Sex-Related Differences in Motor Unit Firing Rates and Action Potential Amplitudes of the First Dorsal Interosseous during High-, but not Low-Intensity Contractions

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Sex-Related Differences in Motor Unit Firing Rates and Action Potential Amplitudes of the First Dorsal Interosseous during High-, but not Low-Intensity Contractions

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ABSTRACT

Despite ample evidence that females are weaker and possess smaller muscle cross-sectional areas (CSAs) compared to males, it remains unclear if there are sex-related differences in the properties of motor units (MU). Eleven males (age=22±3 yrs) and twelve females (age=21±1yrs) performed isometric trapezoid muscle actions at 10% and 70% of maximal voluntary contraction (MVC). Surface electromyography signals were recorded and decomposed into MU action potential (AP) waveforms and firing instances. Average MUAP amplitudes ($\text{MUAP}_{\text{AMPS}}$), mean firing rates (MFRs), initial firing rates (IFRs) and recruitment thresholds (RT) were calculated for the 10% MVC while $\text{MUAP}_{\text{AMPS}}$, IFRs, and MFRs were regressed against RT for the 70% MVC. Ultrasonography was used to measure CSA of the first dorsal interosseous (FDI). Males had greater CSAs ($p<0.001$; males=2.34±0.28 cm^2, females=1.82±0.18 cm^2) and MVC strength ($p<0.001$; males=25.9±5.5 N, females=16.44±2.5 N). No differences existed for $\text{MUAP}_{\text{AMPS}}$, IFRs, MFRs, or RTs ($p>0.05$) during the 10% MVC. For the 70% MVC, the y-intercepts from the $\text{MUAP}_{\text{AMPS}}$ vs. RT relationships were greater ($p<0.05$) for the males (males=-0.19±0.53mV; females=-0.78±0.75 mV) while the inverse was true for the MFR vs. RT relationships (males=31.55±6.92 pps, females=38.65±6.71 pps) with no differences ($p>0.05$) in the slopes. Therefore, smaller CSAs and weaker MVCs are likely the result of smaller higher-threshold MUs for the females.

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TABLE OF CONTENTS

ABSTRACT ....**III**

ACKNOWLEDGMENTS... **IV**

TABLE OF CONTENTS... **V**

1. REVIEW OF LITERATURE **6**

1.1 MOTOR UNIT CONTROL AND RECRUITMENT 6
1.2 MU FIRING RATE BEHAVIOR AND AMPLITUDES 7
1.3 SEX RELATED DIFFERENCES IN STRENGHT AND MHC 13
1.4 MENSTRUAL CYCLE AND THE NEUROMUSCULAR SYSTEM 16
1.5 SEX HORMONES AND THE MOTORNEURON 17
1.6 NEUROMUSCULAR FATIGUE 20
1. REFERENCES 22

2.0 SEX RELATED DIFFERENCES IN MOTOR UNIT FIRING RATES AND ACTION POTENTIAL AMPLITUDES OF THE FIRST DORSAL INTEROSSEOUS DURING HIGH-, BUT NOT LOW-INTENSITY CONTRACTIONS..**29**

2.1 INTRODUCTION 29
2.2 METHODS 31
2.3 RESULTS 37
2.4 DISCUSSION 40
2.5 REFERENCES 46
2.6 TABLES AND FIGURES 49

1.0 REVIEW OF LITERATURE

1.1 MOTOR-UNIT CONTROL AND RECRUITMENT

The control of human movement is determined by motor unit (MU) recruitment and activation. Early work by Henneman adapted the size principle, which states that under load, MU are recruited from smallest to largest (Henneman 1957). Henneman (1957) excited the lumbar ventral roots of decerebrated cats through electrical stimulation of the dorsal root. Progressive stimulation was then applied to elicit two responses, a synchronous discharge of the motor neuron and a rhythmical firing. The second phase of the reflex was analyzed using a low power microscope and impulses of single motor neuron discharges were assessed. The amplitudes of these motor units ($MUAP_{AMP}$) differed according to the diameter of the fibers. In other words, as electrical stimulation increased, larger motor neurons were recruited. Therefore, Henneman concluded that the participation of a motor neuron is dictated by the size of the motorneuron (Henneman et al. 1965). In a follow-up study, Hennemen et al, (1965) concluded that muscles of the medial gastrocnemius and soleus of a cat follow this same “size principle”, and the maximal tension or size of the MU is directly related to the diameter of the MU axon and its conduction velocity. This construct is also present in fatigue, where MUs that generate the largest forces are subject to fatigue at high rates of stimulation (Hunter and Enoka 2001; Hunter 2016a). Therefore, motor neurons are recruited on a linear continuum, where smaller MUs are recruited initially with a high discharge rate, and lower amplitudes, while larger MUs are recruited at higher thresholds, possess slower firing rates and larger amplitudes.

This principle implies that all smaller MUs in the pool are discharging concurrently with larger, later recruited MUs. Later, Milner-Brown et al (1973a) described the orderly recruitment of MUs will vary linearly as a function of voluntary force at recruitment in humans. The findings note that earlier recruited MU had a tendency to contract more slowly than those recruited at higher force levels. The Henneman size principle, therefore, has been observed in a number of muscle groups and under a number of contraction intensities (De Luca and Hostage 2010). Recruitment thresholds of these MU's may adapt due to fatigue (Stock et al. 2012; Dimmick et al. 2018) aging (Miller et al. 2017, 2019; Sterczala et al. 2018), or training intervention (Herda et al. 2015), however, the size principle remains unaltered.

1.2 FIRING RATE BEHAVIOR AND AMPLITUDES

Firing rate behavior of the motor neuron has been under investigation for decades, beginning with the study of firing rate behavior in anesthetized cats (Eccles and Lundberg 1958; Kernell 1965). Eccles et al. (1958) and Kernell et al (1965) reported that when electrically stimulated, higher threshold or larger diameter motor neurons exhibited a shorter afterhyperpolization and greater firing rates than lower threshold or smaller diameter motorneurons. These theories were later examined in human models by a number of laboratories, and have reported that higher-threshold MUs have lower firing rates than lower threshold MUs during greater levels of excitation (Freund et al. 1975; Masakado et al. 1991; Kamen et al. 1995; Holobar et al. 2009; De Luca and Hostage 2010). De Luca and Hostage (2010) later provide justification for these theories in indicating the inverse relationship that exists between mean firing rate (MFR) and recruitment threshold (RT) at any given force level.

It is well established that a relationship exists between MFR and RT during voluntary contractions, termed the “onion-skin scheme” by De Luca and Erim (1994). Deluca and Hostage (2010) later recorded MU of the first dorsal interosseous, vastus lateralis and tibalias anterior and examined MFR regressed against their individual RTs to yield slope and y-intercepts values. These slope and y-intercept values were then used to compare and contrast MFR vs RT of different muscle groups and contraction intensities. Results of De Luca and Hostage (2010) exhibit differences in maximal recruitment threshold values for the first dorsal interossious (FDI), tibialias anterior (TA) and vastus lateralis (VL). The FDI observed the lowest recruitment threshold at 67%, followed by the TA at 90% and the VL at 95%. This theory maintains that the level of excitation to the motorneuron pool will dictate the “operating point” at which the motorneuron responds to voluntary control (De Luca and Hostage 2010).

EMG from the surface of the skin allows for a global perspective of the muscle, rather than single MUs analysis through intramuscular EMG (MU behavior for the surface of the skin via EMG is often debated with the use of intramuscular and single MU analysis (Enoka 2019). Del Vecchio and Farina (2017) reported weak correlations between RT and MUAP (using EMG RMS), however differences in statistical procedures in the handling and classification of MUs, as well as decomposition methodologies present differences in outcomes. Del Vecchio and Farina (Del Vecchio et al. 2017) used three to six channels, and manually selected each $MUAP_{AMP}$ and motor unit root mean square was derived. The slope of the regression from EMG variables and joint torque was then correlated with the slope of the regression lines between MU variables and RTs. Furthermore, all subjects and contraction intensities were collapsed and analyzed, rather than assessing by individual subjects and contraction intensities

Decomposition is a common technique to analyze EMG from the surface of the skin to determine MU behavior (Beck et al. 2011; Colquhoun et al. 2018b; Dimmick et al. 2018; Enoka 2019; Miller et al. 2019) . Recently, Colquhoun et al (2018b) quantified intra and inter-day reliability of the slopes and y-intercepts of the Delsys PDIII decomposition algorithm (PDIII) and found ICC's between 0.766-0.919 for the slopes and 0.780-0.927 for the y-intercepts. These findings provide information for the PDIII system as a reliable method for assessing changes in firing rate behavior and excitation to the motorneuron pool during an isometric contraction at differing intensities (Colquhoun et al. 2018b).

A strong inverse relationship exists between $\mathrm{MUAP}_{\mathrm{AMP}}$ and RT in active human muscle(Milner-Brown and Stein 1975) which is in agreement with the size principle. Milner-Brown et al (1975) observed a strong relationship between MUAP amplitude using Intramuscular and surface electrodes. Analysis of MUs has traditionally used spike trigger average to serve as triggers for the EMG signal for a given epoch, which is then averaged to estimate the peak-to-peak $\mathrm{MUAP}_{\mathrm{AMP}}$. The surface sensor collects on 4 unique EMG channels, which are then decomposed to yield individual action potential trains and firing instances for each individual MU. Waveforms are then calculated through average of the peak-to-peak $\mathrm{MUAP}_{\mathrm{AMPs}}$ (Hu et al. 2013a).

Alternate methodologies in surface EMG decomposition technology allow for the decomposition of signals MUAP single firing instances and their corresponding amplitudes (De Luca et al. 2006; Chang et al. 2008; Nawab et al. 2010). Validity of these measurements including differences in the PDIII software is of interest. A recent paper by Enoka (2019) outlines the differences in decomposition methods for analyzing multiple MUs and their individual firing times. Enoka (2019) suggests the use of other validation tools to further analyze the firing behavior of the decomposition system, including oscillations during steady force, and rate coding characteristics.

