EXAMINATION OF MUSCLE QUALITY AND MOTOR UNIT BEHAVIOR OF THE FIRST DORSAL INTEROSSEOUS OF NORMAL AND OVERWEIGHT CHILDREN

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B.S., University of Kansas, 2015

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ABSTRACT

EXAMINATION OF MUSCLE QUALITY AND MOTOR UNIT BEHAVIOR OF THE FIRST DORSAL INTEROSSEOUS OF NORMAL AND OVERWEIGHT CHILDREN

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Supervising Professor: Dr. Trent J. Herda

It is unknown whether differences in muscle quality or motor unit (MU) behavior exist between normal weight (NW) and overweight (OW) children **Purpose:** Therefore, the purposes of this study were to examine potential differences in the first dorsal interosseous (FDI) between NW and OW for peak maximal voluntary contraction force (MVC force), muscle cross-sectional area (CSA), subcutaneous fat (sFAT), and echo intensity (EI), as well as parameters of motor unit behavior, specifically motor unit action potential size ($MUAP_{SIZE}$) vs. recruitment threshold (RT), mean firing rate (MFR) vs. $MUAP_{SIZE}$, and MFR vs. RT relationships, as well as EMG amplitude (EMG_{RMS}[normalized as %max]). **Methods:** Anthropometric assessments were taken for body mass, BMI, and percent body fat (%BF). Ultrasonography scans of the FDI were completed in order to determine the muscle CSA, sFAT, and EI. MU behavior will be assessed during isometric muscle actions of the FDI at 20% and 50% MVC by the decomposition of the EMG signal from the surface of the skin. **Results:** OW was significantly greater than NW for mass ($P < 0.001$, NW = 30.96 ± 3.68 kg, OW = 41.37 ± 7.31 kg), BMI ($P < 0.001$, NW = 15.96 ± 0.94 kg/m^2, OW = 21.22 ± 2.19 kg/m^2), %BF ($P < 0.001$, NW = 17.01 ± 3.25%, OW = 31.01 ± 4.97%), sFAT ($P < 0.001$, NW = 2.19 ± 0.60 mm, OW = 3.71 ± 0.97 mm), and EI ($P = 0.002$, NW = 47.99 ± 6.01 AU, OW = 58.90 ± 10.63 AU). There were no differences between groups for CSA ($P = 0.688$, NW = 1.138 ± 0.146 cm^2, OW = 1.162 ± 0.156 cm^2), MVC force ($P = 0.790$, NW = 14.81 ± 3.49 N, OW = 14.43 ± 3.87 N) or MVC force/CSA ($P = 0.697$, NW = 13.00 ± 2.84 N/cm^2, OW = 12.52 ± 3.60 N/cm^2). However, NW had significantly greater lean CSA (CSA/EI) than OW ($P = 0.040$, NW = 0.024 ± 0.004 cm^2/AU, OW = 0.020 ± .005 cm^2/AU). For the $MUAP_{SIZE}$ vs. RT relationships, A terms were greater for NW (0.185 ± 0.12 mV) than OW (0.091 ± 0.05 mV) ($P = 0.002$), and for the MFR vs. $MUAP_{SIZE}$ relationships the B terms were less negative ($P = 0.039$) for the NW (-1.98 ± 1.36 pps/mV)

than OW (-2.79 ± 1.46 pps/mV). There were no differences between groups for the slopes and y-intercepts from the MFR vs. RT relationships or for EMG_{RMS}, however, slopes for the 50% MVC were less negative (-0.646 ± 0.18 pps/%MVC) than the slopes for the 20% MVC (-1.46 ± 0.64 pps/%MVC) and EMG_{RMS} was greater for the 50% MVC (86.23 ± 37.7%) than the 20% MVC (32.27 ± 12.4%).

Discussion: It is plausible that alterations in muscle architecture and/or stiffness as a result of greater intramuscular fat allowed the OW group to produce similar isometric MVC strength to the NW while possessing less lean CSA. This is supported by the finding that NW showed greater $MUAP_{SIZES}$ than OW at similar RTs and MFRs, which can be an indication of greater motor unit and muscle fiber size. The MFR vs. RT relationships for both groups agreed with the onion skin scheme, and were similar to what has previously been reported in adults, however, EMG_{RMS} for the 50% MVC was greater for children in the current study than what has been previously reported for adults or aged individuals during similar contractions of the FDI.

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TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

Background

The rising prevalence and issues associated with childhood overweightness and obesity are well documented (Ebbeling et al. 2002; Lobstein et al. 2004; Skinner and Skelton 2014). From 2011 to 2012, 32.2% of children ages 2-19 were considered overweight, while 17.3% were considered obese (Skinner and Skelton 2014). In addition, the prevalence of obesity has increased in children from 1999 to 2012 (Skinner and Skelton 2014). Overweightness and obesity in children are risk factors for serious health complications including type II diabetes, heart disease, increased risk of bone fractures, and non-alcoholic fatty liver disease (Lobstein et al. 2004). However, the impact of childhood obesity on muscle quality and neuromuscular performance has not been fully elucidated. Moreover, it is unknown how differences in muscle quality between normal weight (NW) and overweight (OW) children may affect motor unit (MU) behavior.

Ultrasonography is commonly used to measure cross-sectional area (CSA), or muscle thickness, and echo intensity (EI, a measure of muscle quality). EI has been reported to be sensitive to the infiltration of non-contractile tissues within the muscle (Reimers et al. 1993; Morse et al. 2005; Pillen et al. 2009; Cadore et al. 2012; Fukumoto et al. 2012; Wilhelm et al. 2014; Young et al. 2015; Lopez et al. 2016). For example, greater EI, indicating poorer muscle quality, is associated with lower strength, power, and functional performance in aging men (Wilhelm et al. 2014). Since EI is influenced by intramuscular fat (Pillen et al. 2009; Cadore et al. 2012; Wilhelm et al. 2014; Young et al. 2015), it would be expected that overweight individuals, which have higher percent body fat, would display greater muscle EI. Indeed, muscle EI is correlated with intramuscular fat as measured by MRI in large limb muscles in adults (age 20-61 years) (Young et al. 2015). However, no study has examined EI in healthy NW and OW children or its relation to neuromuscular performance.

In addition to substantially greater fat mass, obese individuals may possess a significantly greater quantity of fat free mass (FFM). This has been reported in adolescents (Maffiuletti et al. 2008; Abdelmoula et al. 2012) as well as adults (Hulens et al. 2001; Rolland et al. 2004; Lafortuna et al. 2005;

Maffiuletti et al. 2007). FFM is correlated with muscular strength independently of whole body mass or percent body fat. Thus, obese individuals have shown greater strength than normal weight individuals (Hulens et al. 2001; Rolland et al. 2004; Lafortuna et al. 2005; Maffiuletti et al. 2007; Maffiuletti et al. 2008; Abdelmoula et al. 2012). However, obese individuals display significantly lower muscle strength and/or power per unit body mass than lean individuals (Hulens et al. 2001; Lafortuna et al. 2005; Maffiuletti et al. 2007; Abdelmoula et al. 2012) including obese adolescents (Blimkie et al. 1990) and obese children (Blimkie et al. 1989). Blimkie and colleagues (Blimkie et al. 1989) reported obese children (age 9-13 years) had significantly reduced maximum isometric and isokinetic knee extension and elbow flexion strength when normalized for body weight. Similarly, obese adolescents (age 15-18 years) had reduced maximum isometric and isokinetic knee extension strength when normalized for body weight, as well as reduced voluntary activation during isometric MVCs in comparison to lean adolescents (Blimkie et al. 1990). Taken together these data suggest there may be deficiencies in the neuromuscular system in obese children.

It has been suggested that differences in strength between obese and normal weight individuals may be due to the training stimulus of increased body weight (Hulens et al. 2001; Hulens et al. 2002; Lafortuna et al. 2005; Maffiuletti et al. 2007; Tomlinson et al. 2014). However, this training stimulus does not appear to occur in the muscles of the hand, likely because the hand muscles do not operate in a way that either supports or lifts a significant portion of body mass. Rolland et al. (2004), reported differences in knee and elbow extension but not in hand-grip strength between lean and obese elderly women. Therefore, the FDI was chosen for this study in order to eliminate any training effect of greater body mass. The use of the FDI will also reduce the error caused by increased subcutaneous fat on electromyography (EMG) (Barkhaus and Nandedkar 1994; Roeleveld et al. 1997; Nordander et al. 2003) and EI (Young et al. 2015) measures.

Studies of MU behavior via electrical stimulation of anesthetized decerebrate cats have reported that MUs with higher recruitment thresholds (RT) achieve greater firing rates than MUs with lower RT (Eccles et al. 1958). Similar findings were reported for humans when analysis was performed on pooled data from multiple contractions and subjects (Gydikov and Kosarov 1974; Moritz et al. 2005; Tracy et al.

2005; Barry et al. 2007). In contrast, when MU firing rates are characterized separately for each contraction and subject during voluntary muscle actions in humans, firing rates of earlier recruited lower-threshold MUs are greater than later recruited higher-threshold MUs at targeted forces (De Luca et al. 1982; Stashuk and de Bruin 1988; Masakado 1994; Masakado et al. 1995; Herda et al. 2016). This has become termed as the *onion-skin control scheme* (De Luca and Erim 1994; McGill et al. 2005; De Luca et al. 2006; De Luca and Hostage 2010; De Luca and Contessa 2012; De Luca and Contessa 2015). This scheme places less reliance on higher-threshold MUs to maintain force in order to delay their fatigue (De Luca and Contessa 2015). This is beneficial as higher-threshold MUs are considered to be less resistant to fatigue than lower-thresholds MUs (Stephens and Usherwood 1977). In addition, the RT of MUs is related to their action potential size ($MUAP_{SIZE}$) which is an indication of the size of the MU and its muscle fibers (Pope et al. 2016).

