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Sex Differences in Central and Peripheral Factors of Skeletal Muscle Fatigue

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SEX DIFFERENCES IN CENTRAL AND PERIPHERAL FACTORS OF SKELETAL MUSCLE FATIGUE

A Thesis Presented to the Faculty of the

Graduate Program in Exercise and Sport Sciences at

Ithaca College

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

Sally A. Gore

August 2007

Ithaca College

Graduate Program in Exercise and Sport Sciences

Ithaca, NY

CERTIFICATE OF APPROVAL

_____

MASTER OF SCIENCE THESIS

_____

This is to certify that the Master of Science Thesis of

Sally A. Gore

submitted in partial fulfillment of the requirements for the degree of Master of Science in Exercise and Sport Sciences at Ithaca College has been approved:

Thesis Advisor: _____

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ABSTRACT

Women generally exhibit greater fatigue resistance than men, due in part to differences in muscle mass. Less muscle mass in women results in decreased oxygen demand and increased oxygen delivery at the same relative workload compared to men and yields greater endurance. The purpose of this study was to examine sex differences in muscle fatigue between male and female athletes matched for muscle mass. Twenty four males and females were tested and yielded eight male-female pairs matched for age, training history and thigh muscle volume. Thigh muscle volume was estimated via circumference and skinfold measurements using the truncated cone method. Mean difference in thigh muscle volume between subjects within each pair was 2.13 ml *(SD* = 2.25*)*. Strength was measured as maximum voluntary contraction (MVC), determined by superimposing an electrical stimulus at 3x threshold (26-pulse, 100-Hz, 250-ms train) while subjects performed a 5-second maximum isometric contraction on a Cybex® dynamometer. Central activation ratio (CAR = MVC / MVC + electrical stimulation) was calculated to insure maximum motor unit recruitment. Subjects then completed a fatigue protocol of intermittent, 5-second sustained isometric leg extension at 50% of initial MVC with alternating 5-second rest periods until exhaustion. At termination, a final 5-second MVC was performed with superimposed electrical stimulation and CAR was calculated to quantify the contribution of central factors to fatigue. Sex differences in time to fatigue, rate of fatigue, percent of initial strength at fatigue, and CAR were assessed with paired t-tests. There were no significant differences within matched pairs for time to fatigue, rate of fatigue, or percent of initial strength. There was no significant difference in CAR. The

results of this study suggest that the greater fatigue resistance typically observed in females is probably due to differences in muscle mass.

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Thanks to the members of the 2002-2003 Ithaca College Men's and Women's Crew Teams and their coaches, Dan and Becky Robinson, respectively. They were terrific subjects all along the way. Thanks to Mary Turner DePalma, Ph.D. and the students in her introductory psychology class who volunteered to be pilot subjects for the testing protocol.

As most of my data was collected after I had already vacated my apartment in Ithaca, I wish to thank Janet Wigglesworth for allowing me to stay in her home rent-free while she went off on sabbatical. This helped tremendously in the most practical sense.

Finally, thank you to all of the faculty members of the Graduate Program in Exercise and Sport Sciences at Ithaca College. Each one of them helped in some way to encourage my learning and interest in the field of exercise physiology, something that endures to this day, even as I spend my vocational hours as a medical librarian. My time in Ithaca, the friends I made, the education received in classes, and finally the completion of this thesis were all tremendous gifts and memorable in so many ways.

DEDICATION

In memory of my mom, who taught me the valuable lesson of finishing what you started. In honor of my dad, who ran long distances well before it was fashionable. And in humble gratitude to my spouse and life partner, Lynn Simmons, who kept our home in Maine in order while I went off to pursue the adventure of this degree. Her enduring support and love make most of who I am possible.

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CHAPTER 1

INTRODUCTION

Historically, restrictions were implemented to limit participation in various activities based upon presumed sex differences in muscle strength, endurance and fatigue. Oftentimes these assumptions were based solely upon anecdotal or other non-scientific evidence. In the realm of athletics for example, it was not until 1984 that women were allowed to participate in the Olympic marathon event. The only reasoning behind this exclusion was the unfounded belief that women were incapable of such feats of muscular endurance. The fact that members of the exercise science community adopted this view only served to bolster the claim.

Since the passage of Title IX in 1972 and the subsequent opportunities afforded girls and women to participate in athletics, the landscape has changed. The final match of the 1999 Women's World Cup between the United States and China garnered more than 40 million television viewers making it the most watched soccer match to date. Further, with more than 90,000 fans in attendance, it is the largest spectator event in the history of the Rose Bowl.

Similar changes have occurred in the perception of women in the workforce. Occupations once limited to males based primarily upon physical requirements are more accessible to women as the lack of sex differences in these realms is realized. It is naïve and unsupported to claim no differences exist between sexes in muscular strength and endurance, but a growing body of scientific literature seeks to clarify the issue.

Measuring Sex Differences

When studying sex differences several factors must be considered. Mere comparison of males and females based upon sex differences alone does not account for the plethora of factors that contribute to differences in strength and endurance. Despite this, much research on sex differences in skeletal muscle fatigue has not taken into account, nor controlled for factors such as age, anthropometrics, lean body mass, oxygen consumption relative to lean body mass, diet, testing time, training status, and menstrual status (Tarnopolsky, 1999). As any and all of these can affect outcome measures, the need to control for them in study design is imperative.

Measurement of muscular fatigue is typically determined as the change in maximal voluntary contraction (MVC) force over repeated or sustained contractions. A MVC assumes recruitment of all available motor units during contraction. Fatigue is defined as that point when MVC can no longer be maintained and/or produced. Therefore, an accurate assessment of fatigue must insure that the initial MVC recorded is a true measure of complete motor unit recruitment.

Other factors that contribute to individual differences in fatigue include training status and athletic participation history. Further, societal factors that promote gender differences in these areas can affect the outcome measures. For example, does societal encouragement of competition amongst males compared to that amongst females alter how an individual approaches a test for maximal effort? Do males and females differ in how they approach a time-oriented or endurance task? If so, then the resulting impact on MVC is a factor that must also be controlled.

Sex Differences in Muscle Fatigue

Muscle fatigue is generally defined as a decrease in or inability to sustain the maximum force-generating capacity of the muscle (Gandevia, 2001). In contrast, the ability to resist fatigue is termed muscular endurance. Skeletal muscle fatigue has been the subject of much study and more recently, sex differences in muscle fatigue examined (Hicks, Kent-Braun, & Ditor, 2001). The research represents a wide range of purpose, methodologies, and control variables with varied results about individual differences in fatigue.

In a review of sex differences in muscle fatigue, Hicks, Kent-Braun, & Ditor (2001) suggested that females may have greater resistance to fatigue than males. Since fatigue is a multi-factor event, the authors note that the exact mechanism(s) accounting for this difference is unclear. Still, they identify the four areas most studied to account for any differences to be (1) muscle mass, (2) substrate utilization, (3) muscle morphology, and (4) neuromuscular activation. Other areas of research have focused on the role of estrogen (Bunt, 1990; Hackney, 1999; Lebrun, McKenzie, Prior, & Taunton, 1995; McCracken, Ainsworth, & Hackney, 1994; Phillips, Gopinathan, Meehan, Bruce, & Woledge, 1993; Sarwar, Niclos, Beltran, & Rutherford, 1996), variations in perceived exertion (West, Hicks, Clements, & Dowling, 1995), and sociological factors as determinants of gender and sex difference (Hicks, Kent-Braun, & Ditor, 2001). However, most research considers multiple variables when examining the subject.

The majority of research on muscle fatigue focuses on peripheral factors such as those occurring within the muscle itself. Fatigue at this level is associated primarily with the neuromuscular junction, the cell membrane, or within excitation-contraction coupling.

Fatigue originating from some point in the central nervous system (central activation failure) has been studied much less and the overall contribution of central factors to muscular fatigue is controversial. However, such factors have been shown to account for up to 20% of total fatigue (Kent-Braun, 1999). Research examining sex differences in central fatigue factors is limited.

Statement of the Problem

The primary purpose of this study was to determine the magnitude of sex difference in fatigue when controlling for lean body mass (LBM), training status, training history and estrogen. Sex differences in muscle fatigue have been attributed to each of these variables, thus controlling for each in the research design presupposes that any remaining differences are due to some other variable(s).

A second purpose was to examine sex differences in central fatigue. Previous studies quantifying central fatigue used mixed-sex subject pools (Kent-Braun, 1999; Kent-Braun & Le Blanc, 1996; Latash, Yee, Orpett, Slingo & Nicholas, 1994; Stackhouse, Dean, Lee, & Binder-MacLeod, 2000); however the research designs did not specifically examine sex differences, nor did they control for the above-mentioned variables.

Hypothesis

Based on the research to date, there were two hypotheses for this study. First, it was hypothesized that there is no inherent sex difference in muscle fatigue. Sex differences in fatigue are generally attributed to differences in muscle mass, therefore,

matching males and females for muscle mass should eliminate the difference. Further, it was hypothesized that there are no sex differences in central nervous system contribution to muscle fatigue.

Significance of the Study

The results of this study will add to the current body of knowledge regarding sex differences in skeletal muscle fatigue, further differentiating between those differences that are physiologically based from those derived from sociological and/or historical bias. Scientific findings benefit female athletes in training and performance. Research opens doors for participation in events once and/or currently prohibited due to false or unproven assumptions regarding female physical limitations and capabilities.

Similarly, females seeking employment in physically demanding careers such as the military, police work or firefighting can successfully argue against gender discrimination when it is proven that no biological differences exist preventing them from effectively performing the requirements of these vocations. Size and strength demands may well exclude an individual from certain employment, due to his or her inability to perform specific work-related tasks, but societal and historical gender bias can be addressed and hopefully eliminated as research provides a clearer understanding of sex-related differences in physical capability.