Collapsing MUs across contraction intensities and subjects is a technique used by various laboratories (Farina et al. 2009, 2010, 2014; Del Vecchio et al. 2017). Using this technique, differences in firing rate and amplitude behavior are lost in the sample due to an averaging effect of the high and low intensity contractions across multiple subjects. Investigations that separately analyze contractions on a subject by subject basis find strong correlations of the MUAP vs RT relationships (Kamen and Knight 2004; Beck et al. 2011; Stock et al. 2012; Pope et al. 2016; Colquhoun et al. 2018a)

Regression analysis of MU behavior through MUAP vs RT and MFR vs RT has allowed the tracking of MU through intervention or disease states, in the case of atrophy, hypertrophy or adaptation to training (Herda et al. 2015, 2018; Trevino et al. 2016; Miller et al. 2017; Sterczala et al. 2018; Dimmick et al. 2018). Statistical comparison of the slopes would indicate change in the MU size at a given RT, and change in the y-intercept value would indicate a change in the lowest observed MU or initial RT. Pope et al, (2016) analyzed the size of $MUAP_{AMP}$ and muscle cross-sectional area (size) of the vastus lateralis to determine the effect of an 8 week resistance training intervention. Subjects were trained three times per week for 8 weeks, and assessed pre and post training. Outcomes of the study included a linear relationship between $MUAP_{AMP}$ and RT, to analyze differences in pre and post training. An exponential fit to the data was more appropriate post training intervention (Pope et al. 2016), because MU of the highest recruitment threshold exhibited greater $MUAP_{AMP}$, while the lower threshold MU remained similar to pre-training values. The authors conclude that these changes were due to hypertrophy of the highest threshold MUs with little changes in the low threshold MUs, with marked increases beginning above 30% MVC. There were no changes in slopes of the lower contraction intensities, however the high contraction intensities observed an increase in slope values. Notably, these increases in slope values were highly correlated (r^2= 0.836) with increases in the muscle cross-sectional area of the vastus lateralis muscle, further pointing to overall muscle hypertrophy caused by resistance training. Therefore, changes in slope values of the MUAP_{AMP} vs RT relationships after training may provide a non-invasive measure of recruitment threshold specific hypertrophy, or in this case, muscle hypertrophy as well as MU specific hypertrophy of the type II muscle fiber types.

A direct relationship is also hypothesized between fiber-type composition (myosin heavy chain [MHC]) and MFR vs RT relationships (Beck et al. 2007; De Luca and Contessa 2012; Trevino et al. 2016). These relationships are supported by a number of studies involving differing training backgrounds and controls. De Luca et al (1982) first examined the regression coefficients of the MFR vs RT of detected MU of the first dorsal interosseous and deltoids in Olympic caliber weightlifters, swimmers and concert pianists compared to healthy control subjects during high force-level contractions. De Luca et al (2012) hypothesized that training and genetic factors effecting muscle fiber type may account for differences in MFR vs RT relationships between subjects. Additional evidence to support this theory was later provided by several studies (Herda et al. 2015, 2018, 2019; Trevino et al. 2016, 2018). Herda et al (2015) observed greater (less negative) y-intercepts during isometric contractions at 40% and 70% MVC in endurance-trained compared to chronic resistance trained individuals. These differences may be the result of fiber-type distribution differences, where resistance trained individuals possesses greater muscle size, and higher type II MHC content than endurance-trained individuals, who possess a greater type I MHC. Training status, therefore may influence muscle phenotype and seemingly influence MFR behavior, or vice versa. The influence of Type I MHC content was examined in further detail by Trevino et al (2016), and direct evidence was collected. The authors reported all subjects displayed evidence of the onion skin scheme, however, less negative slopes were reported for those with higher Type I MHC content were. Therefore subjects with less negative slopes for the MFR vs RT would suggest a higher type I MHC content and a smaller difference between MFR at lower contraction intensities compared to higher contraction intensities. In this same construct, those with greater type II MHC content would have more negative slopes (Trevino et al. 2016).

There is a positive relationship between $\mathrm{MUAP}_{\mathrm{AMP}}$ and muscle fiber diameter (Hakansson 1956). Although, specific fiber types cannot be directly linked to an observed RT or RT range, lower threshold MU have been associated with Type I muscle fibers and higher threshold MU associated with Type II muscle fibers (Carpentier et al. 2001; Pope et al. 2016). These larger- high thresold MU and associated muscle fibers are more suciptible to hypertorphy and atrophy than the lower threshold MU (Carpentier et al. 2001). Higher threshold MU have greater reported fatige during reapeated contractions, (25-50% MVC of the FDI) (Carpentier et al. 2001) and have an inverse relationship between RT and twitch durination (Milner-Brown et al. 1973a). Type II muscle fibers have been shown to display greater cross-sectional area (Van Wessel et al. 2010) compared to type I muscle fibers. Sterczala et al. (Sterczala et al. 2019) recently found that 8 weeks of resistance lower body resistance training increased muscle size and $\mathrm{MUAP}_{\mathrm{AMP}}$ of the VL. The slopes and y-intercepts of the $\mathrm{MUAP}_{\mathrm{AMP}}$ vs. RT relationships were analyzed and compared pre and post resistance training intervention in resistance training naive subjects. 8 weeks of a lower body focused linear periodization model, with decreasing volume and increasing intensity each week for 8 weeks. Subjects improved 1 repetition maximum strength, isometric muscle actions and muscle CSA after 8 weeks of training. A novel finding in this study, is that changes in the slopes of the $\mathrm{MUAP}_{\mathrm{AMP}}$ vs RT relationships demonstrate changes in the MU seizes from pre to post training. Thus increases in isometric knee extension post training and was primarily the result of MU hypertrophy.

These are important implications in sex related differences of MU properties, due to biological and physiolgical differences in males and females. Males have been shown to have greater muscle cross-sectional area, with a greater percentage of type II muscle fiber area (Staron et al. 2000) and possess larger $\mathrm{MUAP}_{\mathrm{AMP}}$ of the higher threshold MU (Trevino et al. 2018). This has great implications when assessing differences in males and females. Disparities in sex have been widely criticized, yet little is known about the mechanisms that cause the disparity.

1.3 SEX RELATED DIFFERENCES IN STRENGTH AND MHC

Sex related differences in muscle strength and power are well documented (Schantz et al. 1983; Maughan et al. 1983; Miller et al. 1993, p. 19; Trevino et al. 2018; Herda et al. 2019). Although there is no debate that males are physiologically stronger and have greater muscle cross-sectional area and specific tension than females, the underlying evidence for these differences is equivocal. Active males typically display larger muscles that possess a greater amount of type II fiber area (Staron et al. 2000), while females display smaller muscle size, with a greater type I fiber area (Staron et al. 2000; Hunter 2016a). This evidence is congruent with the data on fatigability and maximal strength, where females display lower MVCs (Trevino et al. 2018; Herda et al. 2019), but are less fatigable than their male counterparts(Hunter and Enoka 2001; Hunter 2016b, a). Greater type II fiber is associated with greater muscle size, and more negative slopes of the MUAP vs. RT when assessed on a subject by subject basis (Trevino et al. 2018; Herda et al. 2019, p. 20). The strong negative slopes of these regressions represent a rapid rise in MUAP_{AMP} size accompanied with greater contraction intensities. Trevino et al (2018) found type I MHC is associated with less negative slopes of the MUAP_{AMP} vs RT relationships during a moderate intensity contraction of the VL. Conversely, greater MHC II was associated with more negative slopes of the MUAP vs. RT relationship and greater maximal force generation. In a follow up study, Herda et al (2019) found a greater proportion of type I and less fast type II fibers in the VL of females compared to males. Therefore, continuing to add to the literature that type II fibers possess greater twitch forces, larger MUAP_{AMP} and the ability to generate greater forces with contribution to greater muscle size (Herda et al. 2019).

There are many examples of indirect evidence of smaller MUAP_{AMP} in females, as they characteristically contribute to greater endurance capacity by association to type I fibers. A study by Hunter and Enoka (2001) found that at low to moderate contraction intensities (of the elbow flexors) women are capable of longer endurance times than males. A caveat of Hunter and Enoka (2001) is that previously collected data of the FDI is presented in the paper. Contrary to current work, no sex related differences in endurance time for the FDI were found at three contraction intensities (20, 35 and 65% MVC. However, men were found to have greater target forces than females.

Although methods for this particular analysis are not presented in the paper, this may be due to pooling data across contraction intensities. However, a strong exponential relationship existed between target force and endurance time for men and women whereas greater target forces were associated with more brief endurance times. Consequently, sex related endurance differences have been observed in a number of muscle groups, including adductor pollicis(Ditor and Hicks 2000), extrinsic finger flexors (Petrofsky et al. 1975) and knee extensors (Maughan et al. 1986). Although the mechanism is unclear, it is hypothesized that due to the higher output in force from the males, they may sustain greater absolute force and therefore fatigue faster at greater contraction intensities (percentages of MVC). In other words men have greater targeted forces for a 70% contraction intensity due to greater force outputs during MVCs. Therefore, an inverse relationship was present between maximal force generation and endurance time in a fatiguing contraction(Hunter and Enoka 2001).

Fiber type discrepancies may attribute to a greater proportion of fatigue-resistant muscle fibers in females compared to males (Hunter 2016a) and causing less maximal force generation capabilities. Although many studies have examined MFR and $MUAP_{AMP}$ with male and female subjects, most have neglected to analyze this data separately to account for sex related differences (Stock et al. 2012), opting to pool the male and female data or pool the data across contraction intensities and losing the unique motor unit data from each subject.

1.4 MENSTRUAL CYCLE AND THE NEUROMUSCULAR SYSTEM

Females exhibit differences in FR behavior during different phases of the menstrual cycle. Peng et al (2018) found differences in FR behavior of the leg extensors using fine wire electrodes. Females were assessed in the late-follicular phase of the menstrual cycle, (day 8-14 of a 28 day cycle). Contraction intensity was set at 75% MVC for Females and males. Females observed faster firing rates than males after controlling for force, and even after controlling for menstrual cycle in the late follicular phase. Sex therefore was a confounding factor for differences in rate-coding of the vastus medialis. Peng attributes the differences in males and females to a greater percentage of slow twitch fibers in the VL than male subjects even after assessing females in the late follicular phase when they are hormonally similar to men (Tenan et al. 2013). This is in conflict with data by Tenan (2013) who found no significant differences in sexes at a (30%) lower contraction intensity.

Tenan et al (2013) examined differences in MU recruitment of the vastus medialis (VM) and vastus medialis oblique (VMO) during different phases of the menstrual cycle. Using basal body temperature as an indicator of menstrual cycle phase, Tenan collected MVC's for each subject, followed by isometric contractions at 30% MVC. Results of the study conclude that females have a significant difference in vastus medialis and vastus medialis oblique firing rates in the ovulation and midluteal phase. These changes are suggested to be caused by progesterone with a secondary action of estradiol. Estradiol binds to the estrogen receptor α sites on γ-aminobutyric acid (GABA) releasing neurons, which has been shown to decrease GABA transmission and increase cerebellar neuron discharge in rodents (Smith et al. 1989). Also of note, no differences were observed between males and females at 30% MVC, (2013) while significant differences were observed at a higher contraction intensity >70% MVC (Peng et al. 2018). Unpublished data from our lab has found sex related differences in the FDI between males and females, only at high intensity contractions (70%) with no differences observed at lower contraction intensities (10%). This data fits nicely with the results of Tenan (2013) and Peng (2018) and further identifies sex related differences at higher contraction intensities, and little differences between sexes at higher contraction intensities.