While MU behavior has been extensively studied in healthy adults, to the author's knowledge, no data has been published reporting EMG amplitude (EMG_{RMS}) MU mean firing rates (MFR), RT patterns, or $MUAP_{SIZE}$ via EMG decomposition techniques for either obese individuals or children. In addition, lower muscle quality is associated with reduced maximal and functional force production (Cadore et al. 2012; Fukumoto et al. 2012; Wilhelm et al. 2014; Lopez et al. 2016). Miller et al. (2017) reported aging individuals with greater EI display increased neural cost (EMG amplitude) during submaximal contractions and, consequently, greater MU recruitment. Examining MU behavior in children, which may be altered in overweight children as a result of lower muscle quality, is warranted.

Purpose

Therefore, the purposes of this study are to examine potential differences in the first dorsal interosseous (FDI) between normal weight and overweight children for (1) maximum voluntary contraction force ([MVC force] absolute, and normalized to FDI CSA), (2) CSA and EI to elucidate the contribution of muscle quality on force production and MU behavior, and (3) MU behavior, specifically $MUAP_{SIZE}$ vs. RT, MFR vs. $MUAP_{SIZE}$, and MFR vs. RT relationships, as well as EMG_{RMS}. MU behavior will be assessed via isometric muscle actions of a small hand muscle, the FDI, from low to moderate intensity (20% – 50% of MVC). We hypothesize that (1) OW children will show decreased muscle quality which will result in lower

strength per unit CSA, (2) OW will show differences in MUs recruited, or altered behavior of MUs to reach targeted forces in the FDI. Differences may become more apparent at higher contraction intensities (50% MVC). It is unclear if RTs, MFRs, or $MUAP_{SIZE}$, or a combination of these will be altered to reach the targeted forces.

Hypothesis and Specific Aims

Hypothesis

Overweight children will show decreased muscle quality which will result in lower MVC force per unit CSA, and differences in MUs recruited, or altered behavior of MUs to reach targeted forces in the FDI.

Specific Aim #1

To assess differences in FDI muscle quality, via EI, between NW and OW children.

Specific Aim #2

To determine differences in FDI isometric MVC force, and MVC force per unit CSA between NW and OW children.

Specific Aim #3

To assess differences in MU behavior, specifically MFRs, MU recruitment patterns, $MUAP_{SIZES}$, and EMG amplitude during submaximal isometric muscle actions of the FDI.

Operational Definition of Terms

Isometric MVC force (MVC force) – peak force recorded from an isometric maximum voluntary contraction. Expressed in terms of Newtons (N).

Echo Intensity (EI) – The mean greyscale of a selected area (muscle cross-section) of an ultrasound image. Expressed in terms of arbitrary units (AU) on a scale from 0 (black) to 255 (white).

Surface Electromyography (sEMG) – a recording of the MUAPs that move across the sarcolemma and are detected by a surface electrode. The signal amplitude and frequency contain physiological information concerning the recruitment and firing rates of motor units during muscle actions.

Delimitations

Twenty-eight children between the ages of 8 and 10 years were recruited for this study. All participants gave verbal assent to participate, and their guardians completed a written informed consent form prior to any testing. Subjects reported no current or ongoing injuries or neuromuscular diseases related to the hand or wrist.

Assumptions

Theoretical assumptions

1. Subjects gave full effort in all isometric MVC tests.
2. Subjects accurately achieved and maintained force during isometric submaximal muscle actions.
3. Echo intensity is a valid measure of muscle quality and extraneous factors affecting echo intensity (excluding fat and fibrous tissue) did not significantly alter echo intensity measurements.
4. There were no significant training status differences in the FDI between NW and OW groups due to increased body weight or lifestyle.
5. All equipment was calibrated and functioning properly throughout all tests and testing sessions.

Statistical assumptions

1. The population from which the samples were drawn is normally distributed.
2. The data meet the assumption of sphericity, which requires that repeated measures data have both homogeneous variance and covariance.
3. The data was based on a parametric scale, either interval or ratio.

Limitations

Subjects were recruited by flyers and by word of mouth, and as such, cannot be considered completely random. In addition, surface EMG cannot detect every active MU during muscle actions; consequently, information from many MUs that contributed to muscle force were not observed.

CHAPTER II

REVIEW OF LITERATURE

Strength and Functional Performance in Normal and Overweight or Obese Populations

Hulens et al. (2001)

The purpose of this study was to determine whether differences exist in peripheral muscle strength between lean and obese women. Lean and obese groups were controlled for age and phyiscal activity levels using an allometric approach, and the study design was cross-sectional. 173 obese (age = 39.9 ± 11.4 years, BMI = 37.8 ± 5.3 kg/m^2) and 80 lean (age = 39.7 ± 12.2 years, BMI = 22.0 ± 2.2 kg/m^2) participated in this study. Weight and height were measured and recorded in addition to body composition via the bioelectrical impedence method (Bodystat 1500, Bodystat Ltd. Douglas, United Kingdom). Isometric handgrip maximum voluntary contractions were performed (JAMAR Dynamometer), as well as isokinetic leg flexion and extension, and trunk flexion, extention, and rotation, strenth tests (Cybex 350 dynamometer, Lumex Inc., Ronkonkoma, NY). Absolute isokinetic strength was greater in obese women in comparison to lean women, for all strength test excluding knee flexion and isometric handgrip. Pearson correlation coefficients between strength tests and fat-free mass were low or moderate in both lean ($r = 0.28 – 0.53$, $P < 0.05$) and in obese ($r = 0.29 – 0.49$, $P < 0.001$) women. Fat mass was not correlated with measures of strength in the lean, but was for obese women in all isokinetic tasks excluding knee flexion ($r = 0.21 – 0.39$, $P < 0.01$). Although obese women displayed greater isokinetic back extensor, oblique abdomial, and quadricep strength, when strength measurements were normalized by fat-free mass all measures of strength were no less than 6% lower for obese women in comparison to lean women. When measures of strength are corrected for fat-free mass obese women have lower strength per unit of fat-free mass than lean individuals.

Lafortuna et al. (2005)

The purpose of this study was to evaluate differences in body mass (BM) and composition, in addition to muscle strength (ST) and power output ($\dot{W}$) between obese (OB) and normal weight (NW) males and females, and to determine whether variations in body composition influence muscular

performance in OB. 95 OB subjects (28 male, 67 female; age = 29.3 ± 7.0 years; BMI = 41.2 ± 4.4 kg/m^2) and 18 NW subjects (8 male, 10 female; age = 30.3 ± 5.3 years; BMI = 22.6 ± 2.1 kg/m^2) participated in the study. A two-component bioelectric impedence analysis (Human IM, Dietosystem, Italy) was used to determine body composition for all subjects. Overall upper limb and lower limb (antigravity muscles) strength were measured using isotonic machines (Technogym, Cesena, Italy) and the sum of the two tests was considered ST. Testing consisted of a 1 repitition maximum test (S_{1RM}) for both leg press and chest press. Lower limb maximum ($\dot{W}$) was measured during a maximal vertical jump test consisting of 5 consecutive maximal vertical jumps. $\dot{W}$ was derived from the flight time and contact time of the subjects feet by an optical acquisition system (Optojump, Microgate, Italy). Both male and female OB subjects had significantly greater ($P < 0.001$) fat mass (FM) and fat-free mass (FFM) than NW. Male OB subjects differed from male NW subjects by an equal contribution of excess FM and FFM; whereas female OB subjects mainly differed from female NW subjects in the quantity of FM. Because there was a correlation between FFM and ST (ST (N) = 64.4 FFM (kg) – 190.0, $R^2 = 0.612$) OB exhibited greater strength than NW ($P < 0.05$). As expected male subjects had greater ST than female subjects for both upper and lower body tests when collapsed across group ($P < 0.05 – 0.001$). When S_{1RM} was analyzed to measure strength separately for upper and lower body, it was observed that OB had significantly greater lower body strength ($P < 0.01$) than NW, but the groups did not differ significantly for upper body strength ($P > 0.05$). OB showed similar $\dot{W}$ to NW subjects; however, when normalized to body weight OB had significantly lower ($P < 0.001$) $\dot{W}$ than NW subjects. The results of this study indicate there are differences in OB body composition between males and females. ST is positively correlated with FFM, thus OB subjects showed greater ST, although the upper body S_{1RM} was similar between OB and NW. Finally OB subjects produced $\dot{W}$ similar to that of NW, but when normalized for body mass it was revealed that NW subjects produced more $\dot{W}$ per unit body mass than OB, suggesting that the less favorable ratio of the contractile element to fat mass in OB dissallowed them from using the force-velocity curve advantageously.

Blimkie et al. (1989)

The purpose of this study was to determine whether the inferior strength and power performance displayed by overweight and obese children in activities that involve support of or lifting of body weight could be attributed to a lower strength to muscle area ratio in the obese. 11 nonobese (age = 11.1±1.6 years, body fat percentage = 16.6% fat) and 13 obese (age = 11.2±1.2 years, body fat percentage = 35.5±3.1% fat) participated in the study. Isometric peak torque was measured at 4 joint angles for elbow flexion (80°, 90°, 120°, and 150°) and knee extensions (90°, 120°, 140°, and 160°) using a custom-made dynamometer. Isokinetic peak torque was measured at 4 angular velocities for elbow flexion and knee extenstion (0.52, 1.05, 2.09, and 3.14 rad/s) using a cybex II isokinetic dynamometer (Lumex Inc, Ronkonkoma, NY). Nonvoluntary twitches were performed for the elbow extensors to obtain measures of twitch torque, time to peak torque, and half-relaxation time before any maximal strength tests were performed. In addition, elbow flexor and knee extensor cross-sectional areas (CSA) were examined using computed axial tomography. CSA scans were performed at the mid-upper arm and the mid-thigh level. When compared to lean subjects, obese subjects had significantly greater ($P < 0.05$) mass (Obese = 56.4 ± 13.7 kg, lean = 38.0 ± 11.5 kg), BMI (Obese = 24.6 ± 3.8 kg, Lean = 17.2 ± 1.8 kg), and percent body fat (Obese = 35.5% ± 3.1%, Lean = 16.6% ± 1.7%). Peak voluntary isometric and isokinetic torque were no different between groups; however, when normalized for body weight there was a significant main effect for group ($P < 0$ 0.5), where nonobese boys displayed larger torque relative to body size for both the knee extension and elbow flexion tests. No differences were seen between groups for twitch torque, time to peak torque, or half-relaxation time. The results of this study indicated because differences in strength did not appear until normalized for body weight the differences in performance between obese and nonobese children in tasks that involve the weight of the body must not be attributed to a strength deficit. Rather, differences in motivation, skill, coordination, motor unit behavior, and the presence of extra fat mass more likely account for these differences.