CHAPTER 2

LITERATURE REVIEW

Introduction

Muscle fatigue is a multi-factor event defined as a decrease in or inability to sustain maximum force-generating capacity of the muscle. Resistance to fatigue enhances muscular endurance, therefore understanding fatigue has been the subject of much study.

Early studies that focused on muscle fatigue used male subjects only and, as such, were not generalizable to the entire population. A number of factors produced a change in this bias. The rising participation of females in athletics and physically demanding occupations, the growing number of female exercise physiologists, the awareness of a need for findings applicable and relative to females, and the general trend to include females subjects in scientific study are but a few. Further, this changing landscape produced an interest in identifying inherent biological (i.e., sex-related) differences in muscle fatigue, as opposed to those socially constructed (i.e., gender-related differences). As such, the body of literature continues to grow.

For the purpose of this study, the review of literature will highlight research on factors most closely associated with sex differences in skeletal muscle fatigue. These include muscle mass, substrate utilization, muscle morphology, and hormonal factors. Research addressing differences associated with neuromuscular factors, and particularly the contribution of central factors to muscle fatigue, will also be reviewed.

Muscle Mass

Of the factors highlighted in Hicks et al. (2001), the difference in muscle mass between sexes has garnered the most attention. Studies examining this difference state that greater muscle mass in males prompts several conditions leading to an increase in muscle fatigue. The assumption of similar specific tension in muscles between sexes results in an increased demand and delivery of oxygen to muscle. This causes a delay in clearance of metabolic byproducts that inhibits muscle function. Both of these are due in part to increased vascular occlusion in males. However, methodologies that compared males and females based on relative workloads had females performing less absolute work than males, which caused less vascular occlusion and yielded greater oxygen supply to the working muscle. Ultimately, females demonstrated slower time to fatigue (Hicks, Kent-Braun, & Ditor, 2001).

When muscle cross-sectional area (CSA) is correlated with force production, males display greater strength relative to CSA compared to females. Kanehisa, Okuyama, Ikegawa and Fukunaga (1996) examined sex differences in fatigability during repetitive maximal contractions. Torque output was expressed relative to CSA. Subjects performed 50 isokinetic maximal leg extensions and torque was recorded for each set of five contractions. Males produced significantly higher torque in all trials, but no sex difference existed when torque was expressed relative to CSA, or in percent decline in torque per CSA. The authors concluded that torque generation capacity in repeated maximal contractions was correlated to CSA regardless of sex.

In contrast, Maughan, Harmon, Leiper, Sale, and Delman (1986) compared fatigability in untrained males and females and found sex differences in both isometric

and dynamic exercise trials. Two protocols with different subject groups were used: (1) leg extension sustained at varying percent of maximal voluntary contraction (% MVC), and (2) elbow flexion to failure at varying % MVC. Males were stronger than females in all categories of absolute strength. There were sex differences in endurance capacity at 20% leg extension MVC with females exhibiting 29% longer time to fatigue, however this difference disappeared at higher intensities (50%, 80% MVC). Females also had longer time to fatigue during elbow flexion at 50%, 60%, and 70% of one repetition maximum (1 RM) but not at higher % MVC (80%, 90%). These results initially support the hypothesis that lower absolute force generated by females compared to males results in decreased vascular occlusion, increased blood flow to working muscles, and slower time to fatigue. However, the authors also note that in contrast to this notion, there was no significant correlation between endurance time at 20% MVC and absolute tension between males and females. Further, the brief contraction time associated with elbow flexion was not likely to compromise blood flow. These factors negate the assumption of vascular occlusion being the cause of the observed differences.

Substrate Utilization

In an earlier study of skeletal muscle fatigue, Komi and Karlsson (1978) examined differences in skeletal muscle characteristics, metabolic profiles and functional performance between male and female twins. Measurements were extensive and included anthropometrics, strength and power, oxygen consumption, EMG, muscle morphology, and substrate utilization. Relative to substrate utilization, males exhibited higher activity of glycolytic enzymes than females when performing maximal quadriceps extensions.

Expanding on these findings, Bell and Jacobs (1989) examined the effect of strength training on glycogen utilization during isokinetic leg extension in males and females. Groups of trained and untrained subjects performed three sets of 50 maximal isokinetic exercises. Muscle biopsies following the exercise yielded no significant difference in overall glycogen content between trained and untrained subjects nor between glycogen content of specific fiber types (slow twitch or fast twitch) of either group. The authors hypothesized that strength training would increase muscle fiber recruitment due to increased glycogen utilization. While this was not supported by their results, they did note a sex difference in glycogen utilization. Females both re-synthesized glycogen faster than males during the rest periods, and utilized more non-glycogen substrates than males.

Similarly, Froberg and Pederson (1984) noted differences in glycogen utilization when they examined endurance capacity between males and females matched for physical activity. At 80% maximal oxygen consumption ($VO_{2\ max}$) on a cycle ergometer, females demonstrated significantly greater endurance capacity compared to males (53.8 ± 12.7 minutes versus 36.8 ± 12.2 minutes, respectively). Further, mid-exercise and final respiratory exchange ratios (RER) were significantly lower for females suggesting a greater reliance on fat oxidation than glycogen stores. These differences were not seen at 90 % $VO_{2\ max}$. Concentration of blood lactate was also lower in females at 80% $VO_{2\ max}$ compared to males, but no differences were seen between males and females at 90% $VO_{2\ max}$. The authors concluded that the increased endurance in females was due to spared glycogen stores, and noted that this sparing was countered at high intensity when increased blood lactate concentration caused fatigue.

Hicks and McCartney (1996) compared fatigability in older males and females (age 60-80 years) and found females to display greater endurance than males in both voluntary and evoked contractions. It is important to note that this study was carried out on older adults, thus any hormonal factor, particularly the role of estrogen, was minimal as all females were post-menopausal and not undergoing hormone replacement therapy. All subjects performed work at intensities high enough to elicit similar occlusion of blood flow, thus differences in oxygen delivery to the working muscle due to differences in blood flow was not a factor. The authors attributed the differences in substrate utilization to varying muscle dimensions, primarily muscle length as opposed to cross-sectional area. Cross-sectional area does differ significantly in the elderly population.

Hunter and Enoka (2001) compared pressor response and muscle activation patterns of males and females during submaximal isometric contractions of the elbow flexor muscles. Females displayed a time to fatigue 118% longer than males (1,806 ± 239 vs. 829 ± 94 seconds) at contractions of 20% MVC. Similar relative reductions in MVC force were observed between sexes, however males produced greater MVC (393 ± 23 N vs. 177 ± 7 N) and thus sustained a greater target force during the fatigue protocol. No sex differences were observed in average rectified EMG at fatigue, but heart rate (HR) and mean arterial pressure (MAP) were less in females than males. Based on these findings, the authors concluded that sex difference in fatigability was explained by differences in absolute force and limited by mechanisms distal to the activation of the muscle.

Muscle Morphology

Previous studies of sex differences in muscle strength compared relative to absolute percentages of MVC. This results in a difference in the proportion of oxygen demand between sexes. Matching males and females for absolute strength, Fulco, Rock, Muza, Lammi, Cymerman, et al. (1999) minimized this difference. Males and females matched for absolute strength in adductor pollicis muscle performed static contractions of 50% MVC every 5 seconds until exhaustion. Maximal voluntary contraction force was measured at minute intervals during exercise, at exhaustion, and for each minute of a 3-minute passive recovery. The most notable difference between sexes was seen in the first minute of force production and the first minute of recovery, wherein females fatigued at a significantly slower rate and recovered at a faster rate than males. Noting that Type I oxidative fiber type make-up for the adductor pollicis muscle is similar in males and females (~80% slow twitch fibers), the authors suggested a difference in the distribution of slowly fatigable fast-twitch oxidative fibers between sexes, with females possessing a greater percentage.

Miller, MacDougall, Tarnopolsky, and Sale (1993) examined a variety of muscle parameters in both upper and lower limbs in males and females in an attempt to determine if sex differences in muscle strength were linked to biological limitations or to differences in physical activity. Males and females were matched for total body mass and physical activity, and measurements were made to determine motor unit number, size and activation, and voluntary strength of the elbow flexors and knee extensors. Results of strength measures showed males had greater MVC than females in both absolute measures and when expressed relative to lean body mass. Further, males had greater

twitch torque in elbow flexion. Females displayed greater muscle endurance than males at 60% 1RM of elbow flexion, but no differences were noted in leg extension. A significant correlation was seen between both elbow flexion and knee extension 1RM and cross-sectional area. In relation to this, the authors noted that females demonstrated greater non-contractile tissue proportions in leg (but not elbow) compared to males. Muscle biopsies showed no significant difference in fiber type distribution between sexes, but males did display larger fiber type size. Further, no differences were seen in the number of motor units recruited or in activity of recruitment during contraction.

Hormonal Factors

Studies have used several methods to control and/or account for circulating estrogen levels when examining sex differences related to hormonal factors. Some of these include comparisons between amenorrheic and eumenorrheic females, between pre- and post-menopausal females, between post-menopausal females undergoing hormone replacement therapy and those not, and between females using and not using oral contraceptives. Similarly, protocols were used that obtained variable measurements during different points of the menstrual cycle when levels were known to be higher or lower. Day counts from start of menses, basal temperature measurements, and blood samples for estrogen and progesterone have all served as indicators of cycle phase. Some of these are inherently more accurate than others and thus produce varying results.