1.5 SEX HORMONES AND THE MOTORNEURON

Sex related differences are thought to be estrogen/estradiol dependent. Estrogen is thought to have an inhibitory effect on motor neuron excitability (Hoffman et al. 2018). Reproductive function in females is controlled by the hypothalamic-pituitary-gonadal axis and results in predictable oscillations of steroid hormones that may traverse the blood-brain barrier. Studies using transcranial magnetic stimulation have found increased excitability of the motor neuron during the late follicular phase of the menstrual cycle compared to the early follicular and mid luteal phases (Smith et al. 1999, p. 199; Smith and Adams 2002). Estradiol has an excitatory effect on glutamate receptors, which mediate the opening of Na+ and CA++ channels. Therefore changes in ovarian hormones may affect behavior of the motor neuron (Smith et al. 1999; Smith and Adams 2002; Tenan et al. 2013, 2016). Progesterone however, has an inhibitory effect through binding to the $GABA_A$ receptors. Transcranial magnetic stimulation (TMS), may be used to study the effects of drugs, hormones or disease states on the brain and spinal cord by reflecting the activation of inhibitory and excitatory systems. Smith et al, (1999;

2002) further investigated the effects of ovarian hormones on cortical excitability and found estrogen has an excitatory neuronal effect and progesterone has an inhibitory association.

Hoffman (2018) found that estrogen concentration explained varying levels of presynaptic inhibition of skeletal muscle. With increases in circulating estrogen of the female subjects between menses and ovulation, there is a substantial fall in presynaptic inhibition. This may mean that estrogen attenuates GABA and results in decreased presynaptic inhibition.

MU excitation can also be measured through direct stimulation of the nervous tissue or the recording of single MU activity during voluntary contractions (Tenan et al. 2016). When the motor cortex is directly stimulated through TMS the orderly recruitment of MU is stimulated by isolating the cortiocospinal tract (Bawa and Lemon 1993). Early research suggests that H-reflexes do no change across the menstrual cycle (Hoffman et al. 2018), however, later research supports corticospinal excitability is highest and inhibition is lowest in the late follicular (near ovulation) phase compared to early follicular and mid luteal phases (Smith and Adams 2002). Stimulation research indicates that the corticospinal tract is altered by the menstrual cycle at the late follicular phase (i.e. ovulation) when estrogen and progesterone is at peak. Tenan et al.,(2016) indicates that recording single MU discharges may allow us to understand how the nervous system is able to generate movement.

Maximal strength during the menstrual cycle has been investigated with equivocal outcomes. Sarwar et al, (1996) reported both quadriceps and handgrip strength were elevated in the ovulatory phase of the menstrual cycle, which were attributed to increased estradiol levels. MVC strength was reported to increase in the follicular phase with a large drop in MVC strength after ovulation by Phillips et al (1996).

Phillips (1996) studied the adductor pollicis in young untrained and trained women and oral contraceptive users and found an increase in MVC of about 10% during the follicular phase of the menstrual cycle. The authors cite estrogen as a primary indicator of these increases in strength. Therefore, reported decrease in MVC in the luteal phase may be due to a rise in progesterone and a much lower rise in estrogen. Progesterone's neuroinhibitory effect in the motor neuron and autonomic nervous systems may be a factor in strength decreases.

Studies have also reported no change in muscular strength across menstrual cycle (Dibrezzo et al. 1988; Lebrun et al. 1995; Jonge et al. 2001; Birch and Reilly 2002; Abt et al. 2007; Kubo et al. 2009; Montgomery and Shultz 2010), while others suggest menstrual cycle has a diurnal effect on muscle strength due to circulating hormone concentrations throughout the day (Birch and Reilly 2002). Considering diurnal effects, the luteal phase has been indicated as the lowest strength level for the morning, however afternoon testing suggests strength is increased in the luteal phase. Further research should control for diurnal effects of circulating sex hormones on strength outcomes. Circulating hormone concentrations in females are extremely complex, research has yet to identify or isolate the effects of these hormones on the nervous system and its overall integration to muscle strength and power. Maximal force generation fluctuations correspond to an increased MU discharge in the luteal phase to maintain force output (Tenan et al. 2013). Maximal force, therefore, may be lower in the luteal phase, and firing rate may increase in order to maintain the same force output obtained in an earlier phase of menstruation (Tenan et al. 2016).

1.6 NEUROMUSCULAR FATIGUE

Fatigue is noted as the basis of adaption to performance and neuromuscular overload. Fatigue is vital to the rehabilitation and adaptation of skeletal muscle. Females are usually less fatigable than men during single limb isometric fatiguing contractions at matched intensity. Enoka and Duchateau (2008) describe fatigue as a decrease in maximal force or power that the involved muscles can produce. When a task involves sustaining a maximal isometric contraction the decline in performance parallels the increase in fatigue. However, during a submaximal contraction, the onset of fatigue is not associated with task termination or inability to continue the task. Fatigue, therefore may not be a task induced isolated muscle failure, but may be caused by fatigue of synergistic muscles, inadequate action of the motor cortex, altered cross-bridge dynamics (Enoka and Duchateau 2008). Due to the complexity of muscle fatigue, characteristics of the population assessed must be taken into account. These population parameters include training status and biological sex (Hunter 2016b).

Females are generally less fatigable than males during isometric fatiguing tasks at the same relative intensity (Fulco et al. 1999; Hunter and Enoka 2001; Clark et al. 2005). Contractile mechanisms are hypothesized to cause disparity in sexes during fatiguing contractions. Females possess a greater percentage of slower and more fatigue-resistant muscle fibers, that are generally associated with less fatigable motoneuons (Miller et al. 1993; Staron et al. 2000). Differences in fiber type and fiber diameter would also explain differences in males and females during dynamic muscle actions. Females have been shown to be less fatigable than men at slow, but not high velocity contractions (Hunter 2016a). Therefore fiber type may describe a portion of fatigability in males. Females have smaller type II fibers than males, which also explain differences in muscle size between males and females. Fulco et al., (1999) compared males and females during repeated MVC's of the adductor pollicis muscle. This study was novel because sex comparisons were matched for MVC force. Maximal contractions were then repeated for 5 seconds on and 5 seconds until exhaustion. Females were less fatigable compared to women, MVC force fell less than men after 1 minute of repeated MVC's and time to exhaustion was almost twice as long as males. Hunter and Enoka (2001) further describe differences in males and females during MVCs of the elbow flexors, and describe factors distal to the muscle as factors in endurance contractions. Mean Arterial pressure and heart rate were measured during contractions. Females observed 20% lower MVCs than males, however, during the endurance contractions, women were 118% longer in time to fatigue, and endurance time and target forces were inversely related. Therefore, stronger individuals were the first to fatigue during endurance contractions for both single muscles and an isolated muscle (FDI). These differences were described as pressure response differences between the males and females. Mean arterial pressure and heart rate were less in females compared to males during all treatments. The authors note that this could be due to less muscle mass and therefore lower MVC force for the females which, in conjunction with a decrease in sympathetic outflow would result in greater muscle perfusion.

The compilation of this data challenges the assumption that males and females respond in a similar manner to training and adaption of the neuromuscular system. Mechanisms to the differences in males and females are widely disputed and still unclear. Sex related differences and their effects on the motoneuron and muscular coordination.

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2.0 SEX RELATED DIFFERENCES IN MOTOR UNIT FIRING RATES AND ACTION POTENTIAL AMPLITUDES OF THE FIRST DORSAL INTEROSSEOUS DURING HIGH-, BUT NOT LOW-INTENSITY CONTRACTIONS

2.1 INTRODUCTION

The firing rates of motor units (MU) are orderly in correspondence with recruitment threshold during submaximal isometric muscle actions. Earlier recruited (*lower-threshold*) MUs possess greater firing rates than the later recruited (*higher-threshold*) MUs regardless of recording methods (Kamen et al. 1995; Carpentier et al. 2001; Farina et al. 2009; Holobar et al. 2009). Only recently has it been speculated that females may possess different MU firing rates than males (Tenan et al. 2013; Peng et al. 2018). Accounting for recruitment threshold as a measure of orderly firing rate behavior, Peng (2018) reported females possessed significantly greater initial firing rates (IFR; firing rates at recruitment) during a 75% maximal voluntary contraction (MVC) of the leg extensors. These findings contradict earlier work by Tenan (2013) who reported no differences for IFRs during a lower intensity contraction (30% MVC) of the leg extensors. This discrepancy may be due to examined recruitment thresholds of MUs during the 30% MVC. Tenan et al. (2013) observed MUs possessing recruitment thresholds < 11% MVC, unlike the 70% MVC (higher intensity contraction intensity) where the mean recruitment thresholds of observed MUs were > 11% MVC and up to 70% MVC (Peng et al. 2018). Sex-related differences in IFR, therefore, may be related to recruitment threshold or the intensity of the contraction. Analyzing firing rates at a targeted force rather than at recruitment may provide further insight on possible sex-related differences. Further examination and comparison of firing rates at recruitment and targeted force in males and females during lower- and higher-intensity contractions is warranted.

The Henneman size principle (Henneman 1957) indicates that MUs are recruited in an orderly fashion according to size, with lower-threshold MUs possessing smaller action potential (AP) amplitudes recruited earlier followed by the recruitment of higher-threshold MUs that possessing greater AP amplitudes (Milner-Brown and Stein 1975; Goldberg and Derfler 1977; Martinez-Valdes et al. 2018). Therefore, increases in excitatory synaptic input to the MU pool result in an orderly recruitment of larger MUs (De Luca and Erim 1994). These larger MUs possess larger $\mathrm{MUAP_{AMP}}$ (Milner-Brown et al. 1973a; Tanji and Kato 1973) and greater twitch forces (Goldberg and Derfler 1977) than the earlier recruited smaller MUs. The higher-threshold MUs are reported to differ in AP amplitude between males and females for the leg extensors (Trevino et al. 2018). Specifically, Trevino (2018) reported sex-related differences in the cross sectional-area of the vastus lateralis was explained by the rate of increase in $\mathrm{MUAP_{AMP}}$ in relation to recruitment thresholds during a 40% MVC and the type II myosin heavy chain isoform composition (muscle fiber typing) of the muscle. The authors concluded the greater diameter of muscle fibers that primarily expressed type II characteristics accounted for the greater increase in $\mathrm{MUAP_{AMPS}}$ relative to recruitment thresholds. Therefore, diameters of muscle fibers comprising higher-threshold MU primarily explain muscle cross-sectional areas regardless of sex. In addition, Trevino (2018) suggested that the AP amplitudes may be of similar size for the lowest-threshold MUs between males and females because research has indicated that diameters of fibers that primarily express type I characteristics are more similar in size (Staron et al. 2000) and are believed to encompass lower-threshold MU. The concurrent examination of firing rates and AP amplitudes during lower- and higher-intensity contractions may provide insight on sex-related differences in properties of lower- and higher-threshold MUs.