Blimkie et al. (1990)

The purpose of this study was to determine whether neural and/or muscular factors contribute to the inferior motor performance of obese adolescents. 10 non-obese (14.6% ± 3.1% body fat) and 11 obese (32.3% ± 1.8% body fat) males which were age (15-18 years), maturity (Tanner stages IV and V), lean body mass, and height matched between groups participated in this study. Maximal isometric (IS) knee extensions (KE) were performed on a custom-made dynamometer at knee joint angles of 1.57, 2.09, 2.44, and 2.79 rad (3.14 rad = full knee extension). The torque signal was recorded from a strain gauge as peak torque (PT) which represented isometric knee extension strength the (IS KE strength). Evoked IS contractile properties of twitch torque (TT), time to peak torque (TPT), and half-relaxation time (HRT) were measured at the joint angle of 1.57 rad before any maximum strength tests were performed, and the interpolated-twitch technique was used to assess motor unit activation (MUA). Maximal isokinetic (IK) knee extensions were performed on a cybex II isokinetic dynamometer (Lumex Inc, Ronkonkoma, NY) at angular velocities of 0.52, 1.05, 2.09, and 3.14 rad/s. Torque values were recorded as IK KE strength. A cross-sectional area (CSA) measurement was made at the mid-thigh level using computed tomography. Obese subjects had significantly larger ($P < 0.05$) total CSA and fat CSA than non-obese; however, there was no difference between groups for the CSA of lean mass in the thigh ($P > 0.05$). There were no differences between groups for absolute strength or power, although obese subjects had signifcantly lower ($P < 0.05$) IS and IK KE strength in terms of strength per kilogram body mass. There were no significant differences ($P > 0.05$) between any of the evoked twitch contractile properties (TT, TPT, and HRT). Obese subjects had significantly lower ($P < 0.05$) mean MUA (85.1%) in comparison to non-obese subjects (95.3%) during the IS KE strength tests. Because there were no differences in KE strength between obese and non-obese subjects it is unlikely that motor performance deficits in obese adolescents is due to the intrinsic properties of the muscle. It appears more likely that the deficit in motor performance is due to the inability of the obese subjects to attain high levels of MUA during maximal strength tests. However, the negative influence of extra body weight in all motor activities that involve the movement or support of body weight cannot be discounted as a handicapping factor for obese adolescents.

Motor Unit Behavior and the Onion-Skin Control Scheme

De Luca and Erim (1994)

The purpose of this article was to review original research on motor unit (MU) behavior and discuss the potential MU control schemes that govern the operation of MUs. Joint analysis of firing-rate data from multiple MUs during a single contraction revealed concurrently active MUs modulate firing rates in a highly interdependent fashion, such that all concurrently active MU firing rates vary simultaneously where increases or decreases occur mutually for all MUs. This phenomenon, which has been observed in multiple muscles and in over 300 contractions, suggests that the activation of motor units is controlled by a "common drive." According to the common drive concept the CNS regulates the net sum of excitatory and inhibitory inputs to the motorneuron pool. Motorneurons belonging to the same pool receive the same drive at any given time. Differences in recruitment threshold and firing rates of MUs are determined by the architecture of the motorneuron pool. As excitation to the motorneuron pool increases, increasingly larger MUs are recruited (higher-threshold MUs) and the firing rates of all active MUs increase. As excitation decreases firing rates decrease and MUs are derecruited in reverse fashion, such that higher-threshold MUs become inactive before lower-threshold MUs. Another property of the scheme controlling the operation of MUs during muscle actions is the *"onion-skin"* phenomenon. The onion-skin property of MU behavior is that earlier recruited MUs maintain greater firing rates than later recruited MUs during any point of an isometric muscle action. This results in an orderly nesting of motor unit firing rate curves under one another. The shape of the firing rate curves appears to resemble an onion-skin. This phenomenon represents a paradox in the conventional understanding of MU behavior. It is well known that higher-threshold MUs have shorter duration yet more forceful twitches compared to low threshold MUs, and require greater firing rates to reach tetanus. If the neuromuscular system was designed to maximize force output higher-threshold MUs would be driven to attain higher firing rates than lower threshold MUs. This is not so, which suggests that higher-threshold MUs operate at lower firing rates in order to delay fatigue. This model overall represents the basic rules which control muscle force and explain the behavior of MUs under different conditions.

De luca et al. (2015)

The purpose of this study was to mathematically model and compare the previously widely accepted after-hyperpolarization (AHP) scheme for motor unit (MU) behavior with the more recently observed onion-skin control scheme in order to determine the characteristics and implications of each scheme. Total muscle force is modulated by the number of active MUs and their firing rates. The AHP scheme maintains that MU recruitment threshold is directly related to MU firing rates, which was first observed in electrically stimulated cat motorneurons, but also reported for voluntary contractions in humans in some scenarios. This control scheme for MU behavior would optimize force as the firing properties of MUs would match their mechanical properties. Lower-threshold MUs have slower and smaller amplitude force twitches in comparison to higher-threshold MUs, and require lower firing rates to tetanize. Thus if all MUs fired at frequencies that would produce twitch fusion the force would be optimized for each active MU. However, it has been more recently shown that at any time during an isometric voluntary constant-force contraction in humans, an inverse relationship exists between MU recruitment threshold and MU firing rates, such that lower-threshold MUs consistently maintain higher firing rates than higher-threshold MUs. This has been referred to as the onion-skin control scheme. Based on previous data for both MU control schemes mathematical models were created in which the firing rate of all MUs from a theoretical human first dorsal interosseous (FDI) and vastus lateralis (VL) were plotted against percent maximal input excitation. Based on these models it was noted that higher-threshold MUs in the AHP scheme display greater minimum and maximum firing rates in comparison to higher-threshold MUs in the onion skin scheme. Force output is greater from 0-60% maximum input excitation for the onion-skin scheme, and maximal theoretical force is ~20% greater for the FDI and ~30% greater for the VL for the AHP scheme. To examine the fatigability of the muscles based on the two different schemes a simulation was performed in which both muscles sustained a contraction at 50% maximum voluntary contraction (MVC) and the time until fatigue was observed. The AHP simulation showed that both the FDI and the VL fatigued faster for the AHP than the onion-skin scheme. Thus, the onion-skin scheme of MU control has better initial force production and is superior in delaying the fatigue of the motorneuron pool. The onion-skin scheme also maintains a reserve capacity to increase force as

high-threshold MUs are not tetanized at MVC which aligns with the fight-or-flight reflex response and is more advantageous from an evolutionary standpoint.

Herda et al. (2016)

The purpose of this study was to investigate the change in motor unit (MU) firing rates (FR) at de-recruitment (DEREC) relative to recruitment (REC) and the relationship between these properties of the MUs to % type I myosin heavy chain isoform content (type I %MHC) of the vastus lateralis (VL). 6 men (age = 20 ± 1 years) and 4 women (age = 20 ± 1 years) participated in this study. Participants performed isometric 50% maximum voluntary contractions (MVC) of the knee extensors, which consisted of a 12 second force plateau preceded and followed by 10% MVC/s ramp up and ramp down phases. Muscle actions were completed on an isokinetic dynamometer (Biodex System 3; Biodex Medical Systems, Shirley, NY, USA). Force was measured by a load cell (LC; Omegadyne, Sunbury, OH, USA) and surface EMG of the vastus lateralis (VL) was recorded via a 5-pin electrode (Delsys, Boston, MA, USA) during each muscle action. A muscle biopsy of the VL was collected from each subject and analyzed for type I %MHC using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). When firing rate at recruitment (FR_{REC}) was regressed against REC for each subject the mean y-intercept was 9.67 ± 3.79 pulses per second (pps) and the slope was –0.28 ± 0.54 pps/%MVC. Thus, in agreement with previous modelling of MU behavior. When the change in firing rate from from firing rate at recruitment to firing rate at derecruitment (ΔFR) was regressed against FR_{REC} for each subject the mean y-intercept was 6.38 ± 2.69 pps and the slopes were –0.68 ± 0.39 pps/%MVC. Thus, FR_{DEREC} was greater than FR_{REC} for MUs that had lower FR_{REC}. The negative slope indicates that the ΔFR will decrease as FR_{REC} increases, and at a certain point will cross zero, such that lower threshold MUs which display higher FR_{REC} will display a negative ΔFR. Type I %MHC was negatively correlated to the slope of the ΔFR vs. FR_{REC} relationship ($P = 0.006$, $r = –0.793$). Therefore, the rate of change in MU firing rates from recruitment to derecruitment in an isometric 50% MVC knee extension was related to type I %MHC isoform content of the VL. In addition the majority of MUs displayed greater FR_{DEREC} than FR_{REC} which

was likely an indication of fatigue related decreases in twitch forces. This effect was less pronounced in individuals with greater type I %MHC isoform content.

Trevino et al. (2016)

The purpose of the study was to correlate MU firing rates at recruitment, targeted force, and decruitment with the contractile properties, specifically the percent type I myosin heavy chain isoform content (type I %MHC), of the muscle *in vivo*. 12 participants (age = 20.7 ± 2.35) participated in this study. Participants performed isometric 40% maximum voluntary contractions (MVC) of the knee extensors, which consisted of a 12 second force plateau preceded and followed by 10% MVC/s ramp up and ramp down phases. Muscle actions were completed on an isokinetic dynamometer (Biodex System 3; Biodex Medical Systems, Shirley, NY, USA). Force was measured by a load cell (LC; Omegadyne, Sunbury, OH, USA) and surface EMG of the vastus lateralis (VL) was recorded via a 5-pin electrode (Delsys, Boston, MA, USA) during each muscle action. The EMG signal was decomposed in order to observe the firing events of individual motor units (MUs). Using the decomposed firing instances of individual MUs, regressions were created for each subject's contraction for (1) firing rate at recruitment vs. recruitment threshold, (2) mean firing rates at steady force vs. recruitment threshold, and (3) firing rates at derecruitment vs. derecruitment threshold. Type I %MHC was quantified from muscle biopsies of the vastus lateralis (VL) using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Correlations were examined between type I %MHC and the slopes and y-intercepts of the three previously mentioned relationships. Type I %MHC was correlated with MU firing rates at recruitment (y-intercepts: $r = –0.577$; slopes: $r = 0.741$) and target force (slopes: $r = 0.853$) vs. recruitment threshold, and the MU firing rates at derecruitment (y-intercept: $r = –0.597$; slopes: $r = 0.701$) vs. derecruitment threshold relationships. Despite this, the majority of the MU firing rates vs. recruitment and derecruitment relationships were not significant ($P > 0.05$). However, MU firing rates during the steady force behaved consistently with the onion-skin control scheme for MU behavior, such that lower-threshold earlier recruited MUs consistently displayed higher firing rates than lower-threshold later

recruited MUs. This study supports the hypothesis that there exists a relationship between type I %MHC isoform content and the behavior of MUs during isometric contractions *in vivo*.