There are a number of literature reviews examining the role of estrogen during exercises. Bunt (1990) reviewed the metabolic effects of estradiol on acute exercise and the effects of chronic exercise on estrogen status and related clinical issues. The authors

noted that examining the role of estrogen in vivo is problematic due to complexities of measuring hormone levels. Further, normal range of estradiol is wide and varies greatly both between and within individuals, and estrogen is countered by progesterone with wide variability. Generally though, estradiol levels are lower in early follicular phase and higher in luteal phase, and progesterone is higher during the luteal phase. The role of estradiol on lipid and carbohydrate metabolism is well established and occurs either directly via liver, muscle and adipose cells or indirectly via alterations in metabolic hormones such as insulin, glucagon, cortisol and growth hormone. These findings describe sex differences in aerobic activities, but offer little explanation regarding differences in muscle strength and fatigue.

Review of the few studies on muscle strength, muscle fatigue, and estrogen levels show conflicting results. For example, some show detrimental effects of progesterone during luteal phase (primarily an effect of increased muscle temperature) but others do not substantiate this claim. Differences in methodologies, particularly in measurement of estrogen and progesterone levels, may be a factor in these results.

Lebrum, McKenzie, Prior, and Taunton (1995) examined the effects of menstrual cycle phase on strength and endurance. These authors investigated the effects of menstrual cycle phase on (1) aerobic capacity, (2) anaerobic capacity, and (3) high-intensity endurance activity. This study used basal body temperature as a measure for increased progesterone concentrations, and validated with serum levels because a eumenorrheic cycle may occur without ovulation or normal luteal phase. Testing took place in early follicular (3-8 days) and mid-luteal (4-9 days post-ovulation) phases. Results showed no significant difference between phases for body composition,

expiratory ventilation, maximum heart rate, maximum respiratory exchange ratio, anaerobic speed, and isokinetic strength. A slight increase in $VO_{2\ max}$ (L/min) was seen during the follicular phase, but increased relative $VO_{2\ max}$ (L/min) was not seen. Conversely, a slight decrease in $VO_{2\ max}$ (L/min) occurred during the luteal phase.

To examine the effects of menstrual phase on muscle glycogen stores and rate of muscle fatigue, Hackney (1999) measured muscle glycogen levels at rest and in response to exercise in women under conditions of high and low circulating estrogen levels. The author hypothesized reduced utilization of glycogen during periods of high circulating levels of estrogen which was supported by the results. Subjects consisted of eight healthy, physically active, eumenorrheic women. Testing took place during the mid-follicular (low estrogen) and mid-luteal (high estrogen) phases. Daily oral temperatures were recorded for two months prior to the study to confirm cycle phase and blood samples analyzed to validate hormone levels. The exercise protocol consisted of ergometer cycling for 60 minutes at 70% $VO_{2\ max}$. Results showed progesterone and estrogen levels were significantly greater before and after exercise in the luteal phase compared to follicular phase. Resting glycogen levels were higher in the luteal phase. Glycogen levels decreased with exercise in both phases, but were greater during the follicular phases (46.4% decrease follicular phase; 21.2% luteal phase). These findings confirm previous research suggesting estrogen enhances the activity of lipoprotein lipase, increases circulating growth hormone levels, and decreases circulating insulin levels, the result of each being increased lipolysis.

Muscle fatigue is related to production and clearance rates of metabolic by-products, particularly lactate, thus studies examining the relationship between menstrual

cycle phase and blood lactate levels are warranted. The literature to date suggests that resting lipid oxidation and glycogen storage are higher in the luteal phase, and the corresponding higher level of circulating estrogen attributes to observed differences. Greater reliance upon fat oxidation and lower blood lactate level with activity during the luteal phase is expected, yet study results are contradictory.

McCracken, Ainsworth and Hackney (1994) examined differences in blood lactate levels in response to intensive running during mid-luteal and mid-follicular phases of menstrual cycle. Results of this study showed no changes in time to exhaustion or no differences in lactate levels with cycle phase. However, lactate levels during recovery were significantly different between cycle phases with lower levels during mid-luteal phase. These findings support the notion that menstrual cycle phase does affect lactate response to exercise. The exact mechanism for the difference is unclear, but the authors suggest they result from an estrogen-mediated enhanced lipid metabolism during the luteal phase.

Sarwar, Niclos, Beltran, and Rutherford (1996) examined the effect of menstrual cycle phase on muscular strength in muscle groups used in sporting events and activities of daily living. Measurements of MVC, relaxation times, force-frequency relationship and fatigue during leg extension and handgrip isometric contractions were taken weekly during early and mid-follicular, mid-cycle (ovulatory) and mid-late luteal cycle phases through two complete menstrual cycles. Results found strength differences were greatest and fatigue resistance lowest at mid luteal-ovulatory phase, not mid follicular-ovulatory phase when estrogen is highest. This finding raises the question as to the inhibitory role of progesterone. The lower fatigue resistance seen in luteal phase could be attributed to

increased body temperature associated with progesterone activity, thus increasing blood supply to muscle and reducing fatigue. Further, glycogen stores are greater during luteal phase, thus being a factor for increased endurance time.

Phillips, Gopinathan, Meehan, Bruck, and Woledge (1993) saw similar findings. They measured maximum and varying voluntary force production during isometric thumb adduction contractions. Force was measured on approximately days 1, 14, and 21 of one menstrual for seven eumenorrheic females. Results found force was significantly greater on day 14 than on day 1, whereas there was no difference between day 21 and day 1. The authors hypothesized that the increase in muscle strength occurred mid-cycle when estrogen levels were expected to be highest, though the exact mechanism for the differences remains unclear.

Central Factors in Muscle Fatigue

Muscle fatigue is defined as the inability to sustain maximal voluntary contraction and assumes the voluntary activation of all motoneurons (Gandevia, 2001). Imposing a supramaximal electrical stimulus during maximal voluntary contraction (twitch interpolation) has been shown in some cases, though, to increase contraction force; suggesting that the initial MVC did not recruit all motoneurons. The inability to voluntarily recruit all motor units, or a decrease in discharge rates, is attributed to some failure in central activation and the progressive decline in voluntary activation called “central fatigue” (Gandevia, 2001).

During submaximal contraction, an individual can counter fatigue by increasing motor unit recruitment. At maximal contraction this is not possible since all motor units

are already recruited. St. Clair Gibson, Lambert, and Noakes (2001) suggest that some changes in neural control mechanisms occur during maximal isometric contractions to account for alterations in force production. Specifically, they note changes in the firing patterns to motor units as a key factor. The role of the central nervous system in producing this change, or central fatigue, is believed to serve as a protective measure against fiber type damage from metabolic substrates.

Central fatigue is measured by examining changes in central activation. Central activation ratio (CAR) is determined by comparing fatigue from voluntary muscle contraction to that occurring from electrical stimulation. Central factors affect voluntary muscle contractions only, whereas peripheral factors affect both voluntary and electrically induced contractions (Latash et al., 1994).

Merton (1954) introduced the use of twitch interpolation to compare voluntary contraction with tetanus. He superimposed a single electrical stimulus to the ulnar nerve and compared the tension produced with that achieved during MVC of the adductor pollicis muscle. Results showed no evidence of the superimposed twitches during contraction, indicating no increased recruitment of motor units with electrical stimulation. Further, the decline in interpolated twitch amplitude was proportional to the decline in voluntary force. Based on these results, Merton concluded that muscle fatigue was due to peripheral factors (Merton, 1954).

Similarly, Bigland-Ritchie, Furbush, and Woods (1986) found that the reduction in force-generating capacity of the quadriceps femoris during submaximal contractions was due to peripheral factors alone. Ten subjects (male and female) performed a fatiguing protocol of repeated intermittent, sustained (6-second) contractions followed by rest (4-

second) periods until the target force of 50% MVC was no longer maintained. Measures of force production were taken under the following conditions; 1) superimposed trains on resting muscle, 2) single twitch on submaximally contracted muscle, 3) single twitch on relaxed muscle, and 4) MVC with superimposed twitch. The results of EMG measures displayed no superimposed twitches during the protocol and at the fatigue limit, suggesting voluntary effort was capable of complete motor unit recruitment. Based on these results, the researchers concluded that fatigue induced by intermittent submaximal contractions was due to peripheral factors alone and not inadequate motor drive.

In contrast, McKenzie, Bigland-Ritchie, Gorman, and Gandevia (1992) found support for central factors in their study examining fatigue in the elbow flexors of healthy males. Subjects underwent a fatiguing protocol of ten 10-second MVCs, each followed by 10-second relaxation. The regimen was repeated three times until a total of 30 MVCs had been performed. A superimposed twitch (100-ms, 15-20% above the voltage for maximal response) was applied at random during one of the first four MVCs and then again during one of the last four MVCs of each series. The mean “voluntary activation index” (the ratio of evoked potential during maximal contraction to that during a rested state) decreased significantly with fatigue. Based upon these results the researchers concluded that central and peripheral factors contributed to fatigue.

Kent-Braun and LeBlanc (1996) and Kent-Braun (1997) first proposed calculating the central activation ratio (CAR) as a means of quantifying central activation failure. The ratio represents the force associated with a maximal voluntary contraction related to the total force produced (stimulated plus voluntary forces). A ratio of 1.0 indicates

complete activation of all motor units whereas a CAR less than 1.0 denotes incomplete recruitment of all available motor units.

Kent-Braun (1999) measured central activation failure during sustained MVC of dorsiflexors in healthy males and females. Results showed a significant decline in CAR during the 4-minute fatigue protocol, as well as a decrease in MVC/tetanic force ratio. These results suggested that central factors contributed to some portion (~20%) of muscle fatigue.