Sex-related differences in muscle cross-sectional area (CSA) and strength are well documented within the literature for large muscle groups, such as the vastus lateralis and biceps brachii (Maughan et al. 1983; Miller et al. 1993; Monica, J Hubal et al. 2006). Although untested, sex-related differences in CSA and strength of the first dorsal interosseous (FDI), a mixed fiber type muscle (Johnson et al. 1973), would likely exist similar to the leg extensors. The FDI is the only muscle involved in the abduction of the index finger (Thomas et al. 1986) and the majority of MUs are recruited prior to 50% MVC with a few more recruited up to 70% MVC (Thomas et al. 1986; Kamen et al. 1995; De Luca and Hostage 2010). Therefore, the examination of the higher-threshold MUs is more conducive for the FDI than large limb muscles that have greater recruitment threshold ranges, such as, the vastus lateralis where difference in muscle size and strength are more prominent. Examination of the FDI could provide further understanding of the potential sex-related differences in the properties of the lowest- and highest-threshold MUs of a mixed fiber type muscle. We hypothesize that MU action potential amplitudes and firing rates during a low-intensity contraction will be similar between males and females, but will differ during a high-intensity contraction. Therefore, the purposes of the present study were to examine differences in CSA and strength of the FDI between males and females and, subsequently, to determine if the amplitudes and firing rates of the lower- and higher-threshold MUs recorded during 10% and 70% MVCs differ between sexes. Superimposition of action potential amplitudes does not allow for the recording of the smallest lower-threshold MUs during the 70% MVC and, therefore, the 10% MVC was also included in the study to examine the smallest lower-threshold MUs.

2.2 METHODS

Participants

Eleven males (mean ± SD: age=22 ± 3 yr, height=181.1 ± 6.0 cm, weight=72.9 ± 9.4 kg, BMI=22.2 ± 1.8 kg/m ^{2}) and twelve females (age=21 ± 1 yr, 164.2 ± 3.7 cm, weight=63.6 ± 5.3 kg, BMI=23.6 ± kg/m^2) volunteered for this investigation. None of the participants reported any current or ongoing neuromuscular disease or musculoskeletal injury. Each participant completed a pre-exercise health history questionnaire and signed a written informed consent document. The participants reported participation in recreational activities, and were not competitive athletes. These individuals may best be classified as moderately-active or recreationally-trained. This study was approved by the university's institutional review board for human subjects.

Research Design

Participants were asked to visit the laboratory twice, separated by at least 24 hours. The first visit consisted of familiarization to procedures, which included practicing each of the isometric trapezoidal muscle actions several times. During the experimental visit, participants completed the isometric muscle actions as well as ultrasound scans of the FDI. For the isometric muscle actions, the subject's right forearm was pronated and positioned on a table with the hand open. The forearm, wrist and third through fifth fingers were immobilized with a Velcro strap. The thumb was resisted with a Velcro strap that would allow for a 90º angle between the index finger and the thumb during the isometric contractions. The muscle action of the FDI was isolated and measured during abduction of the index finger against a small flat piece of metal connected to the force transducer (MB-100; Interface, Inc. Scottsdale, AZ), amplified with the Bangnoli-16 EMG Amplifier Unit (Delsys Inc., Natick, MA, US), and converted digitally with the NI PCI-6220 (National instruments, Austin, TX, US). Subjects performed 3 maximal voluntary contractions (MVC) that were 5 seconds in duration, with 2 minutes of rest between each contraction. Strong verbal encouragement was given during each MVC. Target force for the 10% and 70% MVCs was determined from the peak force from the three MVCs. The 10% and 70% MVCs were completed in random order. Each participant completed one 10% and one 70% tracing. Subjects were asked to complete an additional contraction if tracings were not satisfactory. Satisfactory tracings were considered when the force output was within 2% of the template as reviewed by an experienced investigator (MEP). For each isometric trapezoidal muscle action (Figure 1), force was increased at a rate of 10% MVC/second to the target force which was held for 10 seconds, then decreased at a rate of 10% MVC/second back to baseline. Therefore, the 10% and 70% MVCs lasted 12 and 24 seconds, respectively. Five minutes rest was given between each submaximal isometric trapezoidal muscle action to ensure potentiation had diminished (Miller et al. 2017a, b). During each contraction participants maintained their force output as close as possible to the force template displayed digitally on a computer monitor. Subjects were required to refrain from caffeine consumption on the day of the experimental trial.

Electromyography Recording

During the trapezoid muscle actions, surface EMG signals were recoded from the FDI using a 5-pin surface array sensor (Delsys, Inc, Natick, Massachusetts). The sensor array consisted of 0.5 mm pins placed at the corners of a 5x5 mm square, with a fifth pin in the center of the square. The surface of the skin was prepared by shaving, removing superficial dead skin with adhesive tape, and sterilizing with an alcohol swab before sensor placement. Dead skin layers were removed by using hypoallergenic tape (3 M, St. Paul, Minnesota) which was repeatedly applied to the skin, then peeled back to remove contaminants (Delsys, Inc., dEMG User Guide). The sensor was then placed over the belly of the FDI muscle and secured with adhesive tape, and a reference electrode was placed over the elbow.

EMG Decomposition

Action potentials were extracted into single firing events of MUs from the 4 separate EMG signals, sampled at 20 kHz, via the Precision Decomposition (PD) III algorithm as described by Deluca (2006). This algorithm was designed for decomposing surface EMG signals into their individual MUAP trains. The accuracy of the decomposed firing instances was initially tested with the reconstruct-and-test produce (Nawab et al. 2010). In addition, a secondary validation of the accuracy of the MU firing times and AP waveforms generated by the PD III algorithm was performed via spike-trigger average (STA) protocol. Briefly, the PD III algorithm derived firing times were used as triggers for averaging the four raw EMG signals recorded from the surface sensor array (Hu et al. 2013a, b; McManus et al. 2016; Herda et al. 2019; Miller et al. 2019). A window length of 4 seconds was used and shifted over the surface EMG signal using a *step size* of 1 second (Hu et al. 2013a, b). For a MU to be included for further analyses it must have met the 90% accuracy level from the reconstruct and test procedure and met the following criteria from the STA procedure: 1) a high correlation (*r* > 0.70) between the PD III algorithm and STA derived AP waveforms and 2) a low coefficient of variation (< 0.30) of the STA derived AP peak-to-peak amplitude across the contraction time (Hu et al. 2013a).

For each MU, five parameters were extracted from the firing rate data: (1) the recruitment threshold expressed as a percentage of MVC, (2) $\mathrm{MUAP_{AMP}}$ (mV), (3) $\mathrm{MUAP_{DUR}}$ (ms), (4) initial firing rate (IFR) at recruitment (pulses per second [pps]), and (5) the mean firing rate (MFR) at the targeted contraction level (pps). The RT was the average of 0.01 s epoch of force that began at the first discharge of the MU. Four unique action potential waveform templates were derived from the four EMG channels. The average of the peak-to-peak amplitude ($\mathrm{MUAP_{AMP}}$) and average duration ($\mathrm{MUAP_{DUR}}$) from the four unique action potential waveforms were used for further analysis (Figure 2). The IFR was the average of the first three interpulse intervals (Trevino et al. 2016). The MFR was calculated as the average value of the interpulse intervals during the 10 second epoch at the targeted force level. For the 10% MVC, an average of the RT, IFR, MFR, $\mathrm{MUAP_{AMP}}$, and $\mathrm{MUAP_{DUR}}$ for all MUs for each subject were used for further statistical analysis. The IFR, MFR, $\mathrm{MUAP_{AMP}}$, and $\mathrm{MUAP_{DUR}}$ vs. RT relationships could not be calculated for the 10% MVC as a result of the small range of RTs for each subject. For the 70% MVC, linear regressions were applied to the IFR, MFR, $\mathrm{MUAP_{AMP}}$, and $\mathrm{MUAP_{DUR}}$ vs. RT and relationships with the slope and y-intercept values used for statistical analysis (Figure 2). In the present study, the recorded MU RT range from the 70% MVC for each subject exceeded > 19% MVC. A small recorded RT range of MUs for a subject could result in an outlier or physiologically irrelevant coefficients from the regressions. After close inspection of the data by experienced investigators (MEP and TJH), it was deemed there were no outlier or physiological irrelevant coefficients from the regressions.

Ultrasound Measurement

Ultrasound images were taken of the right FDI in order to determine the muscle cross-sectional area using a LOGIQe ultrasound-imaging device (GE Healthcare UK, Ltd., Chalfont, Buckinghamshire, UK) (Herda et al. 2016; Trevino et al. 2016; Miller et al. 2017a, 2018; Sterczala et al. 2018). Subjects were examined while sitting at a table with the right hand lying open and pronated on top of foam pads formed into an L-shape. The foam pads were to standardize the thumb in a relatively neutral position at 70º. For each scan ultrasound brightness mode (B-mode), the musculoskeletal preset, and a GE 12L-RS Linear Ultrasound Transducer (5-13 MHz), with a 42 x 7 mm footprint were used. The scan depth was set to 2 cm, gain was 38 dB and transducer frequency was 12 MHz. The origin and insertion of the FDI were located by longitudinally scanning the muscle. The origin and insertion were marked and measured. The midway point between the two was used as the site of the CSA measurement. Once the mid site was determined, a cross section of the muscle belly was scanned with the length of the probe head oriented perpendicular to the 2nd metacarpal. The 2nd metacarpal was used as a reference for the orientation of the probe as the FDI runs along its lateral side. Much care was given to ensure adequate amounts of gel were used in order to create uniform pressure with the skin, and that the probe was perpendicular to the surface of the skin. An image was captured and saved when the muscle was properly focused. Images were saved for each subject and exported as a jpg format image to a personal computer for subsequent analysis.

Muscle CSA (cm^2) and subcutaneous fat thickness (mm) were determined using the image analysis program ImageJ (National Institutes of Health, Bethesda, Maryland) (Figure 3). The scale of each image was calibrated using the centimeter marks inlaid in the image. Subcutaneous fat thickness was measured using the center of the cross-section of the muscle as a reference point. The periphery of the muscle was carefully outlined using the polygonal tool and the CSA.