Hu et al. (2013)

The purpose of this study was to characterize the recruitment and firing rate organization of multiple concurrently active motor units (MU) of differing action potential amplitude in the first dorsal interosseous (FDI) muscle, and to assess the relation between putative MU size and the firing rate and recruitment threshold properties of these MUs. 4 males and 4 females participated in this study. Surface electromyography (EMG) signals were recorded from the FDI during submaximal isometric trapezoidal muscle actions of the right index finger. Muscle actions were performed at 20%, 30%, 40%, and 50% of each subject's maximum voluntary contraction (MVC) strength. Subjects performed said contractions while seated on a Biodex chair (Biodex Medical Systems, Shirley, NY, USA) with a custom made set-up for isometric abduction of the index finger where a 6 degrees-of-freedom load cell (ATI; no. 3226) was used to measure the force signal. Spike trigger averaging (STA) was performed on each of the four surface EMG signals to determine motor unit action potential (MUAP) amplitude, which was then used to estimate the size of the MU itself. The results of this study indicated that for MUs in the FDI the peak-to-peak amplitude of the MUAPs increased in an orderly manner as recruitment threshold increased. This finding agrees with the size principle of MUs. The mean firing rate (MFR) during steady force segments also decreased as a function of MUAP amplitude which agrees with the onion-skin property of MUs. The fit properties of these relationships were averaged across subjects, but contraction intensities were kept separated for analysis. All recruitment threshold vs. MUAP amplitude relationships had slopes significantly different than zero ($P < 0.05$) and r^2 values for the four different contraction intensities ranged from $0.46 \pm 0.03 – 0.55 \pm 0.05$. The r^2 values for the MFR vs. MUAP amplitude relationships for the four different contraction intensities ranged from $0.41 \pm 0.03 – 0.52 \pm 0.04$. MUAP amplitude, which is here used as an estimate of MU size is related to recruitment threshold and mean firing rates. These findings are in agreement with the reported size principle and onion-skin properties of MUs.

Composition of Muscle: Echo Intensity, Muscle Quality, and Fiber Type

Pillen et al (2009)

The purpose of this study was to examine the correlation between muscle ultrasound echo intensity (EI) and muscle structure, specifically the percentage of interstitial fibrous tissue and fat, via muscle biopsy. 14 muscles of 2 golden retriever dogs (GRMD) which suffered from muscular dystrophy were analyzed for this study. The muscles analyzed were rectus femoris, adductor magnus, Sartorius, tibialis anterior, deltoid (in its three separate parts), supraspinatus, and trapezoid. Ultrasound images were taken using a phased array real-time scanner (Sonos 2000 Phased Array Imaging System; Hewlett-Packard Co., Andover, MA, USA), at the muscle belly (defined as the location of mamimal muscle diameter) and at 1 cm proximal to the muscle belly. Images were analyzed using greyscale analysis on a scale from 0 (black) to 255 (white) in arbitrary units (AU). Following the ultrasound measurements the dogs were euthanized and cross-sectional muscle specimens were collected at the site of the muscle belly. The percentage of fibrous tissue and fat were measured using digital image analysis. Muscle EI was significantly and positively correlated to fibrous tissue ($r = 0.87$; $P < 0.001$). However, no significant correlation between fat content and EI was observed ($r = –0.05$; $P = 0.82$). In conclusion EI is related to the infiltration of fibrous tissue in dog muscle, and EI is also related to fat infiltration based on previous literature.

Young et al. (2015)

The purpose of this study was to compare muscle ultrasound echo intensity (EI) with MRI, and to establish calibration equations to estimate percent intramuscular fat from EI. 31 men and women (age = 20 – 61 years) participated in this study. Participants completed either 2 or 3 visits. The first visit consisted of ultrasound scanning of 4 leg muscles (1) rectus femoris (RF), (2) biceps femoris (BF), (3) tibialis anterior (TA), and (4) medial gastrocnemius (MG) of the dominant leg. Ultrasound images were taken using a LOGIQ e ultrasound-imaging device (GE Healthcare UK, Ltd., Chalfont, Buckinghamshire, UK). Muscle ultrasound EI was examined using greyscale analysis on the image analysis program ImageJ. Based on a regression between subcutaneous fat thickness, which was altered by applying

different amounts of pressure to the skin via the ultrasound probe, and muscle EI a correction factor for subcutaneous fat in the EI measurement was created according to the following equation

Correction factor (*cf*) = -39.887 (1.0 cm) + 80.4148 = 40.5278

Subsequently, the *cf* (*cf* = 40.5278) was used in the following equation to correct for the influence of subcutaneous fat thickness on the EI measurement.

$$y_2 = y_1 + (x * CF)$$

The second visit consisted of the MRI experimental protocol. T1-weighted MRI images (TR = 800 ms, TE = min full) were obtained using a 3.0-Tesla whole-body MR system (GE Healthcare, Waukesha, Wisconsin). MRI images were taken at the same location of the ultrasound images. MRI images were also analyzed using ImageJ. Voxel intensities were determined (DVI) for the different tissue types: fat, connective tissue, and muscle. DVI values for the tissue types were then used to calculated the percentage of fat within the muscle. The third visit was optional and used for ultrasound reliability purposes. Correction for subcutaneous fat thickness yielded the strongest correlations between the corrected EI and the MRI percent fat content of all 4 muscles (r = 0.91 in RF, r = 0.80 in BF, r = 0.80 in TA, r = 0.76 in MG). The calibration equation for the quantification of intramuscular fat in EI images was:

$$y – (0.114 * [40 * z] + x) + 5.231$$

when muscles and genders were grouped, where x = raw EI, y = percent intramuscular fat, and z = subcutaneous fat thickness. In conclusion calibration equations which quantify percent intramuscular fat from muscle EI images have been created, and a correction factor has been established to correct for the potential influence of subcutaneous fat thickness on ultrasound EI measurements.

Lee et al. (2012)

The purpose of this study was to examine the relationships between insulin sensitivity (IS), skeletal muscle (SM) mass and SM quality assessed from magnetic resonance imaging (MRI). 40 obese adolescent boys (age = 12 – 18 years, BMI = 35.0 ± 4.6 kg/m^2) participated in this study. IS was measured in a fasted state by a 3-hour hyperinsulinemic (80 mU/m^2/min) – euglycemic clamp. A 2-hour

oral glucose tolerance test (OGTT(1.75 g/kg, maximum 75 g)) was completed on subjects in a fasted state and results were expressed as glucose and insulin area under the curce (AUC). Whole body MRI data were collected using a 3.0 Tesla magnet (Siemens Medical Systems, Erlangen, Germany), or a 1.5 Tesla MRI system (GE Medical Systems, Milwaukee, WI, USA) for subjects where dental braces were a concern. Intramuscular adipose tissue (IMAT) and SM mass values were determined using MRI data. IMAT was defined as the adipose tissue between bundles of SM below the fascia, and MRI IMAT and SM volumes were converted to mass using assumed density values of those tissues. Muscular strength was assessed by calculating the sum of a 1 repitition maximum bench press test as well as a seated leg press test using a weight stack machine (Life Fitness, Schiller Park, IL, USA). SM mass was not related ($P > 0.1$) to IS or any OGTT parameters. However, IMAT was significantly inversely related to IS ($r = -0.53$, $P < 0.01$) and positively related OGTT-insulin AUC ($r = 0.31$, $P < 0.05$). Muscle strength was associated with IS ($r = 0.39$, $P = 0.01$) and OGTT-insulin AUC ($r = -0.32$, $P = 0.04$). After normalizing muscular strength for race and Tanner stage, IMAT and muscular strength together explained 41% of the variance in IS in obese adolescent boys. SM quality, as assessed by IMAT and muscular strength, is an important factor of IS in obese adolescent boys. SM quality may be more important than SM mass in determining IS in obesity. Together, these findings suggest that there may be a link between metabolic and mechanical muscle functions.

Fukumoto et al. (2012)

The purposes of this study were to determine whether muscle quality, assessed via ultrasound echo intensity (EI, measured via greyscale analysis), is associated with muscle strength and to examine the relationship between muscle EI and body composition. 92 healty women (age = 70.4 ± 5.5 years) participated in the study. Muscle thickness (MT), subcutaneous fat thickness (FT), and EI of the right quadriceps muscles were assessed from cross-sectional images taken by an ultrasound machine (LOGIQ e; GE healthcare UK Ltd., Chalfont, Buckinghamshire, England). Percent body fat (%BF) was estimated via bioelectrical impedence analysis using an impedence instrument (Muscle-α; Art Haven 9, Kyoto, Japan). Maximal isometric strength of the right knee extensors was measure during 3 second maximal

voluntary contractions via an isometric dynamometer (Isoforce GT-330; OG GIKEN Co., Okayama, Japan). EI was not significantly related to body composition. Both MT and EI were significantly correlated with muscle strength when using age as a control variable, such that the partial correlated coefficient between EI and strength was -0.33 ($P < 0.01$) and was 0.40 ($P < 0.01$) between MT and strength. This study was the first to investigate the relationship between strength and EI using greyscale analysis, and reports muscle quality assessed via EI was related to quadriceps strength independently of age in middle-aged to elderly persons.