Russ and Kent-Braun (2003) compared sex differences in skeletal muscle fatigue under two conditions: free-flow circulation and ischemia. Muscle fatigue of the dorsiflexor muscles was produced by intermittent maximal voluntary isometric contractions (MVIC). Subjects performed a series of 5-second MVICs with 5-second rests between contractions for 4 minutes (24 total contractions). Central and peripheral activation measurements were determined using CAR and compound muscle action potential (CMAP), respectively. Both males and females exhibited greater fatigue under ischemic versus free-flow conditions. This was attributed to greater reductions in CAR, CMAP and stimulated tetanic force in both sexes. Under conditions of normal blood flow, males experienced greater fatigue than females, indicated by greater decreases in MVIC and CAR. No sex differences were observed in stimulated tetanic force, low-frequency fatigue, or CMAP in either condition. The authors concluded the sex difference in fatigue is both dependant upon blood flow and related to a greater impairment of central activation in males than females.

Hunter, Butler, Todd, Gandevia and Taylor (2006) found no difference in supraspinal fatigue between males and females during sustained isometric MVCs of the

elbow flexor muscles. Supraspinal fatigue, a part of central fatigue, is associated with activity of the motor cortex. Fatigue develops at sites both proximal and distal to the motor cortex, thus the purpose of this study was to better determine the location of failure in males and females during intermittent muscle contraction. Supraspinal fatigue was measured by using transcranial magnetic stimulation (TMS) of the motor cortex while subjects performed MVCs. Subjects performed a series of six 22-second MVCs with a 10-second rest between each bout. TMS was delivered to the cortex at the beginning and end of each contraction. Both males and females experienced decline in torque during the fatigue protocol, with males exhibiting significantly greater absolute decline (65 ± 3% versus 52 ± 9%, $p < 0.05$). Voluntary activation was measured using the amount of torque responses to the stimuli. There was no sex difference in decline of voluntary activation. Based on their findings, the authors concluded the mechanism(s) for failure were associated with peripheral factors and thus not supraspinal fatigue.

Sex Differences in Neuromuscular Factors

To determine if fatigue is related to failure of neural activation or to factors within the muscle contractile apparatus itself, West, Hicks, Clements, and Dowling (1995) measured electromyogram (EMG) activity in males and females during varying intensities of forearm, wrist and hand flexion. Following assessment of MVC, subjects performed sustained, isometric contraction on a handgrip dynamometer at a predetermined intensity (30%, 50%, 75% MVC) until failure while EMG readings were recorded throughout the testing protocol. Visual cues as well as constant verbal feedback were employed to help subjects maintain effort. Fatigue was determined as that time

when the prescribed level of intensity could no longer be maintained. The results showed that females displayed longer time to fatigue at all three intensity levels but endurance time declined with increased intensity. Neuromuscular activity measured with EMG also increased with intensity, suggesting a relationship between the variables.

Semmler, Dutzscher, and Enoka (1999) found sex differences in muscle fatigue while seeking to identify the mechanisms responsible for the intensity-dependent effect of immobilization on endurance time of a fatiguing contraction. Following four weeks of immobilization of the non-dominant arm via fiberglass case, MVC and fatigue rates during isometric elbow flexion were measured. Fatigue was defined as the inability to sustain a contraction of 15% MVC for 3 seconds and surface EMG was used to measure muscle activity of biceps brachii, brachioradialis, and triceps brachii. A significant difference was seen between endurance time of sustained contraction immediately following four weeks of immobilization (220% increase) in seven of 12 subjects. Of these seven, six were female. Increased endurance time was related to short, alternating bursts of motor unit activity rather than the recruitment of additional motor units. An increase in discharge rate of recruited units might also explain the difference. Female subjects in this study were tested at the same points (different phases) in two consecutive menstrual cycles with no differences between tests, suggesting that hormone flux did not contribute to differences in the EMG pattern.

Hunter, Critchlow, Shin and Enoka (2004a) found no sex difference in time to fatigue in strength-matched males and females performing sustained submaximal contractions of the elbow flexor muscles (819 ± 306 vs. 864 ± 391 seconds). No sex differences were observed in mean arterial pressure (MAP), heart rate (HR), torque

fluctuations, or rating of perceived exertion (RPE). EMG activity increased for both sexes during the fatigue protocol, but was less for females than males. The authors proposed this difference in activity was explained by either less activation of muscles by the spinal cord during sustained contractions or more transient motor unit recruitment.

The same authors found males more fatigable than strength-matched females during intermittent submaximal contractions of the same muscle group (Hunter, Critchlow, Shin, & Enoka, 2004b). Time to task failure was longer for females than males (1,408 ± 1,133 vs. 513 ± 194 seconds), despite both performing the fatigue protocol at the same relative force (50% MVC). Unlike the previous findings, females in this study displayed a lesser increase in MAP, HR and RPE than males. As with the earlier study, females had a slower rise in EMG activity both during contractions and at the end of the fatigue protocol than males.

Hakkinen (1993) examined acute neuromuscular fatigue and short-term recovery patterns in males and females during and after a bout of strenuous heavy resistance exercise. Major findings of the study demonstrated that both males and females experienced considerable acute fatigue as a result of this type of exercise, though males exhibited larger decreases in maximum force production compared to females. At the same time, females exhibited faster recovery from fatigue. Differences in substrate use were likely not a factor because energy requirements were met via immediate sources, primarily the ATP-phosphocreatine pathway. The author attributed the difference in rate of fatigue to some aspect of neuromuscular function and contractile properties of muscle, though noted that the exact mechanism remains unknown.

Clark, Manini, Thé, Doldo, and Ploutz-Snyder (2003) evaluated endurance capacity in 10 males and 10 females during isometric versus isotonic contractions of the back extensor muscles. Additionally, the authors compared neuromuscular activation patterns and EMG changes during the fatigue protocol. Females showed longer time to task failure than males during isometric but not isotonic exercises. EMG data was similar for both groups, however differences in shifts in median frequency between sexes were noted. A difference was observed between males and females as related to specific muscle groups. The authors attributed the differences to type of contraction and frequency shifts in EMG, but not to changes in activation patterns.

Clark, Collier, Manini, and Ploutz-Snyder (2005) examined sex differences in fatigability and neuromuscular activation patterns of the quadriceps femoris. Subjects performed sustained knee extensions at 25% MVC under conditions of normal and occluded blood flow. EMG recordings for activity in the four different muscles of the quadriceps femoris group were measured during contractions to evaluate if differences exist. Females displayed greater fatigue resistance in non-occluded tasks, but not during occluded tasks. A difference in activation of the rectus femoris muscle was also detected, with females having greater relative activation than males at the time of fatigue. The findings further demonstrated the role of muscle blood flow and substrate metabolism in explaining the sex difference in muscle fatigue, but leave the issue of activation pattern differences in need of further study.

In studying differences in knee injury patterns between males and females, Pincivero, Gear, Sterner, and Karunakara (2000) examined sex differences in quadriceps work and fatigue rates during high-intensity isokinetic exercise. Male and female subjects

performed 30 maximal concentric contractions at a preset velocity, and quadriceps fatigue was measured as a decline in force production throughout the contractions. Results showed a greater reduction in work over time in males compared to females. The authors attributed the difference to reduced glycolytic capacity as well as lower level of neuromuscular control in females compared to males. While this data is concurrent with other findings, limitations in design including lack of data on training status, failure to match subjects on body mass, and limited time for familiarization with testing protocol on the isokinetic dynamometer, leave the results open to question.

Summary

The variety of research described demonstrates clearly that multiple factors contribute to muscle fatigue. In order to understand the proportionate contribution of each to the observed differences between males and females, control of as many factors as possible is required. Differences in LBM between males and females appears in the literature to account for the greatest percentage of difference observed, however limitations in study design leave this conclusion open for debate. Differences in substrate utilization, either inherent or due to differences between males and females in body type or muscle morphology, is also proposed as a factor for any observed difference. The suspected glycogen-sparing effect of estrogen in females is also not fully understood. Multiple adaptations occur with training including neuromuscular changes, changes to muscle morphology, and even psychological advantages. The purpose of this study then was to determine the magnitude of sex difference in fatigue when controlling for as many of these factors as possible, including lean body mass (LBM), training status, and training

history. Females' self-reporting of menstrual cycle activity also allowed estimating estrogen levels to correlate any possible hormonal contribution to differences observed.

CHAPTER 3

METHODS

Introduction

The purpose of this study was to determine the magnitude of sex difference in central and peripheral factors of skeletal muscle fatigue. Sex differences in muscle fatigue have been attributed to lean body mass, training status, training history and estrogen. Controlling for these variables in the research design presupposes that any remaining observed differences are due to some other variable(s).

Subjects

Subjects were recruited from the Ithaca College men's and women's varsity crew teams. Subjects were placed into male-female matched pairs based on training status and lean muscle volume of the thigh.

Training Status - All subjects were engaged in formal team training during the course of the study. The two teams trained together, providing subjects with similar training status and conditioning level.

Limb Volume - Sex difference in force production may be related to a difference in vascular occlusion during muscular contraction. The relationship between body size and force production varies, however studies controlling for these variables between sexes show no significant difference (Keller, 1989). Male and female subjects in the study were matched for lean muscle volume of the right quadriceps using the following formula (Tothill & Stewart, 2002):

$V_L = (A_1 + A_2) * h/2$, where

V_L = lean muscle volume of the thigh

A_1 = area at the proximal measure of the thigh

A_2 = area at the distal measure of the thigh

h = length of thigh between proximal and distal measurement points

All subjects signed an informed consent approved by the Ithaca College Review Board for Human Subjects Research (Appendix A). Completion of a Health History Questionnaire (Appendix B) prior to participation identified any risk factors that may lead to injury. Subjects possessing any such factors (e.g., orthopedic injury) were dismissed from the subject pool. A Training History Questionnaire ascertained both current and past physical activity level of subjects (Appendix C). Additionally, female subjects completed a Menstrual History Questionnaire to determine for each their respective point in the monthly menstrual cycle (Appendix D). This allowed approximation of estrogen level for each female subject during testing.