Signal Processing

Channel 1 of the 4 bipolar EMG channels from the 5-pin surface array sensors were selected for amplitude analysis. The force (N) and the EMG (mV) signals from channel 1 were recoded with a NI cDAQ (National instruments, Austin, TX, US) during each isometric muscle action. Data were stored on a personal computer (Dell Optiplex 9010; Dell, Inc., Round Rock, TX) for further analysis. The sampling frequency was 2000 Hz for all signals. The EMG signals were band-pass filtered (zero phase fourth-order Butterworth filter) at 10-500 Hz, while the force signal was low-pass filtered with a 10-Hz cutoff (zero-phase fourth order Butterworth filter). All EMG amplitude calculations were performed on the filtered signals. The EMG_{RMS} values during the 10% and 70% MVCs were normalized (%max) to the amplitude (N-EMG_{RMS}) recorded during the highest 0.25 s average of force (N) that occurred during the three MVCs.

Statistical Analysis

Independent samples t-tests were performed to examine potential sex-related differences in BMI, CSA, sFAT, and MVC force. For MU data from the 10% MVC, the average of

IFRs, MFRs, $MUAP_{AMPS}$, $MUAP_{DURS}$ and RTs were calculated for each subject and independent samples t-tests were used to examine potential differences between sexes. For MU data from the 70% MVC, possible sex-related differences in the calculated slopes and y-intercepts from the IFR, MFR, $MUAP_{AMP}$, and $MUAP_{DUR}$ vs. RT relationships were examined with independent samples t-tests. In addition, a two-way mixed factorial ANOVA (sex [male vs. female] x contraction intensity [10%MVC vs. 70%MVC]) was used to examine possible differences in N-EMG_{RMS} during the 10 second epoch at steady force were MFRs were selected between groups and contraction intensities.

2.3 RESULTS

Voluntary Force and Ultrasound

Independent samples t-test indicated significant differences between males and females for MVC force ($p < 0.001$; males=25.9 ± 5.5 N, females=16.6 ± 2.6 N) and CSA ($p<0.001$; males=2.34 ± 0.28 cm^2, females=1.83 ± 0.19 cm^2). However, there were no significant differences between groups for sFAT thickness ($p=0.168$, males=0.167 ± 0.03 cm, females=0.182 ± 0.03 cm) or BMI ($p=0.174$; male=22.26 ± 2.82 kg/m^2, female=26.03 ± 8.64 kg/m^2).

Motor Units

Descriptive information regarding the number and RTs for recorded MUs from the 10% and 70% MVCs is presented in Table 1. During the 70% MVC, RTs were observed from 18-70% MVC, however, only MUs with RTs from 36% to 42% MVC were recorded for every subject. Therefore, predicted MU data presented in figures 4 and 5 reflect this common RT range among all subjects. For the 70% MVC, each subject's IFR vs. RT (r=0.85 ± 0.09), MFR vs. RT (r =-0.90 ± 0.08), and $MUAP_{AMP}$ vs. RT (r = 0.80 ± 0.19) relationships were significant, however, only 2 of 11 and 1 of 12 $MUAP_{DUR}$ vs. RT relationships were significant for the males and females respectively.

10% MVC

Independent samples t-tests indicated no significant differences between the males and females for average RTs (p=0.165), IFRs (p=0.625), MFRs (p=0.328), $MUAP_{AMPS}$ (p=0.152), and $MUAP_{DURS}$ (p=0.176) from the 10% MVC (Figure 6).

70% MVC

For the IFR vs. RT relationships, there were no significant differences between sexes for the slopes (p=0.603) or the y-intercepts (p=0.132) (Figure 4). For the MFR vs. RT relationships, there were no significant differences between sexes for the slopes (p=0.211), however, there was a significant difference for the y-intercepts (p=0.021). The y-intercepts of the MFR vs. RT relationships were greater for females (38.65 ± 6.71 pps) than males (31.55 ± 6.92 pps) (Figure 5).

An additional statistical test was performed on the IFRs to better correspond with the data presented in Peng et al. (2018). A separate two-way mixed factorial ANOVA (sex [male vs. female]) x RT [36% vs. 40% vs. 42% MVC]) calculated on the IFRs presented in figure 5 did indicate there was a main effect for sex (p=0.035) with females having greater IFRs than males, which does tentatively provide support to the findings of Peng et. al, (2018).

For the $MUAP_{AMP}$ vs. RT relationships, there was no significant difference (p=0.323) for the slopes, however, the y-intercepts were significantly different (p=0.044) between the males and females. The males (-0.19±0.53mV) had less negative y-intercepts than the females (-0.78±0.76 mV) (Figure 5). The negative y-intercepts were a result of not observing lower-threshold MUs (i.e., <18% MVC) in combination with the rapid rise of AP_{AMPS} of the higher-threshold MUs. Figure 7 illustrates the $MUAP_{AMP}$ vs. RT relationships for a male and female subject. Of note, the y-intercepts are negative despite all action potential amplitudes being positive.

For a clearer interpretation, predicted $MUAP_{AMPS}$ were calculated for each individual for MUs within the observed RT ranges for every subject (36% to 42% MVC) (Figure 5). It is evident that the males had greater $MUAP_{AMPS}$ within the recorded RT ranges from 36% to 42% MVC, unlike at lower RT ranges during the 10% MVC (Figure 5).

For the $MUAP_{DUR}$ vs. RT relationships, statistical procedures were performed despite few significant relationships. There were no significant differences between the male and females for the slopes (p=0.300) or y-intercepts (p=0.153). Therefore, it is likely that the depth of the recorded MUs within the muscle did not contribute to differences in the amplitude of MUs recorded at the surface of the skin (Fuglevand et al. 1992; Hu et al. 2013a).

N-EMG$_{RMS}$

There was no significant contraction intensity × sex interaction (p=0.432), nor was there a significant main effect for sex (p=0.430). However, there was a significant main effect for contraction intensity ($p<0.001$) and, as expected, greater N-EMG$_{RMS}$ was observed at steady force during the 70% MVC (87.1 ± 19.6%) in comparison to 10% MVC (10.7 ± 3.6%).

2.4 DISCUSSION

MVC force and anatomical CSA were significantly greater for males, which is similar to large mixed fiber type limb muscles (Miller et al. 1993; Staron et al. 2000). A noteworthy finding in the present study was that the MUs with RTs 1.72% to 2.76% MVC had similar characteristics (IFRs, MFRs, and MUAP$_{AMPS}$) for males and females, however, the higher-threshold MUs (RTs > 18% MVC) recruited during the 70% MVC possessed smaller MUAP$_{AMPS}$ and greater MFRs when expressed relative to RT for the females. Therefore, sex-related differences in firing rates at the targeted force and MUAP$_{AMPS}$ were dependent on recruitment threshold and/or intensity of the contraction. There were few significant MUAP$_{DUR}$ vs. RT relationships as expected (Hu et al. 2013a; Miller et al. 2018; Sterczala et al. 2018) and tentatively indicates that variations in MU depth was not a confounding variable in the interpretations of the MUAP$_{AMPS}$ in relation to RT. In addition, there were no differences in MUAP$_{DURS}$ between sexes and suggests that recording distance was not arbitrarily leading to larger action potential amplitudes of the higher-threshold MUs for the males.

Low-Intensity Contraction

Females had smaller CSAs, however, there were no sex-related differences in the MUAP$_{AMPS}$ for the lowest-threshold MUs (RTs from 1.72% to 2.76% MVC) recorded during the 10% MVC, unlike for MUs recorded during the 70% MVC. Muscular contractions were performed at relative forces, therefore, the absolute targeted force for the females was lower. Despite this lower targeted force, the recorded MUs for the females had similar characteristics (IFRs, MFRs, and $\text{MUAP}_{\text{AMPS}}$) to the males at the lower intensity. Previously, the MUAP_{AMP} has been reported to be associated with the force generation capability of the MU (Milner-Brown and Stein 1975; Goldberg and Derfler 1977). It may be expected that the females would create similar forces to the males if the sizes and the firing rates of recruited MUs during the 10% MVC were equivalent. However, due to a very homogenous population (similar RTs and AP_{AMPS}) of MUs recorded during the 10% MVC, linear regressions could not be performed in a manner similar to the MU data from the 70% MVC. Nonetheless, the lowest-threshold MUs (<3% MVC) recorded in this study possessed similar amplitudes despite differences in muscle CSA between the males and females.

Previously, Tenan et. al, (2013) reported no sex-related differences for IFRs during 30% MVCs regardless of menstrual phase. However, the authors indicated that females had higher IFRs during the later phases of the menstrual cycle (ovulation, mid luteal, and late luteal) in comparison to the earlier phases (early follicular and late follicular) for the vastus medialis and vastus medialis oblique muscles. One limitation of this study is we did not record menstrual cycles of the females. Nonetheless, the results of the present study support the findings of Tenan (2013) that firing rates of lower-threshold MUs did not differ between sexes during a low contraction intensity. Together, the $\text{MUAP}_{\text{AMPS}}$ and firing rates displayed in the present study suggest similar MU control strategies at lower intensities as a result of active MUs being of similar action potential amplitudes and strength (Milner-Brown et al. 1973b; Milner-Brown and Stein 1975; Goldberg and Derfler 1977) for the females and males.

High-Intensity Contraction

The y-intercepts of the $MUAP_{AMPS}$ vs. RT relationships from the 70% MVC were significantly different between males and females. Specifically, indicating that the $MUAP_{AMPS}$ of the higher-threshold MUs were smaller for the females than the males. These results contradict the $MUAP_{AMPS}$ of the lowest-threshold MUs (1.72% to 2.76% MVC) observed during the 10% MVC (Figure 5). Therefore, sex-related differences in $MUAP_{AMPS}$ existed as a function of RT and contraction intensity.

It is well understood that males and females differ in the fiber type characteristics of mixed fiber type muscles, such as, the vastus lateralis and biceps brachii (Simoneau et al. 1985; Miller et al. 1993; Staron et al. 2000; Trevino et al. 2018). Males are reported to possess larger fiber diameters of type I and II muscle fibers with a greater disparity in the diameters of the type II fibers (Miller et al. 1993; Staron et al. 2000). Miller et al (1993) reported the total number of MUs or the number of fibers per MU for the biceps brachii and vastus medialis did not differ between males and females and that fiber diameter and area of fiber type are correlated with CSA and force generating capabilities of large muscle groups.

Muscle fibers that comprise a MU do not strictly express characteristics of one fiber type (I vs. II), but rather co-express type I and II characteristics (Carroll et al. 2005; Rowan et al. 2012). It is believed, however, that fibers expressing primarily type II characteristics tend to comprise higher-threshold more so than lower-threshold MUs (Burke et al. 1973; Canepari et al. 2010; Trevino et al. 2018). Therefore, the smaller $MUAP_{AMPS}$ of the higher-thresholds MU for the females in the present study might be the result of smaller diameter and smaller area of fibers that express II characteristics as reported by Trevino (2018). Whereas, the similar $MUAP_{AMPS}$ of the lowest-threshold MUs could indicate these MUs consist of fibers that primarily express type I characteristics, which do not differ in size between females and males to the same extent as type II fibers. Future research should include contraction intensities between 10% and 70% MVC to provide further resolution on the potential sex-related differences in $\mathrm{MUAP_{AMPS}}$ of the FDI.