Tanner et al. (2002)

The purpose of this study to test the hypothesis that muscle fiber type is related to obesity. 8 lean (age = 41.3 ± 2.3 years, BMI = 25.4 ± 1.3 kg/m^2) and 11 obese (age = 42.3 ± 2.3 years, BMI = 33.3 ± 1.3 kg/m^2) African-American women, as well as 20 lean (age = 42.0 ± 1.6 years, BMI = 24.1 ± 0.9 kg/m^2) and 14 obese (age = 39.9 ± 1.8 years, BMI = 34.6 ± 1.1 kg/m^2) Caucasian women who were undergoing voluntary abdominal surgery participated in this study. During their surgery a muscle biopsy was taken of the rectus abdominus for fiber type analysis. Muscle tissue samples were stained for ATPase activity at pH levels of 4.54 and 10.9 in order to distinguish fiber types I, IIa, and IIb. In addition, several morbidly obese women who underwent a gastric bypass were also examined ~1 year post-operation in order to determine the decrease in body mass and if this was affected by fiber type. Subjects that were placed in the lean group because of their lower BMI had a significantly higher ($P < 0.001$) percentage of type I fibers (lean = 54.6% ± 1.8%, obese = 41.5% ± 1.8%), wheras obese subjects displayed a significantly greater ($P < 0.001$) percentage of type IIb fibers (lean = 14.4% ± 1.5%, obese = 25.1% ± 1.5%). BMI was directly correlated ($r = 0.49$, $P < 0.001$) to the percentage of type IIb muscle fibers. African-American women had a significantly lower ($P < 0.01$) percentage of type I muscle fibers in comparison to Caucasian women (African-American = 43.7% ± 2.8%, Caucasian = 51.8% ± 1.8%), but possessed significantly higher ($P < 0.01$) type IIb fibers (African-American = 23.4% ± 2.9%, Caucasian = 16.3% ± 1.2%). Additionally, 14 morbidly obese women who returned ~1 year post-gastric bypass operation revealed positive relationships between the percentage of type I muscle fibers at the time of the

operation and the change in BMI ($r = 0.55$, $P < 0.05$) and body mass ($r = 0.56$, $P < 0.05$) ~1 year post-gastric bypass surgery. Muscle fiber type, specifically types I and IIb, are related to obesity such that obese women were shown to have greater proportions of type IIb muscle fibers and smaller proportions of type I muscle fibers. African-American women also had a lower percentage of type I fibers but a greater percentage of type IIb muscle fibers. A greater percentage of type I fibers was beneficial for weight loss follwing gastric bypass surgery.

CHAPTER III

METHODS

Subjects

Twenty-eight healthy children between the ages of 8 and 10 were recruited to participate in this study. Children were placed into the NW (12 boys, [mean ± SD] age = 8.8 ± 0.7 years, stature = 138.6 ± 7.6 cm, mass = 31.0 ± 3.2 kg; 5 girls, age = 8.8 ± 0.8 years, stature =140.5 cm, mass = 30.9 ± 5.2 kg) or OW (5 boys, age = 9.2 ± 0.8 years, stature = 141.0 ± 5.3 cm, mass = 44.2 ± 4.8 kg; 6 girls, age = 8.8 ± 1.0 years, stature = 137.6 ± 8.8 cm, mass = 39.0 ± 8.6 kg) groups according to BMI and estimated percentage of body fat percentage (%BF). BMI standards were based on Center for Disease Control and Prevention (CDC) growth charts and %BF standards provided by Laurson et al. (2011). Children with BMIs and %BF between the 5^{th} and 75^{th} percentile for their age were considered NW. Children with BMIs ≥ 85^{th} percentile and %BF ≥ 85% were considered OW. Participants reported no current or ongoing neuromuscular diseases or musculoskeletal injuries specific to the hand or wrist. This study was approved by the University Institutional Review Boards for Human Subjects. Participants provided verbal assent and guardians completed a written informed consent form and a Pre-Exercise Testing Health & Exercise Status Questionnaire.

Research Design

The participants visited the laboratory two times separated by at least 24 hours. The first visit was a familiarization trial followed by an experimental trial. During the first visit the participants practiced the isometric trapezoidal muscle action several times. Ultrasonography scans of the FDI were completed during the familiarization trial in order to determine the muscle cross-sectional area (CSA) and echo intensity (EI). In addition, a two-site skinfold test was completed on each subject.

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 to fifth fingers were immobilized with a Velcro strap. The thumb was restrained with a strap that allowed for a 90° angle between the index finger and thumb during the isometric contractions. The muscle action of the first dorsal interosseous (FDI) was isolated and

measured by instructing the participants to abduct the index finger against a small flat piece of metal connected to the force transducer (MB-100; Interface, Inc., Scottsdale, AZ). Subjects performed three maximum voluntary isometric muscle actions (MVCs) with two minutes of rest between each contraction. Strong verbal encouragement was given during the MVCs. The peak force from the three MVCs was used to determine the target force amplitude for the 20% and 50% MVC isometric trapezoidal muscle actions. For each submaximal isometric muscle action force was increased at a rate of 10% MVC/s to the target force, and then decreased at a rate of 10% MVC/s back to baseline. The target force was maintained for 12 s for the 20% and 50% MVCs. Each participant was given at least two minutes of rest between the contractions. During the isometric contractions participants maintained their force output as close as possible to the force template displayed digitally on a computer monitor.

EMG Recording.

During the trapezoid muscle actions, surface EMG signals were recorded from the FDI using 5-pin surface array sensor (Delsys, Inc., Natick, Massachusetts). The diameter of each pin is 0.5 mm that are placed at the corners of a 5 × 5-mm square, with the fifth pin in the center of the square. Before sensor placement, the surface of the skin was prepared by shaving, removing superficial dead skin with adhesive tape, and sterilizing with an alcohol swab. To remove the dead layers of skin, hypoallergenic tape (3M, St. Paul, Minnesota) was applied to the site, then peeled back to remove contaminants (Delsys, Inc., dEMG User Guide). This process was repeated multiple times. The sensor was placed over the belly of the FDI muscle and secured by adhesive tape. The reference electrode was placed over the elbow of the right arm.

EMG Decomposition.

Action potentials were extracted into firing events of single MUs from the 4 separate EMG signals, sampled at 20 kHz, via the precision decomposition (PD) III algorithm as described by De Luca et al. (2006). This algorithm was designed for decomposing surface EMG signals into their constituent MUAP trains. The accuracy of the decomposed firing instances was tested with the reconstruct-and-test procedure (Nawab et al. 2010). Only motor units (MU) that can be decomposed with >90% accuracies were included

for analysis. For each MU, RT, $MUAP_{SIZE}$, and the MFR during the steady force plateau were recorded. A 2000 ms hanning window were applied to the MFR curves. MFRs were determined by averaging firing rates during a 1 s epoch which was manually selected at the onset of the target steady force. Additionally, $MUAP_{SIZES}$ were calculated as previously described by Pope et al. (2016). For each MU, the peak-to-peak amplitude values of each of the four unique action potential waveform templates were averaged using a custom-written software program (LabVIEW 2015, National Instruments, Austin, TX, USA). For the 20% and 50% MVCs, linear MFR vs. RT, exponential $MUAP_{SIZE}$ vs. RT, and exponential MFR vs. $MUAP_{SIZE}$ relationships were calculated for each subject. The slope and y-intercept values from the linear relationships and the A and B terms (discussed in statistical analysis) from the exponential relationships were used for statistical analysis.

Ultrasound Experimental Protocol

Ultrasound images were taken of the right FDI according to our previous methods (Miller et al. 2017) using a LOGIQe ultrasound-imaging device (GE Healthcare UK, Ltd., Chalfont, Buckinghamshire, UK). Subjects were examined while sitting at a table with the right hand lying open and pronated on top of foam pads which form an L-shape. The foam pads were used to elevate the hand from the table as well as provide a guide angle at 70° to standardize the thumb in a relatively neutral position. 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 was used. The scan depth was set to 2 cm, gain was 38 dB and transducer frequency was 12MHz. The origin and insertion of the FDI were located by longitudinally scanning the muscle. The origin and insertion were marked and used to determine the site of the CSA image. The midway point between the origin and insertion was measured and considered the site for the mid CSA. Once the mid CSA site was determined, a cross section of the muscle belly was scanned with the probe 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. Generous amounts of ultrasound gel were applied for each scan to ensure minimal pressure on the skin. An image was captured and saved when the muscle is 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), EI (arbitrary units [AU]), and sFAT (mm) were determined using the image analysis program ImageJ (National Institutes of Health, Bethesda, Maryland). The scale of each image was calibrated using the centimeter marks inlaid in the image. sFAT was measured from the bottom of the cutaneous layer to the top of the superficial fascia of the muscle, using the mid-point 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 and greyscale of the outlined area was measured. Mean greyscale of the muscle was considered raw EI. Raw EI values were corrected for sFAT using the methods of Young et al. (2015) and the corrected EI values were used for subsequent analysis.

Body Composition

For BMI, children wore lightweight shorts and t-shirts during the body mass and height measurements. In addition, a two-site skinfold test (triceps and subscapular) was performed on each subject. Procedures for these skinfold measurements were done according to (Slaughter et al. 1984) and %BF was estimated using the methods of Slaughter et al. (1988).

Signal Processing

Channel 1 of the 4 bipolar EMG channels from the 5-pin surface array sensors were selected for amplitude analyses. The force (N) and the EMG (mV) signals from channel 1 were recorded with a NI cDAQ (National Instruments, Austin, TX USA) during each isometric muscle action. Data was stored on a personal computer (Dell Optiplex 9010; Dell, Inc., Round Rock, TX) for subsequent analysis. EMG amplitude was expressed as root mean square (RMS) amplitude values calculated by custom written software (LabVIEW v 15.0; National Instruments, Austin, TX). The sampling frequency for force and EMG signals was 2,000 Hz for the MVCs, and 20,000 Hz for the submaximal contractions. The EMG signals were bandpass 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 (EMG_{RMS}) calculations were performed on the filtered signals. EMG_{RMS} for the 20% and 50%

MVCs was determined by averaging the EMG_{RMS} during the same 1 s epoch which was manually selected at the onset of the target steady force to measure MFR. The EMG_{RMS} values were normalized (%max) to the amplitude recorded during the highest 0.25 sec average of force (N) that occurred during the three MVCs.