Testing Schedule

Subjects were tested in the laboratories of the Exercise and Sport Sciences Department at Ithaca College. The first day of testing included completion of the informed consent form, Training History and Health History Questionnaires. Female subjects also completed the Menstrual History Questionnaire at this time. Measurements to determine limb volume took place and a brief explanation of the testing protocol was given. Day two involved completion of the fatigue protocol.

Testing Procedure

Limb Volume - A common method of estimating thigh volume is with anthropometry using truncated cone geometry (Jones and Pearson, 1969). The theory is based upon several assumptions; 1) the thigh describes a truncated cone, 2) any superficial adipose tissue forms an annulus, and 3) the amount of inter-muscular fat and bone are negligible in determining muscle volume. Thus, circumference and limb length measures can be used as a non-invasive means of estimating LBM of the limb. Tothill and Stewart (2002) found the prediction of muscle volume using this methodology to be highly correlated with results obtained using magnetic resonance imaging.

Following this method, circumference measures were taken at three levels on the right upper thigh with the subject standing and weight evenly distributed between both legs. A constant-tension tape measure was used and the same tester took all measurements for all subjects.

The proximal site was defined as the highest horizontal circumference obtainable just below the gluteal fold. The distal site was 1 cm above the superior border of the patella. The third measure was taken at the point equidistant between the proximal and distal sites. This site also served as the reference point for the anterior skinfold. Femoral limb length was measured as the distance between the proximal and distal circumference sites, on the anterior aspect of the thigh.

Fatigue Protocol - The fatigue protocol took place on the second day of testing. Prior to testing, the respective menstrual cycle day was recorded for all female subjects based upon self-report. A Cybex® (Cybex Medical, Stoughton, MA) dynamometer

equipped with the NORM™ software system measured knee flexion and extension strength and fatigue.

Following a 5-minute warm-up of moderate intensity on a cycle ergometer, subjects were seated in the dynamometer and positioned per the manufacturer's instructions. The lower leg, thigh, pelvis and torso were stabilized by the force transducer pad, thigh strap and seat belt. Hips were flexed at approximately 85° and the knees flexed at approximately 90°. These angles were checked using a goniometer. The axis of the dynamometer was aligned with the lateral femoral condyle per manufacturer's instructions.

For familiarization purposes, subjects performed a 3 to 5-second isometric knee extension at a perceived effort of 50-75% maximal contraction. Any necessary corrections in position and form were done upon completion of this contraction.

The skin was cleaned with alcohol and two 5" x 7" reusable, self-adhering neurostimulation electrodes (PALS™, Axelgaard Manufacturing Co., Ltd., Fallbrook, CA) were placed on the quadriceps femoris; the anode over the rectus femoris and the cathode over the vastus medialis. A GRASS™ model S88K (Astro-Med, Inc., West Warwick, RI) square pulse stimulator with stimulus isolation unit was used to produce electrical stimulation. Research comparing stimulation of the muscle versus nerve for twitch interpolation showed no significant difference between protocols (Behm, St-Pierre, & Perez, 1996).

Additionally, research examining the use of single, double, and train stimuli has shown the use of trains to be more sensitive in determining the contribution of central factors to muscle fatigue using the central activation ratio (Kent-Braun & Le Blanc,

1996). Based on this, a 26-pulse, 100-Hz, 250-ms train was used to superimpose stimulation. Stimulation pulse was 600 μs and amplitude was set at three times the threshold value that created the smallest visible muscle contraction (Latash et al., 1994; Stackhouse et al., 2000).

To insure maximal motor unit recruitment during the MVC, an evoked potential protocol was used. Subjects performed a 5-second MVC during which time an electrical stimulus was superimposed. Figure 1a shows a sub-optimal MVC display. Figure 1b shows a true maximal effort.

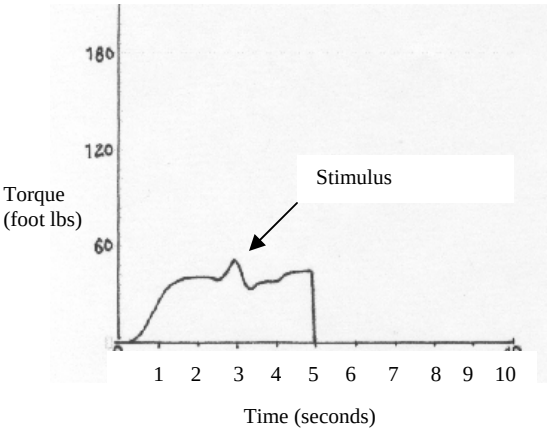

Figure 1a. Five-second sustained, voluntary isometric contraction with superimposed stimulus at 3-seconds showing less than maximum voluntary contraction.

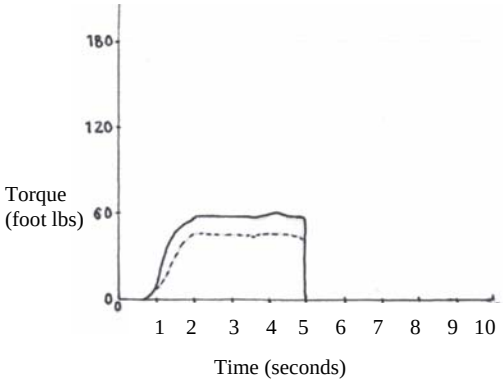

Figure 1b. Five-second sustained, voluntary isometric contraction with superimposed stimulus at 3 seconds, showing maximum voluntary contraction. Dotted line approximates sub-maximal effort performed in first trial (*Figure 1a*).

The CAR was calculated and a value of 1.0 insured that the MVC was a true maximal effort. The test was repeated if a subject produced a CAR less than 1.0, with a 3-minute rest period imposed between attempts. Subjects were permitted three attempts to

meet this criterion. Subjects unable to achieve a CAR of 1.0 were dismissed from the study.

Once the criterion was achieved, a target force of 50% of maximum force production was calculated based on the results from the MVC test. Following a rest period of 5 minutes, the subject performed a fatigue protocol that included repeated 5-second sustained contractions at the target force followed by 5-second relaxation (Figure 2). At each 6th contraction (1 minute), the subject performed a MVC. This protocol was based upon the work of Lewis and Fulco (1998), who proposed interspersing measures of MVC into the periods of static contraction to better measure rate of fatigue.

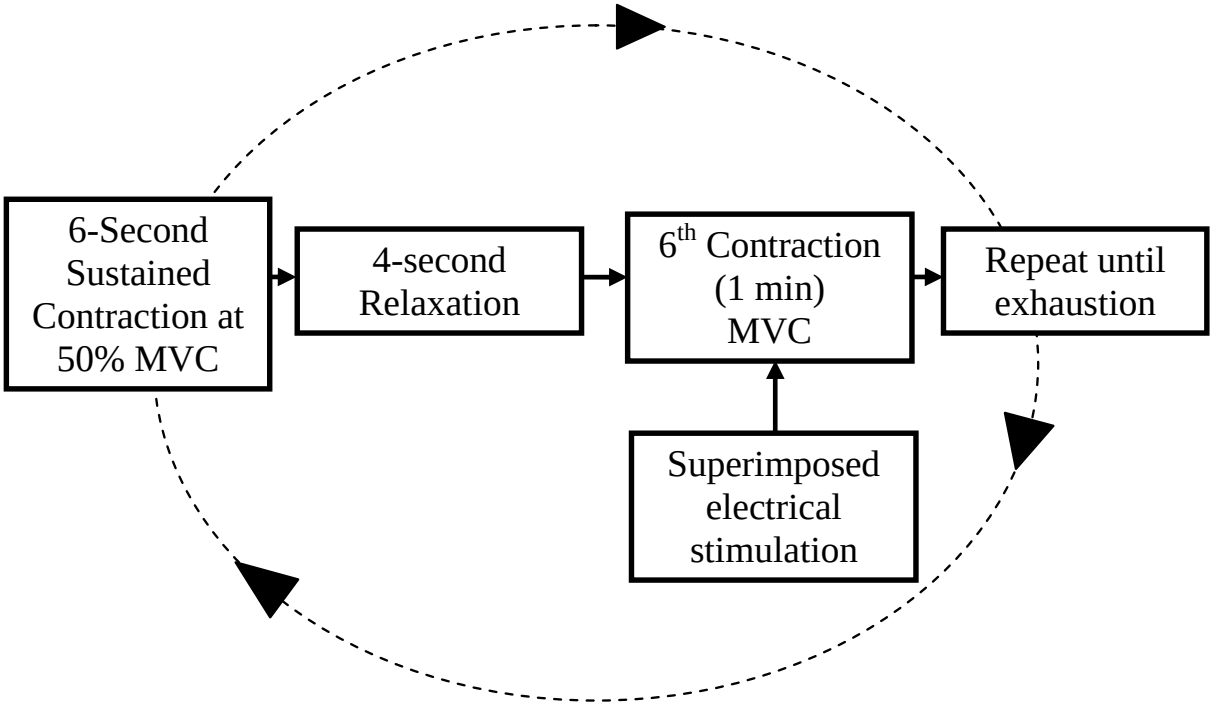

Figure 2. Fatigue protocol: Subjects repeated a cycle of 6-second sub-maximal contractions followed by 4-second rests until exhaustion. An electrical stimulation was superimposed during each minute to monitor rate of fatigue.