The IFR vs. RT relationships from the 70% MVC were strong (r=-0.85 ± 0.09) for the FDI, unlike what was previously reported for the IFR vs. RT relationships of the leg extensors (Trevino et al. 2016; Peng et al. 2018). The negative slopes indicated that the later recruited and larger MUs had lower IFRs as in the present study. As expected, the IFRs for the FDI in the present study were higher than reported for the leg extensors (De Luca and Contessa 2012; Tenan et al. 2013; Trevino et al. 2016; Peng et al. 2018). There was no difference in the y-intercepts or slopes of the IFR vs. RT relationships between sexes. Peng et. al, (2018) provided evidence from 75% MVCs that females possessed greater IFRs than males for the vastus medialis and vastus medialis oblique (pooled data) muscles during the late follicular phase of the menstrual cycle when IFRs were previously reported to be *more similar* between sexes (Tenan et al. 2013). Subsequently, sex-related differences in IFRs reported by Peng et. al, (2018) could be a function of the contraction intensity and/or a function of the recorded MUs (lower- vs. higher-threshold MUs). It should be noted, that in the present study the IFRs during the 70% MVC tended to be higher for the females than males and reflected the sex-related differences for the MFR vs. RT relationships (Figure 5).

The y-intercepts from the MFRs vs. RT relationships were significantly different between males and females. Females possessed greater MFRs than the males for the MUs with RTs ranging from 36% to 42% MVC. It is unclear the mechanism that would result in greater MFRs for the females than males during higher contraction intensities. Although speculative, the females might be at a greater operating point of excitation to the motoneuron pool (Contessa and Luca 2012) to maintain the 70% MVC. Sustaining an isometric submaximal contraction at a greater operating point of excitation would lead to increases in MU recruitment and MFRs. Since the majority of MUs would be recruited prior 50% MVC (De Luca and Contessa 2015), the rightward shift in the operating point of excitation would result in significant increases in MFRs along with recruitment of the few remaining MUs. Therefore, performing the 70% MVC at a greater operating point of excitation for the females might provide an explanation for the significantly higher MFRs of the MU pool (i.e., greater y-intercepts).

A potential explanation is that saturation of the firing rates differed between males and females. Previous studies report that firing rates reach a plateau while voluntary force increases linearly (Bigland and Lippold 1954; Dasgupta and Simpson 1962; Clamann 1970; Milner-Brown et al. 1973b; Monster and Chan 1977). The firing rates of the MU pool may have reached the plateau earlier for the males than females and, therefore, firing rates were higher for the females during the 70% MVC. This could also explain why firing rates were not different between sexes during the 10% MVC.

It is reported that females tend to have greater resistance to fatigue during isometric muscle actions, however, there is less disparity between sexes at higher contraction intensities (Hunter, 2016). Considering the majority of MUs are likely recruited prior to 50% MVC for male and females, the greater MFRs during the 70% MVC for females could increase the neural cost of the contraction and minimize the benefits of possessing a greater percentage of fibers that express type I characteristics in regards to fatigue resistance (Hunter 2016; Trevino et al. 2018). Therefore, the greater MFRs for the females might provide an explanation for the more similar levels of resistance to fatigue at higher contraction intensities between sexes in comparison to lower intensity contractions.

In summary, $\text{MUAP}_{\text{AMPS}}$ of the lower-threshold MUs with RTs from <3% MVC were similar in size between males and females, unlike the higher-threshold MUs where males had greater $\text{MUAP}_{\text{AMPS}}$. MFRs, and potentially IFRs, were elevated for the females during the 70% MVC, which may be the result of performing the contraction at a higher operating point of excitation of the motoneuron pool. Therefore, the non-uniform differences in $\text{MUAP}_{\text{AMPS}}$ (Figure 8), or twitch forces, might partially explain alterations in the overall MU control strategies to perform a high-intensity contraction, but not a lower-intensity contraction. Future research should examine the implications of sex-related differences in MUAP_{AMP} and firing rates of the higher-threshold MUs, such as, resistance to fatigue and functional performance (Lee et al. , 2017).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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2.6 TABLES AND FIGURES.

Table 1. The motor unit (MU) counts, the average recruitment threshold (RT) recorded during the 10% maximal voluntary contraction (MVC), and the range of RT's recorded during the 70% MVC for males and females.

	10% MVC		70% MVC	
	MU Count	RT(%MVC)	MU Count	RT(%MVC)
Male	13 ± 3.1	2.8 ± 2.3	22 ± 6.0	55.6 ± 9.0
Female	11 ± 3.6	1.7 ± 1.0	20 ± 6.3	59.0 ± 9.8

Figure 1.

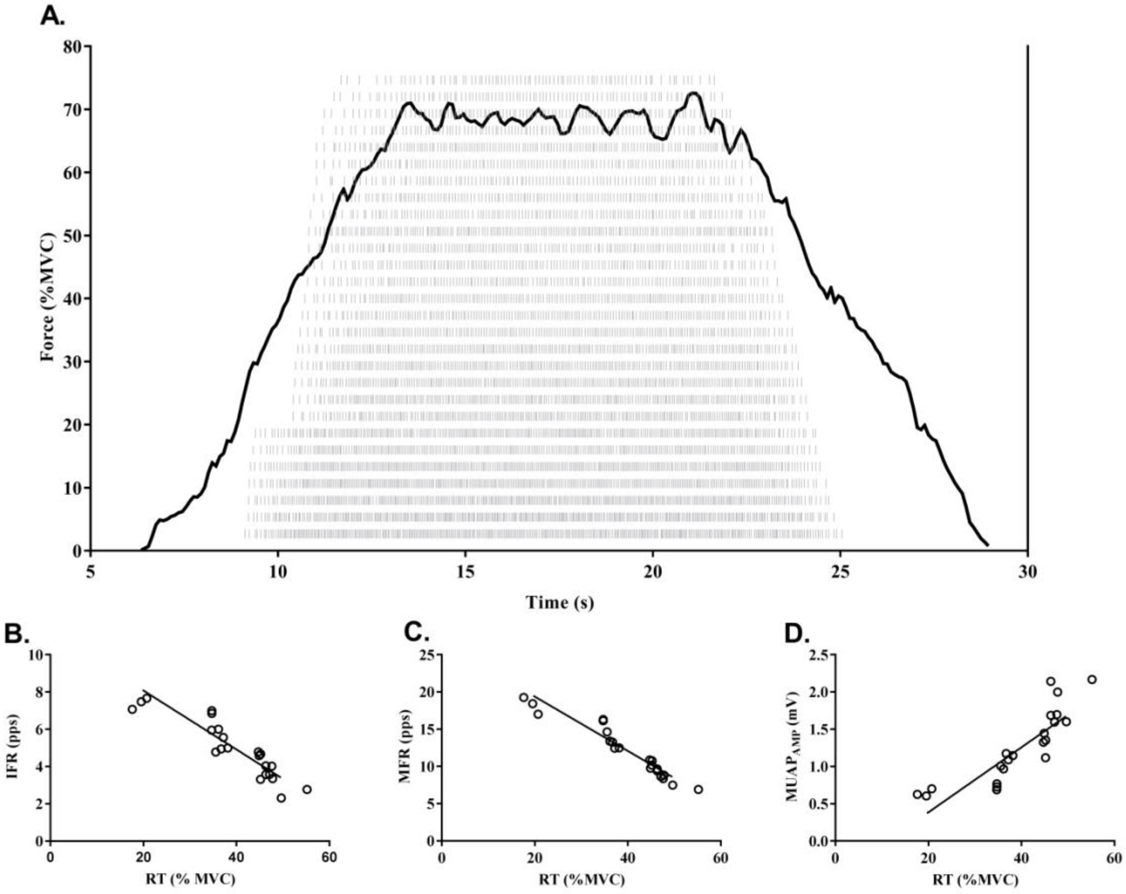

Figure 1. (A) An example of the individual firing instances of motor units of the first dorsal interosseous during an isometric trapezoidal contraction at 70% maximal voluntary contraction (MVC) for a male participant. The black line is the real-time force signal during the trial. The solid gray vertical lines indicate the individual firing times for each individual motor unit recorded during the contraction. (B-D) The 3 relationships analyzed for that subject from the 70% MVC are presented. (B) IFR vs. recruitment threshold (RT, expressed as percentage of MVC [%MVC]). (C) MFR vs. RT. (D) MU action potential amplitude (MUAP_{AMP}) vs. RT.

Figure 2.

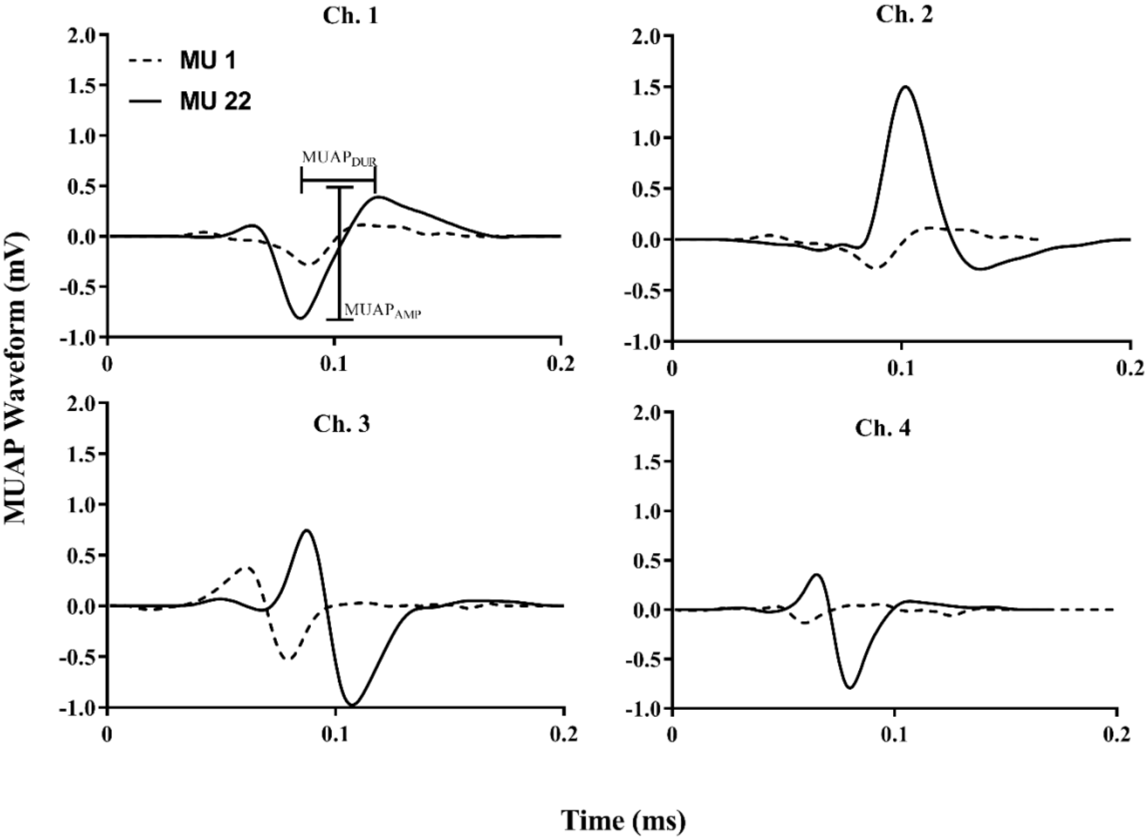

Figure 2. The four unique action potential waveforms of the selected MUs from figure 1. The lower-threshold MU (MU 1) possesses smaller peak-to-peak action potential amplitudes ($\mathrm{MUAP_{AMPS}}$) in all four channels.