Statistical Analysis

There were no sex-related differences for the primary variables of interest, CSA, EI, and the $MUAP_{SIZE}$ vs. RT, MFR vs. RT, and MFR vs. $MUAP_{SIZE}$ relationships. Therefore, sex was excluded as a factor for further analysis to improve clarity of the statistical procedures. To examine potential differences between NW and OW, independent samples t-tests will be performed on BMI, %BF, body mass, MVC force, MVC force/CSA, CSA, EI, CSA/EI, and sFAT. Linear regressions were constructed for the MFR vs. RT relationships. The following exponential models were applied to the $MUAP_{SIZE}$ vs. RT and MFR vs. $MUAP_{SIZE}$ relationships

$MUAP_{SIZE}$ vs. RT: $$MUAP_{SIZE} = Ae^{B(RT)}$$

Where A is the theoretical $MUAP_{SIZE}$ for a MU recruited at 0% MVC, e is the natural constant, B represents the growth coefficient of $MUAP_{SIZE}$ with increments in RT.

MFR vs. $MUAP_{SIZE}$: $$MFR = Ae^{B(MUAP_{SIZE})}$$

Where A is the theoretical MFR of a $MUAP_{SIZE}$ of 0 mV, and B is the decay coefficient of MFR with increments in $MUAP_{SIZE}$.

Nonsignificant relationships were excluded from further analysis. Seven separate two-way mixed factorial ANOVAs (group [NW vs. OW] × contraction intensity [20% vs. 50% MVC]) were used to determine group and contraction intensity related differences in EMG_{RMS}, the slopes and y-intercepts for the MFR vs. RT

relationships, and the A and B terms from the $MUAP_{SIZE}$ vs. RT and MFR vs. $MUAP_{SIZE}$ relationships. Paired samples and independent samples t-tests will be performed as a follow-up to significant interactions. An α of 0.05 was used to determine statistical significance. Statistical analyses were performed using IBM SPSS Statistics v. 24 (SPSS Inc., Chicago, Illinois, USA).

CHAPTER IV

RESULTS

Descriptive data for observed MUs can be found in Table 1, and a summary of the independent samples t-tests can be found in Table 2.

Body Composition, Ultrasonography, And Voluntary Strength

Independent samples t-tests indicated that OW was significantly greater than NW for mass ($P < 0.001$, NW = 30.96 ± 3.68 kg, OW = 41.37 ± 7.31 kg), BMI ($P < 0.001$, NW = 15.96 ± 0.94 kg/m^2, OW = 21.22 ± 2.19 kg/m^2), %BF ($P < 0.001$, NW = 17.01 ± 3.25%, OW = 31.01 ± 4.97%), sFAT ($P < 0.001$, NW = 2.19 ± 0.60 mm, OW = 3.71 ± 0.97 mm), and EI ($P = 0.002$, NW = 47.99 ± 6.01 AU, OW = 58.90 ± 10.63 AU) (Figure 1). There were no differences between groups for CSA ($P = 0.688$, NW = 1.138 ± 0.146 cm^2, OW = 1.162 ± 0.156 cm^2). There were no significant differences in MVC force ($P = 0.790$) or MVC force/CSA ($P = 0.697$) between NW (14.81 ± 3.49 N, 13.00 ± 2.84 N/cm^2) and OW (14.43 ± 3.87 N, 12.52 ± 3.60 N/cm^2). However, NW had significantly greater lean CSA than OW, as there was a significant difference ($P = 0.040$) between groups for CSA/EI (NW = 0.024 ± 0.004 cm^2/AU, OW = 0.020 ± 0.005 cm^2/AU).

MFR vs. RT Relationships

For the slopes there was no significant ($P = 0.401$) two-way interaction or main effect for group ($P = 0.279$), however, there was a main effect for intensity ($P < 0.001$). A paired-samples t-test indicated that the slopes for the 50% MVC were less negative (-0.646 ± 0.18 pps/%MVC) than the slopes for the 20% MVC (-1.46 ± 0.64 pps/%MVC) when collapsed across groups ($P < 0.001$). For the y-intercepts there was no significant two-way interaction ($P = 0.595$) and no significant main effects for group ($P = 0.792$) or intensity ($P = 0.397$) (Figure 2).

Exponential $MUAP_{SIZE}$ vs. RT Relationships

For the B terms there was no significant (P = 0.810) two-way interaction or main effect for group (P = 0.616), however, there was a main effect for intensity (P < 0.001). A paired-samples t-test indicated that the B terms were greater for the 20% MVC (0.103 ± 0.038 mV/%MVC) than the 50% MVC (0.045 ± 0.02 mV/%MVC) when collapsed across group (P < 0.001) (Figure 3).

For the A terms there was no significant two-way interaction (P = 0.440). However, there were significant main effects for group (P = 0.011) and intensity (P < 0.001). Paired-samples t-tests indicated the A terms were greater for the 50% MVC (0.188 ± 0.11 mV) than the 20% MVC (0.114 ± 0.07 mV) when collapsed across group (P < 0.001). In addition, independent-samples t-tests indicated the A terms were greater for NW (0.185 ± 0.12 mV) than OW (0.091 ± 0.05 mV) when collapsed across intensity (P = 0.002) (Figure 3).

Exponential MFR vs. $MUAP_{SIZE}$ Relationships

For the B terms there was no significant (P = 0.448) two-way interaction. However, there were significant main effects for group (P = 0.011) and intensity (P < 0.001). Paired samples t-tests indicated the B terms were more negative (P < 0.001) for the 20% MVC (-3.14 ± 1.35 pps/mV) than the 50% MVC (-1.47 ± 0.99 pps/mV) when collapsed across groups. In addition, independent samples t-tests indicated the B terms were more negative (P = 0.039) for the OW (-2.79 ± 1.46 pps/mV) than NW (-1.98 ± 1.36 pps/mV) when collapsed across intensities. For the A terms, there was no two-way interaction (P = 0.599) or main effects for intensity (P = 0.059) or group (P = 0.698) (Figure 4).

Normalized EMG_{RMS}

For normalized EMG_{RMS}, there was no significant two-way interaction (P = 0.858) or main effect for group (P = 0.969), however, there was a main effect for intensity (P < 0.001). Paired-samples t-tests indicated that EMG_{RMS} was greater for the 50% MVC (86.23 ± 37.7%) than the 20% MVC (32.27 ± 12.4%) contraction when collapsed across group (P < 0.001).

CHAPTER V

DISCUSSION

The results of the current study indicated no differences in MVC force of the FDI between NW and OW children, but greater lean CSA for NW. In contrast, multiple studies have reported greater isometric strength for knee extensors in obese children and adolescents in comparison to their non-obese counterparts (Maffiuletti et al. 2008; Tsiros et al. 2013; Garcia-Vicencio et al. 2016), however, one study, Blimkie et al. (1990), observed no differences in absolute isometric strength between obese and non-obese adolescents (age 15-18 years). It was suggested that increases in strength for the obese was the result of greater body mass acting as a training stimulus similar to resistance training, causing hypertrophy and increases in strength (Hulens et al. 2001; Hulens et al. 2002; Lafortuna et al. 2005; Maffiuletti et al. 2007; Tomlinson et al. 2014). This hypothesis has typically been supported in large limb muscles, especially in the anti-gravity muscles of the legs via the greater FFM reported in obese adolescents (Maffiuletti et al. 2008; Abdelmoula et al. 2012) as well as adults (Hulens et al. 2001; Rolland et al. 2004; Lafortuna et al. 2005; Maffiuletti et al. 2007). However, any body mass-related training stimulus would have little effect on hand muscles (i.e., FDI) in comparison to large limb muscles. This has been previously reported by Rolland et al. (2004), where differences in knee and elbow extension but not in hand-grip strength existed between lean and obese elderly women. In support, there were no differences between NW and OW for MVC force, CSA, or MVC force/CSA, suggesting the hypothesized training effect due to greater body mass does not affect muscle mass or strength in the FDI of OW children. The lack of differences in isometric MVC strength although NW had greater lean CSA may provide further evidence that intramuscular fat-mediated changes in muscle architecture and/or stiffness may result in *pseudo hypertrophy*, such as, increase in CSA and strength independent of growth in lean mass (Tomlinson et al. 2014; Rahemi et al. 2015).

Ultrasonography revealed no differences in CSA between groups, however, OW (58.90 ± 10.63 AU) displayed significantly greater EI than NW (47.99 ± 6.01 AU). Thus, indicating lower muscle quality for OW likely as a result of infiltration of intramuscular fat (Reimers et al. 1993; Young et al. 2015) and would tentatively suggest a smaller amount of contractile tissue (i.e., lean mass) in the OW group. In

support, the NW group had significantly greater CSA when normalized to EI than the OW group. This is unique to the findings of previous literature examining possible differences in muscle composition between obese and non-obese children and adolescents. Previous studies have reported greater CSA for the obese (Maffiuletti et al. 2008; Tsiros et al. 2013; Garcia-Vicencio et al. 2016) or similar lean mid-thigh mass (Blimkie et al. 1990). The present study, however, is the first to report similar CSAs and differences in lean mass (NW > OW) between NW and OW groups.

The $MUAP_{SIZE}$ vs RT relationships indicated that the later recruited higher-threshold MUs were larger in amplitude during the 20% and 50% MVCs for both groups, which conforms to the Henneman size principal (Henneman et al. 1965). Importantly, $MUAP_{SIZES}$ provide insight into the contractile area of the muscle, such as, greater $MUAP_{SIZES}$ of the higher-threshold MUs have been reported following resistance training which was closely associated with increases in CSA (Pope et al. 2016). Subsequently, the growth of the muscle fibers that comprise the higher-threshold MUs increased and resulted in greater $MUAP_{SIZES}$. In theory, if the contractile area or the lean mass was less in the OW group as suggested by the ultrasound measurements, it could result in differences between the groups for the $MUAP_{SIZE}$ vs RT relationships. Indeed, the overall amplitudes (lower A terms) of the $MUAP_{SIZES}$ from the 20% and 50% MVCs were less for the OW than the NW group (Figure 3) and, furthermore, the A terms from the $MUAP_{SIZE}$ vs RT relationship from the 50% MVC were related ($P = 0.050$, $r = 0.423$) to the normalized CSAs (Figure 5). Although speculative, together the ultrasound measurements and $MUAP_{SIZE}$ vs RT relationships tentatively suggested that the contractile area or the lean mass of the FDI was smaller for the OW in comparison to the NW group.