A visual display of force production and verbal encouragement by the tester throughout the fatigue protocol allowed the subjects to maintain the desired intensity and the timing for the contraction and rest. McNair, Depledge, Brettkelly, & Stanley (1996) showed that both male and female subjects displayed a 5% increase in maximal contraction when verbal encouragement was given compared to when it was not, emphasizing the importance of verbal cues in insuring MVC. Similarly, Kim & Kramer (1997) demonstrated that using visual feedback during isokinetic exercise elicits higher and more consistent MVC in male and female subjects.

The subjects repeated the pattern until exhaustion. Exhaustion was defined as the inability to maintain the desired sub-maximal intensity for the full 5-second contraction period for three consecutive contractions, or a MVC less than or equal to 50% of the initial MVC.

After reaching exhaustion, subjects performed a final 5-second sustained MVC with superimposed electrical stimulation. The CAR was calculated to determine the percentage of central factors contributing to fatigue. The decline in voluntary force compared to the decline in tetanic force is indicative of the level of central activation (Bigland-Ritchie, Jones, Hosking, & Edwards, 1978). A decrease in the ratio shows central activation failure while no change supports the conclusion that fatigue is due to peripheral factors.

Statistics and Data Analysis

Males and females were matched based on training status and lean muscle volume of the thigh. Matched pairs met a criterion of < 6.0% difference in thigh muscle volume

between sexes. Closely matching males and females for this variable reduced the variability in sex difference associated with strength (force production), vascular occlusion, muscle cross-sectional area, and subsequently muscle fatigue (Keller, 1989; Tarnopolsky, 1999).

Once matched pairs were established, sex differences in time to exhaustion (endurance), rate of fatigue (decline in MVC·minute^{-1}), contribution of central factors (CAR), and percent of initial strength at fatigue were calculated using dependent t-tests. Alpha was set at $p < .05$ for all calculations.

To determine the contribution of estrogen to fatigue resistance in females, time to fatigue and day of menstrual cycle were correlated in females. Females were ranked on a scale of 1 – 4 for estrogen level based upon the average number of days per cycle reported in the Menstrual History Questionnaire. Subjects who were tested within ± 2 days of their respective estrogen peak (mid-luteal phase) were given a ranking of 4. Those tested between 3-5 days from their peak were given a ranking of 3, between 6-10 days were given a ranking of 2, and those greater than 10 days were assigned a ranking of 1. Pearson product-moment correlation coefficients were calculated for menstrual phase versus fatigue and strength.

CHAPTER 4

RESULTS

Matching Protocol

Twenty four males and females were tested, yielding eight male-female pairs matched for age, training history and thigh muscle volume. Thigh muscle volume was estimated via circumference and skinfold measurements using the truncated cone method. Mean difference in thigh muscle volume between subjects within each pair was 2.13 ml *(SD* = 2.25*)*, and not significantly different *(p* < .05*)*.

Subject characteristics for variables measured per the matching protocol are shown in Table 1. There were no significant differences in age, years of training, and mean hours per training session between matched males and females. Females trained .82 more days per week compared to males (T (7) = -3.05*;* p < .05). There was no significant difference in thigh muscle volume or strength between matched males and females. Raw data for matched pairs in presented in Appendix E.

Fatigue Protocol

Results of the fatigue protocol are shown in Table 2. There was no significant difference in time to fatigue, rate of fatigue, or percent of initial strength. Central fatigue, measured by CAR, did not differ between males and females. Figure 3 illustrates time to fatigue (minutes) for each matched pair. While not statistically significant, the presence of outliers help to explain the large variability observed in this result.

Table 1

Variables for Matching Protocol Measured in Males and Females in Matched Pairs (N=8)

	Mean	SD	SEM	% Difference	p
Age (yrs)					
Male	20.00	1.31	.46	3.10	.22
Female	19.38	1.41	.50		
Training history (yrs)					
Male	6.00	3.42	1.21	4.00	.89
Female	6.25	3.28	1.16		
Training frequency (days/week)					
Male	5.06	.73	.26	13.95	.02*
Female	5.88	.44	.16		
Training frequency (hours/session)					
Male	2.06	.32	.11	.00	1.00
Female	2.06	.18	.06		
Thigh muscle volume (ml)					
Male	96.70	16.50	5.83	.89	.45
Female	95.84	16.22	5.74		

* Indicates a significant difference between sexes for training frequency (T (7) = -3.05; *p* = 02).

Table 2

Results of Fatigue Protocol for Males and Females in Matched Pairs (N = 8)

	Mean	SD	SEM	% Difference	p
Initial MVC (N·m)					
Male	218.29	38.24	13.52	4.27	.14
Female	208.97	29.49	10.43		
Final MVC (N·m)					
Male	134.56	24.47	8.65	11.59	.08
Female	118.97	20.18	7.14		
Time to Fatigue (minutes)					
Male	14.15	5.75	2.03	29.39	.09
Female	20.04	9.44	3.34		
Rate of fatigue (decline in MVC/min)					
Male	6.79	2.86	1.01	10.90	.19
Female	6.05	4.16	1.47		
Percent of initial strength at fatigue					
Male	62.15	7.36	2.60	7.08	.36
Female	57.75	9.24	3.27		
CAR at fatigue					
Male	0.91	.04	.02	3.19	.09
Female	0.94	.02	.01		

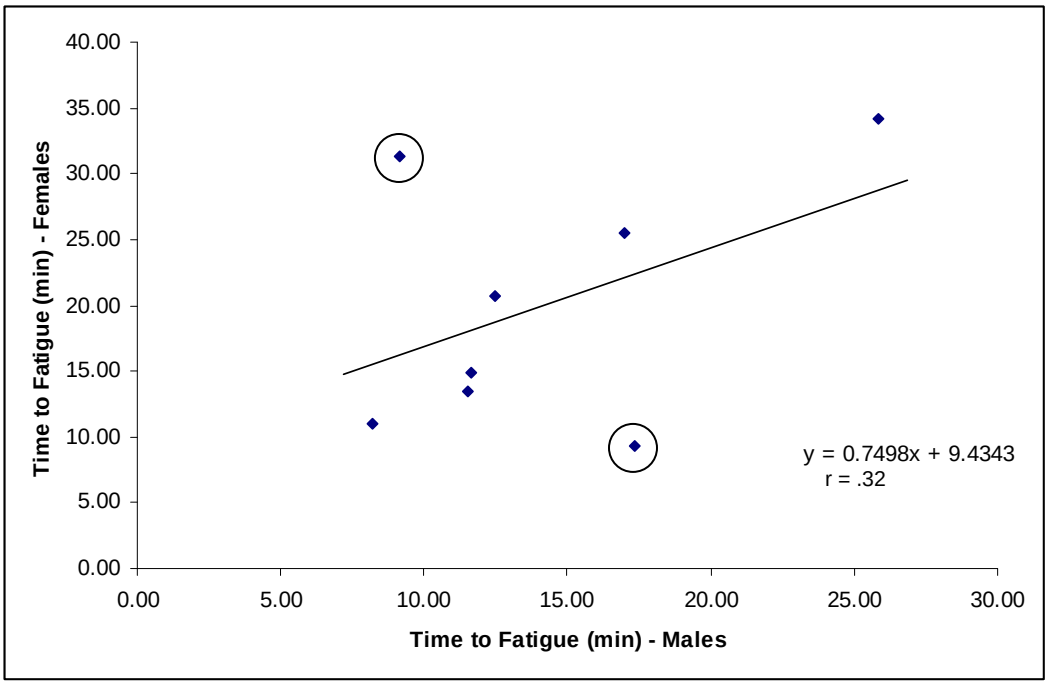

Figure 3. Time to fatigue (minutes) for matched pairs. Outliers that produced difference in measurement are circled.

Pearson product-moment correlation coefficients were calculated for menstrual phase (1 – 4) versus fatigue and strength to determine if hormone activity, presumably estrogen, influenced these measures. Correlations were not significant for either strength (r = .45) or fatigue (r = .32), suggesting that estrogen activity had nominal effect on these variables.

CHAPTER 5

DISCUSSION

Introduction

The majority of factors associated with fatigue occur within the periphery. Peripheral fatigue is related to mechanisms within the muscle itself, such as failure at the neuromuscular junction, the cell membrane or within excitation-contraction coupling. Controlling for certain peripheral-related factors was a goal of this study. Matching male and female subjects for thigh muscle volume, strength, and training history theoretically results in similar oxygen demand, enzymatic activity, and neuromuscular activation. The lack of significant difference observed in muscle fatigue between matched pairs in this study supports the hypothesis that there is no inherent sex difference in muscle fatigue.

Other factors associated with greater fatigue resistance evident in females compared to males such as neuromuscular activation, variations in perceived exertion, and the role of estrogen were also controlled. The results of this study support the hypothesis that after accounting for these factors, there is no sex difference in rate of muscle fatigue.

Muscle Mass

Greater fatigue resistance of females observed in research is most often attributed to a sex difference in muscle mass (Hicks, Kent-Braun, & Ditor, 2001). In the current study, matching males and females for thigh muscle volume produced no significant difference in strength between sexes. Additionally, subjects performed the fatigue

protocol at the same absolute force, presumably experiencing the same degree of vascular occlusion and subsequent oxygen delivery. No sex difference in muscle fatigue was observed between matched males and females.

The results of this study are consistent with some research findings (Clark et al., 2003; Clark et al., 2005; Kanehisa et al., 1996; Russ & Kent-Braun, 2003), despite differences in protocol, most notably the use of relative versus absolute force measurements. Other studies produced contrasting results (Fulco et al., 1999; Maughan et al., 1986). In particular, Fulco et al. (1999) matched males and females for absolute muscle strength yet still found females to have greater fatigue resistance. While their study used a similar fatiguing protocol, the difference in the muscle group(s) tested (adductor pollicis vs. quadriceps, respectively), the type of muscle contraction, and training status of subjects may account for the discrepancy.