Figure 3.

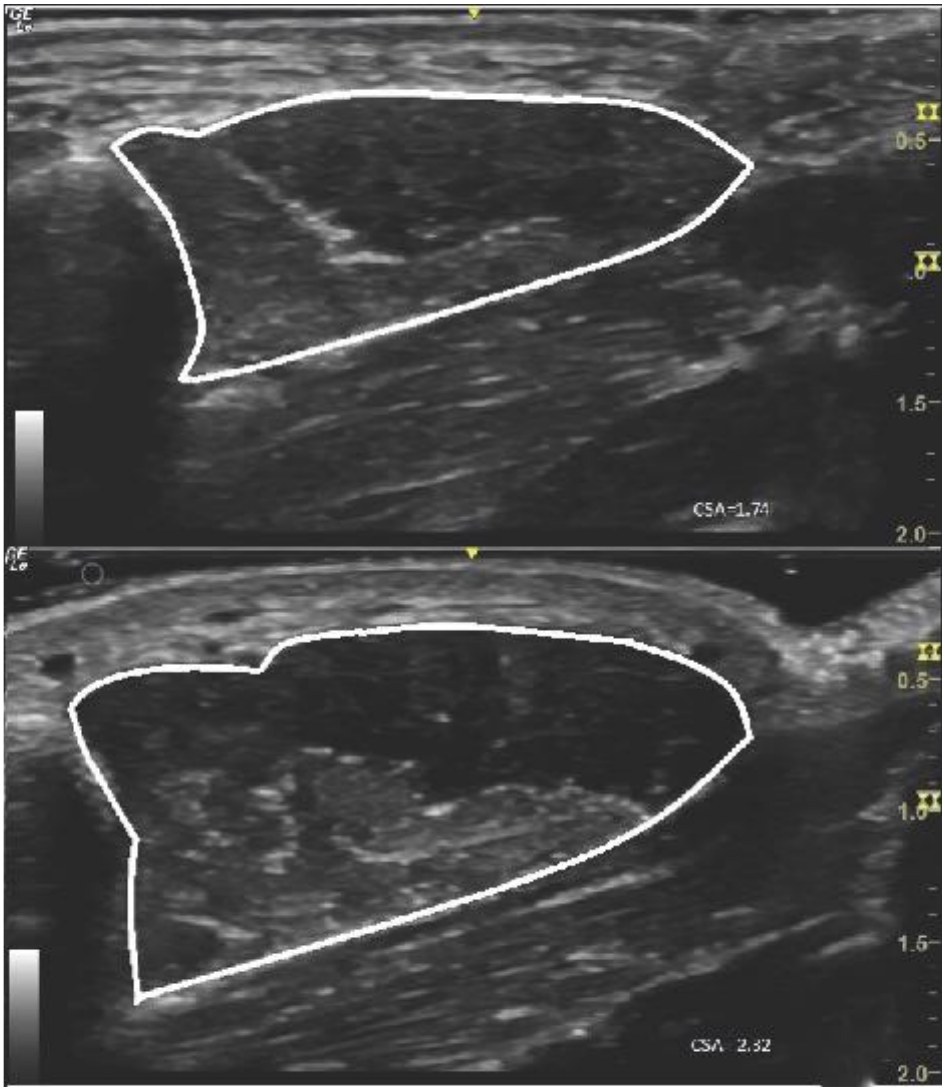

Figure 3. Ultrasonography scans of the cross-sectional area (CSA) of the first dorsal interosseous (FDI) muscle. The white outline indicates the measured CSA of the FDI. The top image is of a female subject and the bottom is a male subject. CSA (cm^2) is presented in the bottom right corner of each scan.

Figure 4.

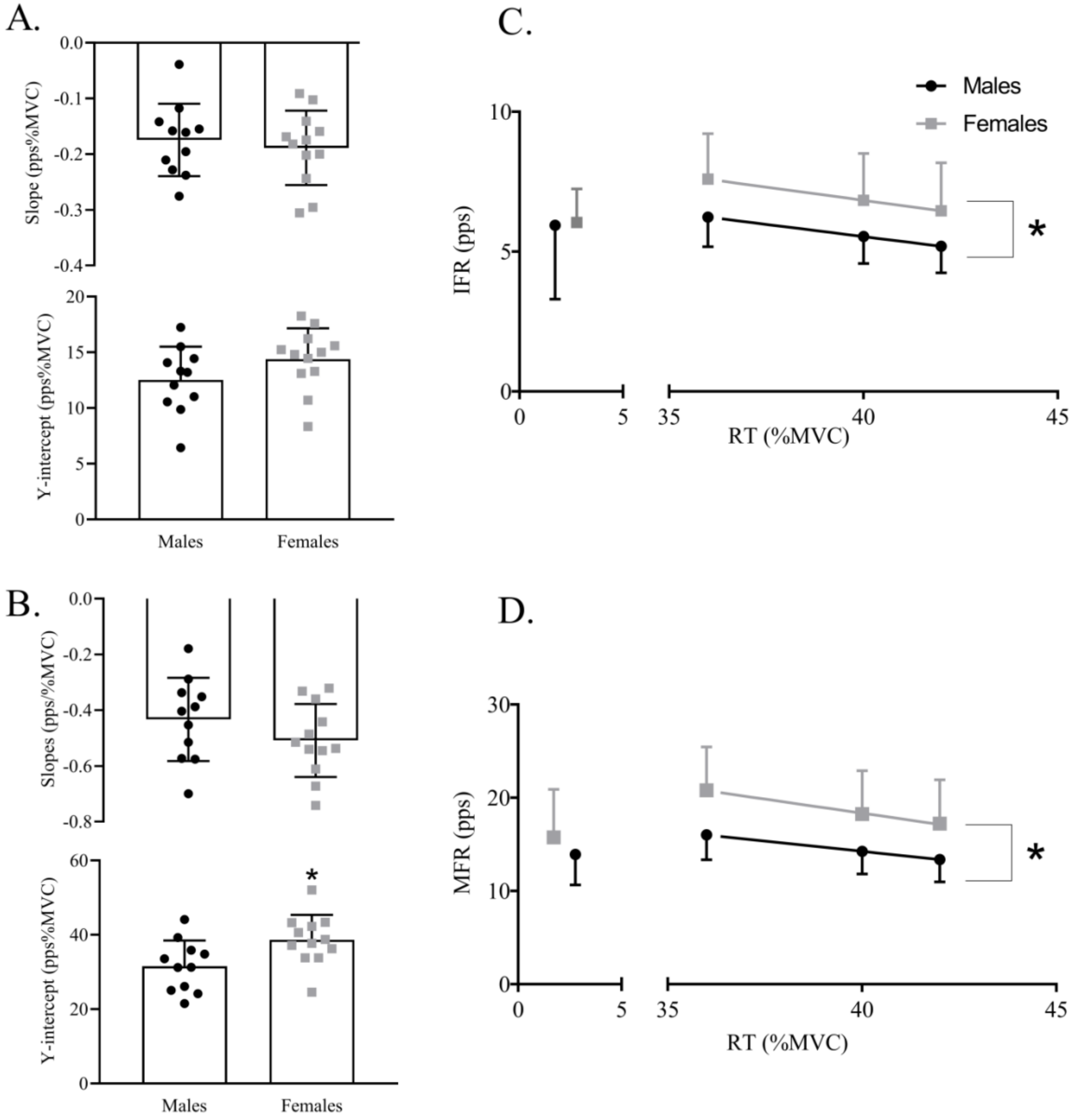

Figure 4. The individual values and mean (SD) for the slopes and y-intercepts from the (A) initial firing rate (IFR) vs. recruitment threshold (RT, expressed as a percentage of maximal voluntary contraction [%MVC]) and (B) mean firing rate (MFR) vs. RT relationships. The (C) IFRs and (D) MFRs expressed as a function of RT from the 10% (left) and 70% MVCs (right). For the 70% MVC, MU recruitment thresholds were observed for every subject within the presented RT range (36% to 42% MVC). The three data points represent predicted firing rates for each subject within the range of RTs that was observed for all subjects. Means (SDs) for each group were developed from each subject's linear regressions and displayed as bar graphs. * indicates a significant difference for the y-intercepts between the males and females.

Figure 5.