The MFR vs. RT relationships for the NW and OW children agree with the onion skin scheme for MU control as described by De Luca and Erim (1994), where earlier recruited MUs express greater firing rates, and with the firing rate behavior described in De Luca and Hostage (2010) where the slope of the MFR vs. RT relationship becomes less negative at greater contraction intensities. Miller et al. (2017) reported similar slopes and y-intercepts from the MFR vs. RT relationships of the FDI in younger (slopes = −0.574 ± 0.17 pps/%MVC, y-intercepts = 33.5 ± 7.1 pps) and older (slopes = −0.637 ± 0.25 pps/%MVC, y-intercepts = 32.8 ± 7.4 pps) adults during a 50% MVC in comparison to the children in the

present study (-0.646 ± 0.18 pps/%MVC, y-intercepts = 33.6 ± 4.8 pps). Of note, there were no significant differences in either the slopes or the y-intercepts between NW and OW, however, predicted MFRs for higher-threshold MUs were marginally lower for the NW children (Figure 2). In addition, Miller et al. (2017) reported greater normalized EMG_{RMS} for the older (~70%) in comparison to the younger (~60 %) adults. The authors suggested that the greater muscle activation was the result of greater MU recruitment needed to match the targeted force for the older individuals, which has been supported in previous studies utilizing nerve stimulation (Scaglioni et al. 2016). Interestingly, normalized EMG_{RMS} was greater for the children (86.23 ± 37.7%) in the present study in comparison to the younger and older adults in Miller et al. (2017) for the 50% MVC. Although speculative, MU recruitment patterns may differ as a function of age, however, muscle activation did not differ between the NW and OW children in the present study.

The MFR vs. $MUAP_{SIZE}$ relationships were negative and indicated that the smallest MUs had greater MFRs at the targeted force than the larger MUs. Thus, providing support that the smaller MUs were recruited at lower RTs. Furthermore, there were differences between the NW and OW groups when MFRs were expressed as a function of $MUAP_{SIZES}$. The OW group had lower MFRs for a given $MUAP_{SIZE}$ in comparison to the NW. In addition, the largest MUs for the NW group, which were not observed in the OW group, displayed the lowest MFRs during the steady force (Figure 4). Another interpretation is that, for the OW group, the MUs with smaller AP_{SIZES} displayed the MFRs that would be expected from the larger higher-threshold MUs of the NW group.

Subcutaneous fat, an anatomical factor, cannot be ruled out as a possible confounding variable in regards to differences in $MUAP_{SIZES}$ between groups. Barkhaus and Nandedkar (1994) reported decreases in $MUAP_{SIZES}$ with increases in skinfold thickness (4 to 18 mm) for MUs located superficially when using surface EMG electrodes. However, Roeleveld et al. (1997) indicated that a 2 mm layer of sFAT had no influence on $MUAP_{SIZE}$ at the surface of the skin. sFAT may reduce EMG amplitude, however, for the current study there was no relationship between peak EMG amplitude from the MVCs and sFAT ($P = 0.144$, $r = -0.283$). In addition, the linear regression between $MUAP_{SIZE}$ and sFAT from Barkhaus and Nandedkar (1994)

demonstrated that a ~1.5 mm difference in sFAT, (i.e., the difference between NW and OW in the present study) would result in a very minor reduction (< 4%) in $MUAP_{SIZE}$ at the surface of the skin. Therefore, the small difference in sFAT between groups likely only had a minor influence on the differences in $MUAP_{SIZES}$ between groups.

The examination of the FDI, a muscle that does not support body mass, provided unique insight into the possible differences in neuromuscular function between normal weight and overweight children 8 to 10 years of age. Differences in MVC force between groups were minimal, however, it is plausible that alterations in muscle architecture and/or stiffness as a result of greater intramuscular fat allowed the OW group to produce similar isometric MVC strength to the NW (Tomlinson et al. 2014; Rahemi et al. 2015). This is supported by the findings from ultrasonography and EMG decomposition methods that revealed similar CSA, greater EI, and smaller $MUAP_{SIZES}$ that tentatively indicates the presence of greater intramuscular fat and smaller lean mass in the OW group. Firing rate behavior was not different between the groups or in comparison to adults from previous studies, however, muscle activation at the same relative targeted force was observed to be higher in the children than previously reported in adults.

Table 1. Mean±SD for the number of motor units (MUs) observed, recruitment threshold (RT) range for observed MUs, Electromyography amplitude (EMG_{RMS}) normalized to max EMG, the A and B terms for the exponential MU action potential size ($MUAP_{SIZE}$) vs. RT, and exponential mean firing rate (MFR) vs. $MUAP_{SIZE}$ relationships, as well as the slopes and y-intercepts of the linear MFR vs. RT relationships.

	NW		OW	
Contraction Intensity	20% MVC	50% MVC	20% MVC	50% MVC
MUs	14.8±4.4	16.5±6.4	15.4±7.8	17.2±5.1
Mean RT range (%MVC)	4±4 - 15±4	10±4 - 35±8	3±3 - 14±5	13±4 - 38±7
EMG_{RMS} (%max)	32.82±13.73	85.98±40.16	31.41±10.53	86.63±35.52
$MUAP_{SIZE}$ vs. RT				
A terms (mV)	0.144±0.07	0.227±0.12	0.062±0.02	0.120±0.05
B Terms (mV/%MVC)	0.105±0.04	0.046±0.02	0.099±0.04	0.044±0.01
MFR vs. $MUAP_{SIZE}$				
A terms (pps)	37.5±7.4	32.7±6.1	35.7±7.9	32.9±7.2
B terms (pps/mV)	-2.91±1.4	-1.05±0.3	-3.48±1.3	-2.11±1.3
MFR vs. RT				
Slope (pps/%MVC)	-1.57±0.69	-0.665±0.21	-1.27±0.53	-0.612±0.11
Y-intercept (pps)	31.0±10.1	33.8±5.4	32.7±7.3	33.4±4.0

Table 2. Significance in terms of P value, and mean±SD for normal weight (NW) and overweight (OW) groups for all independent samples t-tests. Parameters include percent body fat (%BF), subcutaenous fat (sFAT), echo intensity (EI), cross-sectional area (CSA), maximum voluntary contraction force (MVC force).

	Significance (P)	NW	OW
Mass (kg)	<0.001	30.96±3.68	41.37±7.31
BMI (kg/m^2)	<0.001	15.96±0.94	21.22±2.19
%BF	<0.001	17.01±3.25	31.01±4.97
sFAT (mm)	<0.001	2.19±0.60	3.71±0.97
EI (AU)	0.002	47.99±6.01	58.90±10.63
CSA (cm^2)	0.688	1.138±0.146	1.162±0.156
MVC force (N)	0.790	14.81±3.49	14.43±3.87
MVC force/CSA (N/cm^2)	0.697	14.43±3.87	12.52±3.60
CSA/EI (cm^2/AU)	0.040	0.024±0.004	0.020±0.005

FIGURE LEGENDS

Figure 1. Ultrasound images (parallel view) of the first dorsal interosseous of a NW (top) and OW (bottom) child. The yellow outline indicates the cross-sectional area of the muscle. The OW child's image is much brighter (greater echo intensity) and indicates a greater amount of intramuscular fat.

Figure 2. Linear predicted mean firing rate (pulses per second) vs. recruitment threshold relationships based on average slopes and y-intercepts.

Figure 3. Motor unit action potential size ($MUAP_{SIZE}$[mV]) vs. recruitment threshold (% maximum voluntary contraction) relationships for (A) a NW and OW subject for the 20% MVC, and (B) 50% MVC. (C) All subjects exponential predicted relationships for the 20% MVC, and (D) 50% MVC. (E) Mean predictions for NW and OW for the 20% MVC and (F) 50% MVC.

Figure 4. Mean firing rate (pulses per second) vs. motor unit action potential size ($MUAP_{SIZE}$[mV]) relationships for (A) a NW and OW subject for the 20% MVC, and (B) 50% MVC. (C) All subjects exponential predicted relationships for the 20% MVC, and (D) 50% MVC. (E) Mean predictions for NW and OW for the 20% MVC and (F) 50% MVC.

Figure 5. Correlation between first dorsal interosseous cross-sectional area (CSA[cm^2]) normalized to echo intensity (EI[AU]) vs. A terms (mV) from the exponential $MUAP_{SIZE}$ vs. recruitment threshold relationships from the 50% MVC.