Neuromuscular Activation

Neuromuscular adaptations to training vary in individuals but generally occur at a greater proportion early in training. Enhanced motor unit recruitment, increased frequency of activation, and improvement in the elastic properties of muscles account for the greater proportion of strength gains observed at the beginning of training. Thus, males and females matched for training history likely display similar contributions of neuromuscular factors to muscle contraction.

The work of West et al. (1995), Semmler et al. (1999), and Hakkinen et al. (1993) suggest that neuromuscular factors account for some degree of the sex difference

observed in muscle fatigue. Unlike this study, none of these tested highly trained individuals, leaving open the question of how training status affects these factors.

Training History

The matched pairs in this study showed no significant difference in years of training ($p < .05$). Females did train .22 more days/week than males and the contribution of this factor is unknown. The particular training regimens followed were similar as all were involved in the same sport (varsity crew) and trained with coaches using similar routines, exercises, and principles.

Neuromuscular adaptations, muscle fiber hypertrophy, and changes in enzymatic activity all occur with training, and in turn, affect muscle mass, muscle morphology, and substrate utilization. Controlling for differences in training history may account for the lack of sex difference observed in this study.

Effort

Variations in perceived exertion can account for differences in effort and thus differences in time to fatigue. Having subjects perform a maximum voluntary contraction with a superimposed stimulus insured the recruitment of all motor units and thus a “true” maximum effort. This measurement then served as a baseline for determining the percent of force to be used during the sustained contractions. Measured CAR values further suggest males and females were both putting forth maximum effort. Continuous visual feedback of force during these contractions also allowed subjects to maintain the desired output. The ability of subjects to successfully use the feedback system to maintain force

output was consistent with other research (Kim & Kramer, 1997; McNair et al., 1996; Pincivero, Coelho, & Erikson, 2000).

Muscle Morphology

Studies examining sex differences in muscle fiber type of athletes are limited; however, evidence exists suggesting a difference in fiber type, size and number between males and females (Always, Grumbt, Gonyea, & Stray-Gundersen, 1989; Miller et al., 1993). Larger muscle fibers, a greater proportion of type I fibers, and a greater number of fibers per cross sectional area in males are each proposed as determinants of observed strength differences between males and females. A greater number of type I fibers in males, or conversely a greater number of type II fibers in females, could result in differences in preferential metabolic pathways utilized during exercise, thus affecting time to fatigue. Muscle biopsies were not taken in this study and fiber type differences between males and females in the matched pairs is unknown.

Hormonal Factors

Estrogen levels were not measured directly, but the time in each female subject's menstrual cycle was noted and correlated with her respective endurance. No significant correlation was found between estimated estrogen level and muscle fatigue in females. These findings support those of others (Lebrun et al., 1995; McCracken et al., 1994).

Research supporting a relationship between estrogen level and muscle fatigue (Phillips et al., 1993; Sarwar et al., 1996) attributed greater fatigue resistance during the luteal phase to both an increase in glycogen stores and an increase in body temperature

(and thus blood supply to muscle). These results were not supported by the findings of this study, though it must be noted that the sample size and reliance upon subjects' personal reporting of cycle day are limiting factors.

Central Fatigue

It is argued that a proportion of muscle fatigue originates somewhere along the central nervous system (Gandevia, 2001). The percentage of central factors to the entire contribution is less than those observed in the periphery, but still measurable. Calculating the central activation ratio (CAR) is one means of quantifying this percentage (Kent-Braun, 1997; Kent-Braun & Le Blanc, 1996).

In contrast to others who suggest muscle fatigue is an exclusively peripheral event (Bigland-Ritchie et al., 1986; Merton, 1954), the results of this study found a decrease in central activation ratios from the initial strength measurement to post-fatigue protocol. Males showed a decrease from a mean of 1.0 during the initial strength measurement to .91 post-fatigue protocol. The same was demonstrated in females, with a decline from 1.0 to .94. These modest declines differ from other studies reporting up to 20% of contribution to fatigue coming from central (Kent-Braun, 1999), but remain consistent in their suggestion that some contribution is evident. In the present study, there was no sex difference in CAR, supporting the findings of others (Kent-Braun, 1999; Kent-Braun & Ng, 1999).

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The primary purpose of this study was to examine sex differences in muscle fatigue between males and females matched for lean muscle mass. Trained male and female athletes from the Ithaca College varsity men's and women's crew teams were matched for thigh muscle volume, strength, and training history *(N* = 8 pairs*)*. Thigh muscle volume was estimated via circumference and skinfold measurements using the truncated cone method. Maximum voluntary contraction (MVC) was determined by superimposing an electrical stimulus at three times threshold (26-pulse, 100-Hz, 250-ms train) while subjects performed a 5-second maximum isometric contraction on a Cybex® dynamometer. Central activation ratio (CAR = MVC / MVC + electrical stimulation) was calculated to insure maximum motor unit recruitment.

Subjects then completed a fatigue protocol of intermittent, 5-second sustained isometric leg extension at 50% of initial MVC with alternating 5-second rest periods until exhaustion. At termination, a final 5-second MVC was performed with superimposed electrical stimulation and CAR was calculated to quantify the contribution of central factors to fatigue. Sex differences in time to fatigue, rate of fatigue, percent of initial strength at fatigue, and CAR were assessed with paired t-tests.

There were no significant differences ($p < .05$) within matched pairs for time to fatigue, rate of fatigue, or percent of initial strength at fatigue. There was no significant difference in CAR.

Conclusions

The results of this study suggest that the greater fatigue resistance typically observed in females is probably due to differences in muscle mass. When matched for thigh volume, sex differences in strength and time to fatigue disappeared. This is likely the result of similar demands for oxygen at absolute workloads. Similar training histories also produce similar adaptations related to muscle strength, such as muscle hypertrophy and enzymatic activity, and subsequently account for a lack of sex difference in muscle fatigue.

Recommendations

Several factors related to the methodology of this study need to be addressed. As noted by Tarnopolsky (1999), controlling for variables of age, anthropometrics, lean body mass, diet, training history and menstrual status produces a much stronger study design. The use of matched-pairs was imperative to control for some of these variables often associated with research examining sex differences. This protocol proved limiting, however, to the final sample size. A larger sample would produce greater statistical power and is suggested for future studies.

The effect of estrogen level on muscle fatigue remains debatable and was not wholly accounted for in this study. While no significant correlation was found between estimated estrogen status and the variables, the use of a self-reporting method produces questionable results. Further studies examining the role of estrogen level on muscle fatigue need to be performed, utilizing more precise means of hormone level measurement.

Though no sex difference occurred related to central factors, one interesting finding was the modest decline in central activation ratio for these subjects compared to a greater decline observed in other studies (Kent-Braun, 1999; Kent-Braun & Le Blanc, 1996; Latash, Yee, Orpett, Slingo, & Nicholas, 1994; Stackhouse, Dean, Lee, & Binder-MacLeod, 2000). As these other studies used untrained individuals, it would be prudent to more closely examine the effect of training status on the contribution of central factors to fatigue.

Other studies suggest a sex difference in recovery from fatigue (Hakkinen, 1993). While this study did not address this question, a more complete understanding of how or if sex affects fatigue would be revealed by examining recovery from fatigue.

It appears that matching males and females on several variables related to training (e.g., number of years trained, current status, frequency of sessions, and time of sessions) proved to be a key factor related to the results found. Training influences muscle mass, strength, perceived exertion and effort. How and when these effects occur in the training cycle is an interesting question and touches upon the greater sociological questions and issues being addressed. Results from a training study, as opposed to cross-sectional data, would allow one to better observe any changes and perhaps answer some of these questions.

Finally, the subjects for this study were trained, highly motivated athletes. This was beneficial for matching purposes, but limits the ability to generalize the findings. Crew athletes in particular appear a unique group to work with, particularly in a study examining sex differences. However, some of the more general societal factors that promote differences suggested earlier do not apply to this group, especially the females.

They were highly inspired athletes and competitive with both their teammates and the males in the study. This proved a good thing for this individual study, but not necessarily for addressing the larger issue of perceived sex differences that attribute to societal bias.

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APPENDIX A

GENDER DIFFERENCES IN SKELETAL MUSCLE FATIGUE
INFORMED CONSENT DOCUMENT

1. **Purpose of the Study**
   The primary purpose of this study is to measure gender differences in muscle fatigue.

2. **Benefits of the Study**
   The results of the study will add to the current body of knowledge regarding gender differences in muscle fatigue, further differentiating between those differences that are physiologically based from those derived from sociological and/or historical bias.

3. **Your Participation Requirements**
   You will be asked to report to the exercise physiology lab at Ithaca College on two separate occasions for a total of approximately two hours (1 hour each session). You will complete an informed consent form, health history and training status questionnaires, and menstrual history questionnaire (females only). The first day of testing will include body composition assessment for the dominant leg consisting of 1) thigh girth measure, 2) measures of leg length, and 3) measures of lean body mass (LBM). LBM will be measured using bioelectrical impedance analysis. Two electrodes will be attached to your thigh and a small undetectable electric current applied. BIA is safe and it does not hurt. On the second day you will warm-up on a stationary cycle for 3-5 minutes, rest 10 minutes, then complete a test to determine maximal knee extension strength. This test requires you to perform 3 leg extensions as forcefully as you can. Following another rest period of 10 minutes, you will perform a fatiguing protocol of repeat 6-second sustained muscle contraction at 50% of maximal effort followed by 4-second relaxation. During each 6th contraction (1-minute intervals), you will perform a maximal knee extension. During the maximal contractions, your muscle will be electrically stimulated. This type of electrical stimulation is detectable but not painful or dangerous and is typically used in physical rehabilitation. The test will conclude when you are no longer able to maintain the prescribed effort (50% of maximal strength). Knee extension exercises will be performed on a strength device. For familiarization and warm-up purposes, you will perform 3-5 knee extensions at moderate intensity on the dynamometer prior to beginning the testing protocol. For females, this second test day will take place within 1-2 days of your mid-menstrual cycle point.

