBA POST	SST PRE	SST POST
1	C	65	172	74	76	72	74	51	57	21	17
2	C	79	155	80	80	67	67	51	53	16	14
3	C	71	175	76	76	72	69	44	44	28	25
4	C	79	184	93	95	76	76	58	63	18	14
5	C	68	183	98	98	67	67	63	58	4	9
6	C	75	175	71	73	62	62	55	55	8	8
7	C	68	172	92	93	62	62	50	50	12	12
MEAN	**n = 7**	**72 ± 2**	**173 ± 4**	**83 ± 4**	**84 ± 4**	**68 ± 2**	**68 ± 2**	**53 ± 2**	**54 ± 2**	**15 ± 3**	**14 ± 2**
8	P	66	168	79	79	74	74	54	53	20	21
9	P	82	168	67	67	58	54	46	41	12	12
10	P	75	185	95	95	89	92	74	75	16	16
11	P	73	173	80	80	72	72	65	64	7	8
12	P	69	180	81	82	72	69	60	58	11	11
MEAN	**n = 5**	**73 ± 3**	**175 ± 4**	**81 ± 5**	**81 ± 5**	**73 ± 5**	**72 ± 6**	**60 ± 5**	**58 ± 6**	**13 ± 2**	**14 ± 2**

C = creatine, P = placebo, HT = height, WT = weight, TAA = total arm area, MBA = muscle plus bone area, SST = skin plus subcutaneous tissue area

Older Creatine

PRE Contractile Characteristics

SUBJECT CODE	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)	TIME TO FATIGUE (min)	FORCE AT FATIGUE (%)
1	345	97	19	78	54	133	29	56
2	180	85	7	84	45	129	32	90
3	179	99	18	64	65	128		
4	355	100	16	68	62	130	18	54
5	346	100	13	78	77	155	6	65
6	282	99	24	86	68	154	33	64
7	289	97	14	63	41	105	8	58
MEAN	**282 ± 28**	**96 ± 2**	**16 ± 2**	**73 ± 4**	**56 ± 4**	**129 ± 5**	**21 ± 5**	**65 ± 5**
8	215	88	15	79	83	162	31	50
9	216	97	9	81	56	136	22	71
10	416	97	9	71	65	136	6	52
11	247	96	17	58	83	141	37	68
12	291	96	17	77	62	139	18	73
MEAN	**277 ± 37**	**94 ± 1**	**13 ± 2**	**73 ± 4**	**67 ± 6**	**140 ± 7**	**23 ± 4**	**63 ± 5**

MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time,

CD= contraction duration

Older Creatine

POST Contractile Characterstics

SUBJECT CODE	MVC (N)	% ACTIVATION	PT (N)	TPT (ms)	HRT (ms)	CD (ms)	TIME TO FATIGUE (min)	FORCE AT FATIGUE (%)
1	300	92	23	70	50	120	31	65
2	196	95	7	84	43	127	29	86
3	168	64	17	58	71	128		
4	345	100	16	71	63	134	20	55
5	334	97	13	71	80	151	7	62
6	285	99	18	79	77	156	36	62
7	284	99	16	59	39	98	11	60
MEAN	**290 ± 22**	**97 ± 1**	**17 ± 2**	**69 ± 4**	**59 ± 7**	**128 ± 6**	**22 ± 5**	**65 ± 4**
8	222	88	16	78	81	159	24	58
9	262	97	9	71	56	127	30	67
10	374	96	12	72	64	136	6	52
11	248	98	20	67	72	140	35	69
12	281	95	12	72	64	136	16	66
MEAN	**277 ± 26**	**95 ± 2**	**14 ± 2**	**72 ± 3**	**68 ± 4**	**140 ± 5**	**22 ± 4**	**63 ± 4**

MVC = maximal voluntary contraction, PT= Peak Torque, TPT = time to peak tension, HRT = half relaxation time, CD= contraction duration