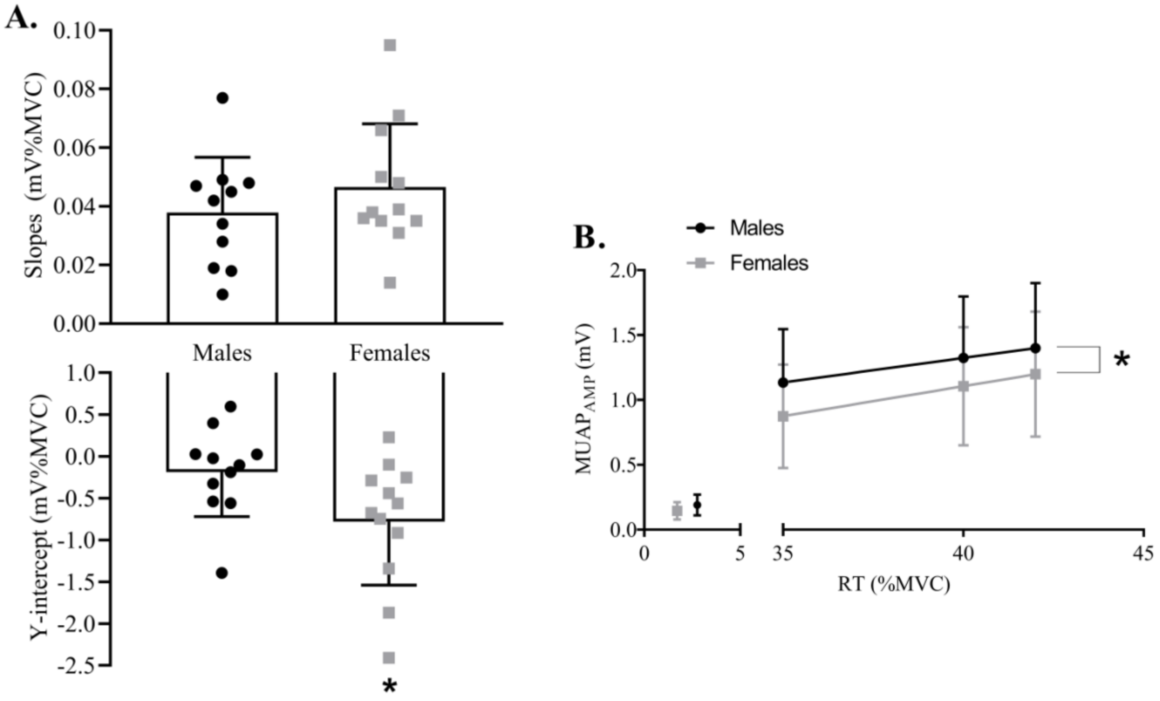

Figure 5. The (A) individual values and mean (SD) for the slopes and y-intercepts from the motor unit action potential amplitudes ($\mathrm{MUAP}_{\mathrm{AMP}}$) vs. recruitment threshold (expressed as a function of percent maximal voluntary contraction [%MVC]) relationships. The (B) $\mathrm{MUAP}_{\mathrm{AMPS}}$ from the 10% (left) and 70% MVCs (right). For 70% MVC, MUs were observed for every subject within the presented RT range (36% to 42% MVC). The three data points represent predicted $\mathrm{MUAP}_{\mathrm{AMPS}}$ for each subject within the range of RTs that was observed for all subjects. Means (SDs) for each group were developed from each subject's linear regressions. * indicates a significant difference for the y-intercepts between the males and females.

Figure 6.

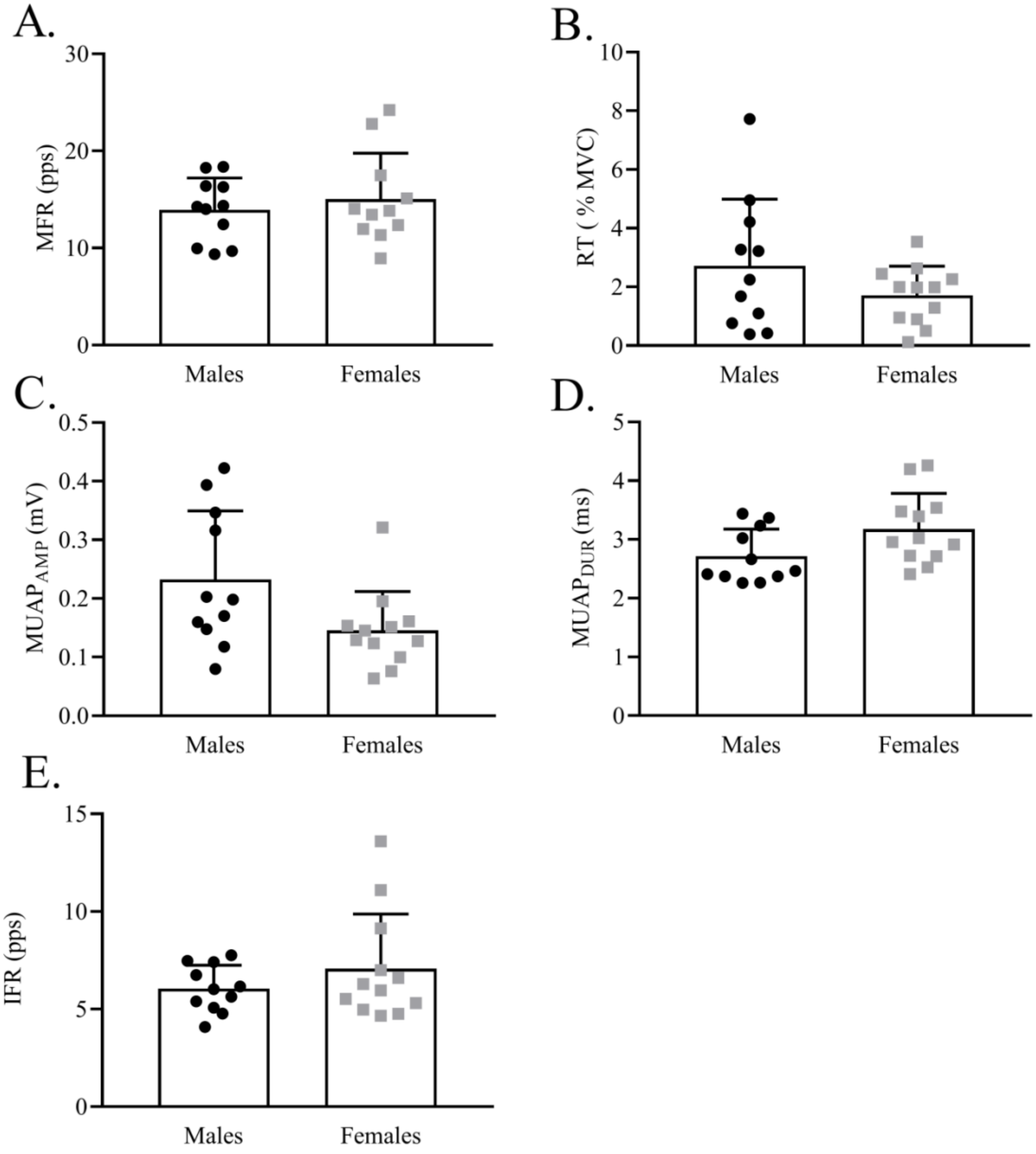

Figure 6. The individual values and mean (SD) for males and females for the 10% maximal voluntary contraction (MVC) for (A) mean firing rate (MFR, pulses per second, pps), (B) recruitment threshold (RT, %MVC), (C) motor unit action potential amplitude ($MUAP_{AMP}$, mV), (D) motor unit action potential duration ($MUAP_{DUR}$, ms) and (E) initial firing rate (IFR, pps).

Figure 7.

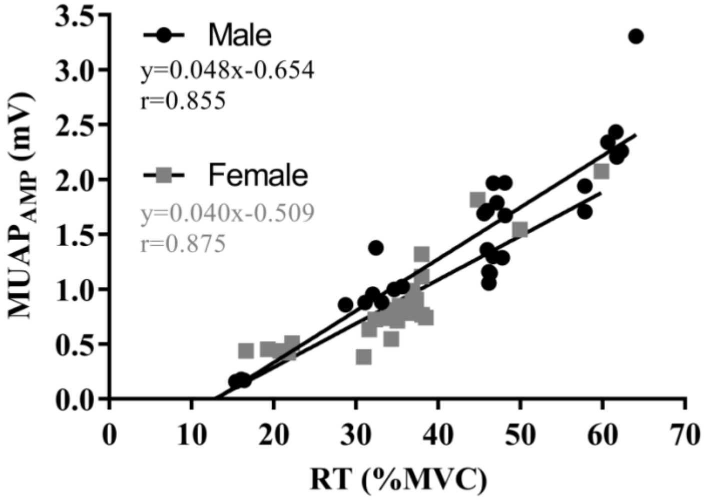

Figure 7. The plotted motor unit action potential amplitude (MUAP_{AMP}) vs. recruitment threshold (RT, expressed as percentage of maximal voluntary contraction [%MVC]) relationships for a male and female participant. The dark circles represent recorded MUs for the male subject and the grey boxes represent recorded MUs for the female subject with corresponding linear equations.

Figure 8.

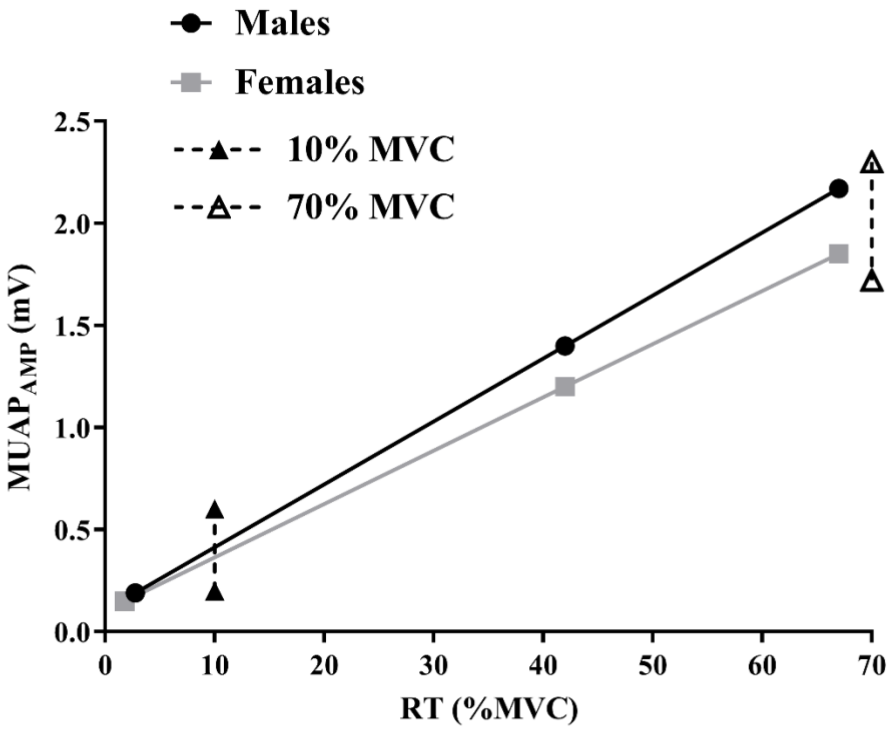

Figure 8. Plotted are the motor unit action potentials (MUAP$_{AMP}$, mV) vs. recruitment thresholds (RT, expressed as percentage of maximal voluntary contraction [%MVC]) relationships for the males and females. RTs were set at 1.7% (Female) and 2.7% (Male) MVC and 42% MVC from the 70% MVC. These RTs were chosen as it was the average RTs from the 10% MVC and each subject had MUs recorded with RTs up to 42% MVC during the 70% MVC. Linear regressions were used to estimate the AP$_{AMP}$ of the last theoretically recruited MU at 67% MVC for the males and females. The vertical dash lines are placed at the %MVC of the lower- and higher-contraction intensity to provide an indication of the sizes of MUs that participated in the respective contraction. It is evident that similar size MUs for males and female were active during the 10% MVC with an overall greater disparity in the sizes of active MUs during the 70% MVC.