4. **Risks of Participation**
   Testing for body composition measures is non-invasive and involves no risk of pain or injury. Knee extension exercises will be performed at maximal and submaximal intensities and pose some risk for delayed onset muscle soreness and/or injury. The risk of injury will be minimized by requiring a warm-up prior to testing. Superimposing electrical stimulus to the muscle is an accepted tool in both research and clinical settings for measuring neuromuscular activity. The stimulus can cause slight discomfort to the subject, but minimal risk of injury. You may experience a temporary slight redness of your skin at the site of electrode placement. All testing will take place under the supervision of the principal investigator and a minimum of one assistant trained in the

testing procedures. In the event of an emergency, first aid and/or CPR will be administered as necessary by the certified investigator; Campus Safety will be summoned (x43333) and/or a physician as needed (x43177). For any non-emergent medical concerns, the subject will be directed to the Health Center.

1. **Withdrawal from the Study**
   You are free to withdraw from participation in this study at any time.

2. **Confidentiality**
   All data collected in this study will be number coded to insure confidentiality of your results. Subject names will not appear in any reports resulting from this study. Data will be presented in a group format.

I have read and understand the above document. I agree to participate in this study and realize that I can withdraw at any time. I also understand that I can and should address questions related to this study at any time to the researcher involved. I also verify that I am 18 years or older.

_____	_____	_____
Name of Subject (print or type)	Signature of Subject	Date

APPENDIX B

HEALTH HISTORY QUESTIONNAIRE

Name _____Date _____ DOB _____ Age (yrs)__

Gender (circle) M F Height (in) _____ Weight (lb) _____

Have you ever had any of the following conditions? (check those that apply)

	Date of Injury
☐ Knee injury	_____
☐ Joint problem(s)	_____
☐ Injury to lower extremities (legs)	_____
☐ Back injury	_____

Please answer Yes or No (circle) to the following and provide explanation where necessary.

Has a doctor ever told you not to participate in any physical activity? Yes No

Why? _____

Are you currently under any restrictions for physical activity? Yes No

Are you currently taking any prescriptions that could limit or affect physical activity? Yes No

Do you know of any other reason why you should not participate in physical activity? Yes No

Please explain: _____

APPENDIX C

TRAINING STATUS AND TRAINING HISTORY QUESTIONNAIRE

Name _____Date _____ DOB _____ Age (yrs)__

Gender (circle) M F Height (in) _____ Weight (lb) _____

Which foot do you kick with? (circle) R L

Select the activity code that best describes your ***current*** level of daily physical activity:

1. You participate regularly (5 or more days per week) in structured physical activity including, but not limited to, formal team practice.
2. You do not regularly participate (no more than 1 day per week) in any structured physical activity including leisure and/or recreational sports (walking, jogging, hiking, swimming, softball, tennis, etc.) or regular fitness workouts.

Activity Code _____

Answer the following questions to the nearest .1 years (if years are requested)

IF YOUR ACTIVITY CODE IS 1, ANSWER ONLY THIS SECTION:

How long have you been training (yrs)? _____ How frequently? (days/wk) _____

How long is a typical training session? _____

_____

Have you missed more than 3 consecutive months of training in the past 3 years? If yes, when and why? _____

_____

Briefly describe your training regimen including any and all sports / activities you participate in and/or train for _____

_____

IF YOUR ACTIVITY CODE IS 2, ANSWER ONLY THIS SECTION:

How long (yrs) have you maintained your current physical activity level? _____

Place a check next to any activities you have done in the past, the age(s) during which you did the activity, and the number of days per week you participated at that time:

Activity	Age(s)	Days/Week	Activity	Age(s)	Days/Week
Walk/jog/run			Racquetball		
Swimming			Tennis		
Cycling			Softball/Baseball		
Weight Training			Basketball		
Aerobics			Other (specify):		

APPENDIX D

MENSTRUAL HISTORY QUESTIONNAIRE

Name _____ Age _____ Date _____

Weight (lb) _____

Height (in) _____

How old were you when your menstrual cycle began? _____

When did your last period begin (month/day)? Approximate if necessary _____

Have you had any irregular menstrual cycles in the past? If yes, please explain.

_____

_____

Have you ever missed 3 periods in a row? Yes No

If yes, when? _____

Do you currently have an irregular cycle? If yes, please explain.

_____

_____

Is your menstrual cycle regulated by oral contraceptives? Yes No

If yes, how long have you been using oral contraceptives? _____

APPENDIX E

ORIGINAL DATA SET

Characteristics						
Pair	M ID	F ID	F Cycle	M Age	F Age	Diff
1	26	63	1	22	19	3
2	2	14	4	19	19	0
3	41	16	3	20	21	1
4	33	11	4	20	18	2
5	4	27	3	19	18	1
6	17	29	4	22	22	0
7	46	60	1	19	19	0
8	56	15	1	19	19	0
Mean			2.63	20.00	19.38	0.88
SD			1.41	1.31	1.41	1.13
SEM				0.46	0.50	0.40
Dep T-Test					0.22	

Characteristics						
Pair	M Train Hist (yrs)	F Train Hist (yrs)	Diff	M Freq (day/wk)	F Freq (day/wk)	Diff
1	4	7	3	5.5	6	0.5
2	6	1	5	5.5	6	0.5
3	8	6	2	4	6.5	2.5
4	7	6	1	6	6	0
5	10	12	2	5	5.5	0.5
6	10	3	7	5.5	6	0.5
7	1	7	6	4	5	1
8	2	8	6	5	6	1
Mean	6.00	6.25	4.00	5.06	5.88	0.81
SD	3.42	3.28	2.27	0.73	0.44	0.75
SEM	1.21	1.16	0.80	0.26	0.16	0.27
Dep T-Test		0.89			0.02	

Characteristics							
Pair	M Hrs Session	F Hrs Session	Diff	M TMM	F TMM	Diff	% Diff
1	2.5	2	0.5	108.08	107.15	0.93	0.86
2	2	2	0	102.29	103.25	0.96	0.92
3	2	2	0	120.45	113.46	6.99	5.80
4	1.5	2	0.5	110.25	114.09	3.84	3.37
5	2	2	0	89.55	89.81	0.26	0.29
6	2.5	2	0.5	76.89	75.13	1.76	2.29
7	2	2	0	92.59	90.92	1.67	1.80
8	2	2.5	0.5	73.51	72.90	0.61	0.83
Mean	2.06	2.06	0.25	96.70	95.84	2.13	2.02
SD	0.32	0.18	0.27	16.50	16.22	2.25	1.82
SEM	0.11	0.06	0.09	5.83	5.74	0.80	0.64
Dep T-Test		1.00			0.45		

Outcome Measures								
Pair	M ID	F ID	M Initial MVC	F Initial MVC	M Final MVC	F Final MVC	M Time	F Time
1	26	63	239.98	233.2007	149.14	123.379	11.50	13.50
2	2	14	230.49	237.2681	141.01	119.312	17.33	9.33
3	41	16	216.93	214.2192	142.36	146.428	17.00	25.50
4	33	11	145.07	159.9865	81.35	84.0607	25.83	34.16
5	4	27	260.32	230.4891	142.36	142.361	12.50	20.67
6	17	29	216.93	203.3727	142.36	119.312	11.67	14.83
7	46	60	253.54	223.71	159.99	101.686	8.17	11.00
8	56	15	183.04	169.4772	117.96	115.245	9.16	31.33
Mean			218.29	208.97	134.56	118.97	14.15	20.04
SD			38.24	29.49	24.47	20.18	5.75	9.44
SEM			13.52	10.43	8.65	7.14	2.03	3.34
Dep T-Test			0.14			0.08		0.09

Outcome Measures								
Pair	M ID	F ID	M Rate	F Rate	M % Init Str	F % Init Str	M CAR	F CAR
1	26	63	7.90	8.13	0.62	0.53	0.97	0.94
2	2	14	5.16	12.64	0.61	0.50	0.93	0.92
3	41	16	4.39	2.66	0.66	0.68	0.91	0.96
4	33	11	2.47	2.22	0.56	0.53	0.81	0.92
5	4	27	9.44	4.26	0.55	0.62	0.93	0.95
6	17	29	6.39	5.67	0.66	0.59	0.91	0.93
7	46	60	11.45	11.09	0.63	0.45	0.92	0.96
8	56	15	7.10	1.73	0.64	0.68	0.91	0.95
Mean			6.79	6.05	0.62	0.57	0.91	0.94
SD			2.86	4.16	0.04	0.08	0.04	0.02
SEM			1.01	1.47	0.01	0.03	0.02	0.01
Dep T-Test			0.62		0.19		0.09	

Key
F Cycle (scale) = Day of menstrual cycle on day of testing
Train Hist (yrs)= Number of years participating in structured physical activity
Freq (days) = Days per week currently training
Hrs Session = Average hours of training session
TMM (ml) = Thigh muscle mass
MVC (N) = Initial strength (MVC w/ elec stim)
Time (minutes) = Time to fatigue during protocol
Rate (deline in MVC / min) = Rate of fatigue (slope)
% Initial Strength = Final MVC / Initial MVC (Nm)
CAR (percent) = Central Activation Ratio (MVC / MVC + stim) at fatigue