Figure 1

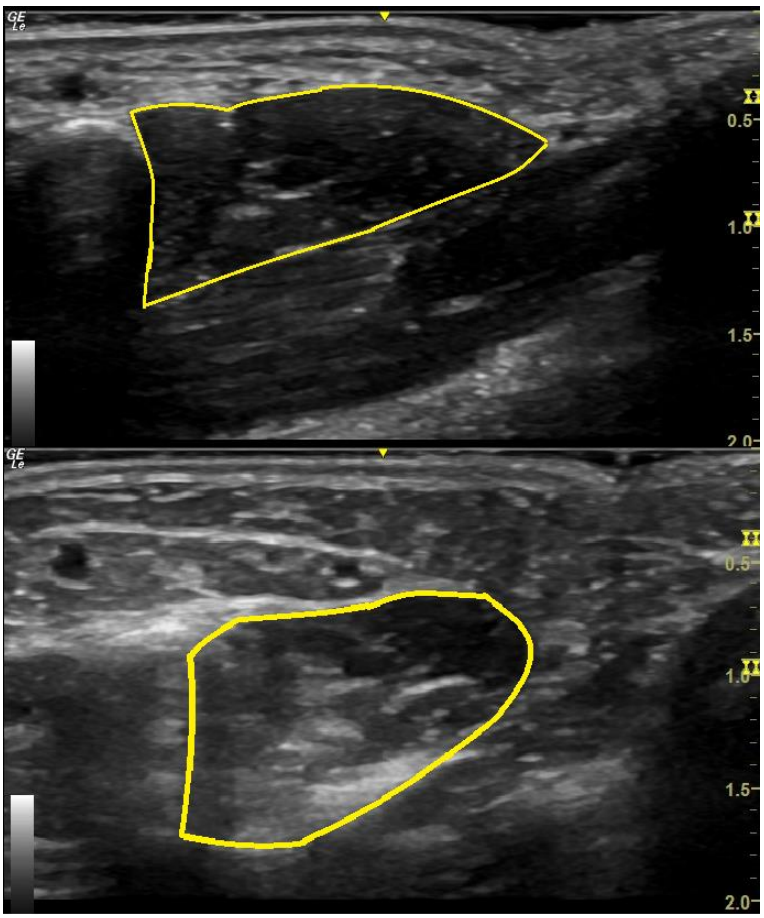


Figure 2

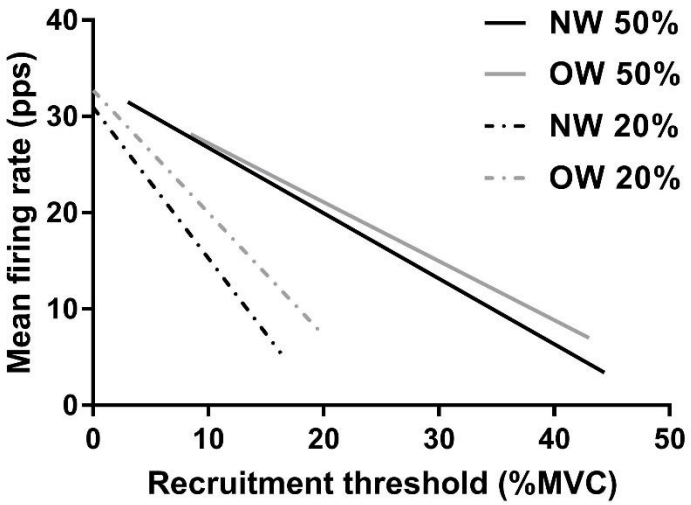

NW 50%
OW 50%
NW 20%
OW 20%
Mean firing rate (pps)
40
30
20
10
0
0
10
20
30
40
50
Recruitment threshold (%MVC)

Figure 3

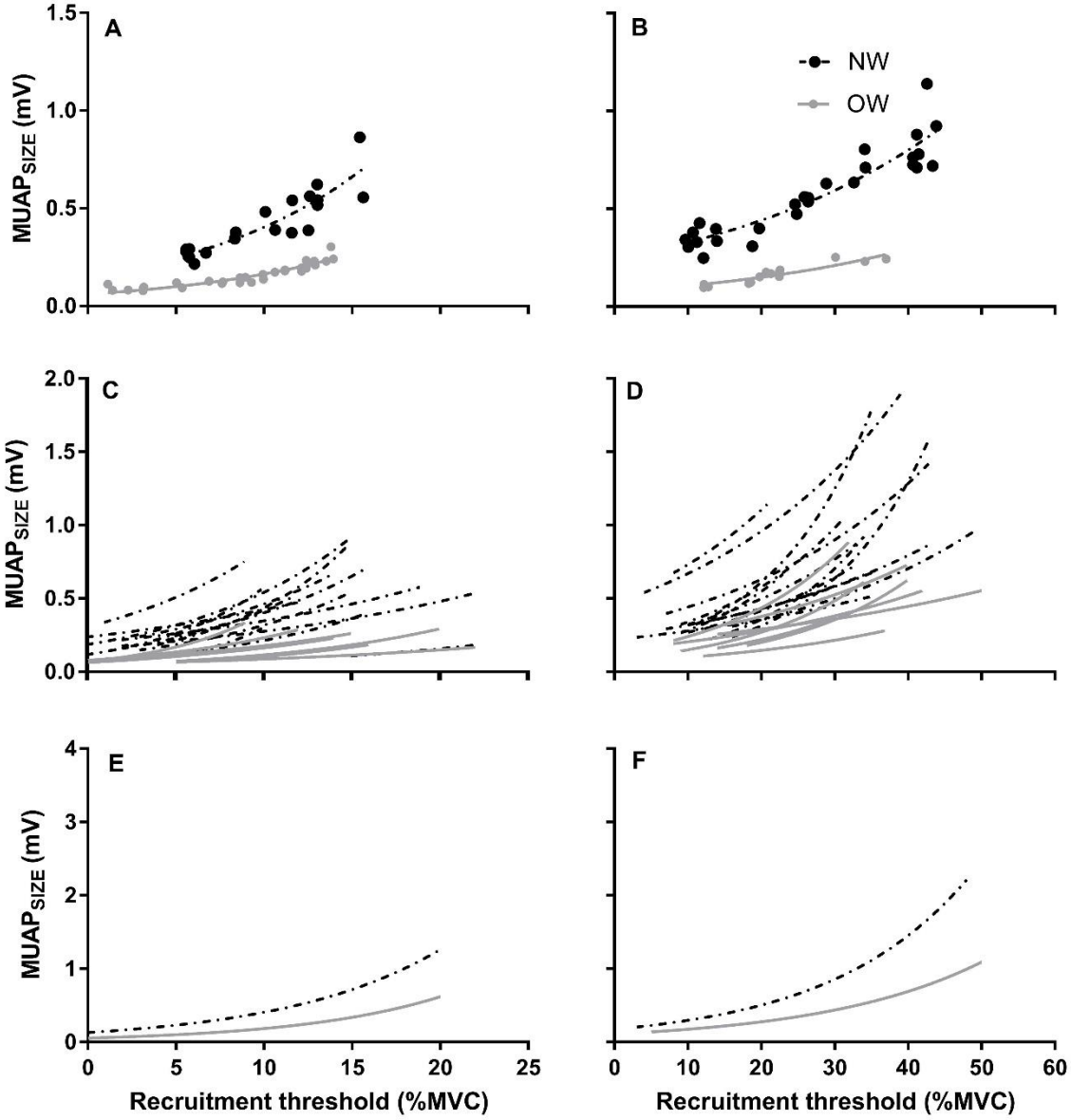


Figure 4

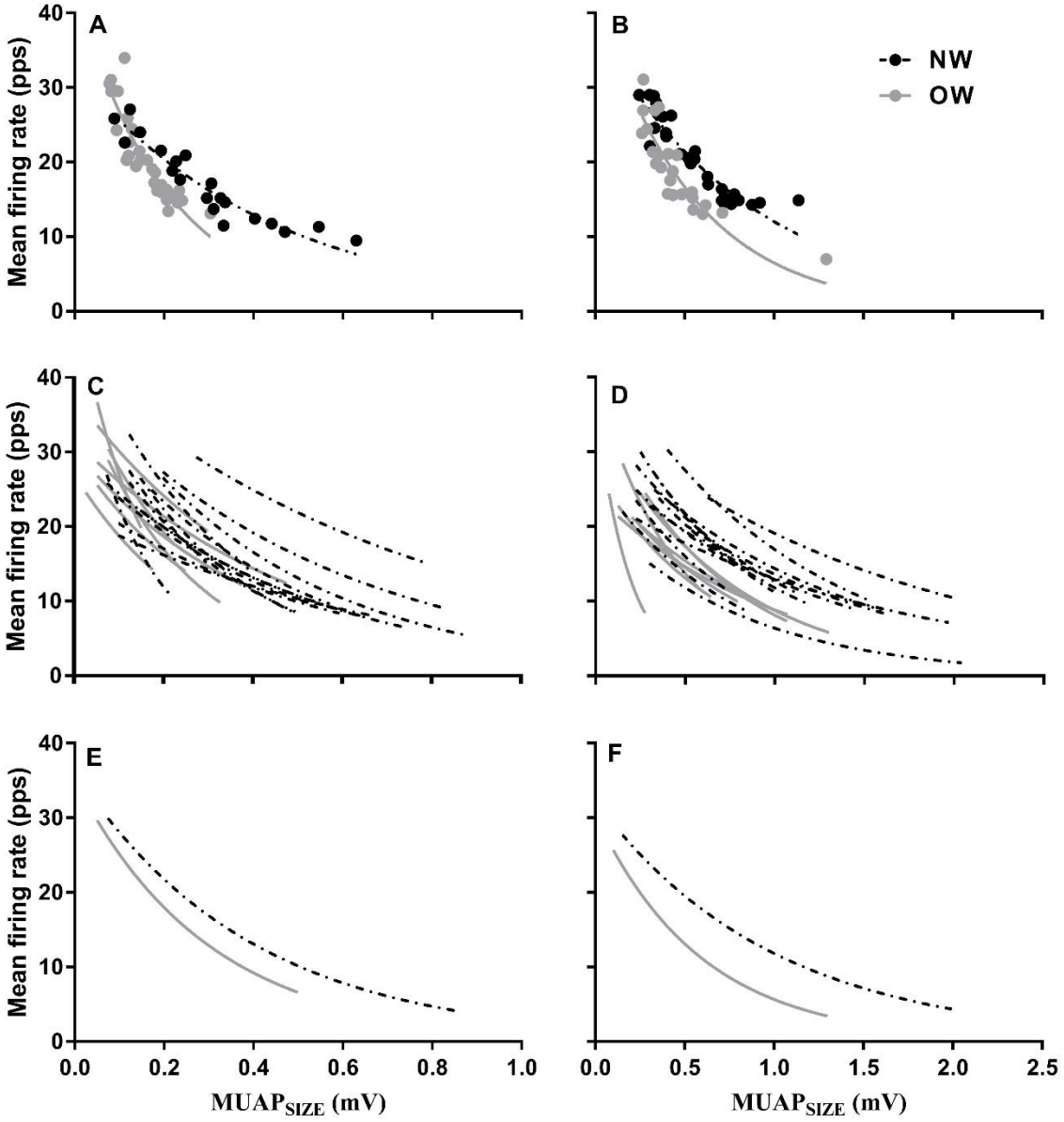
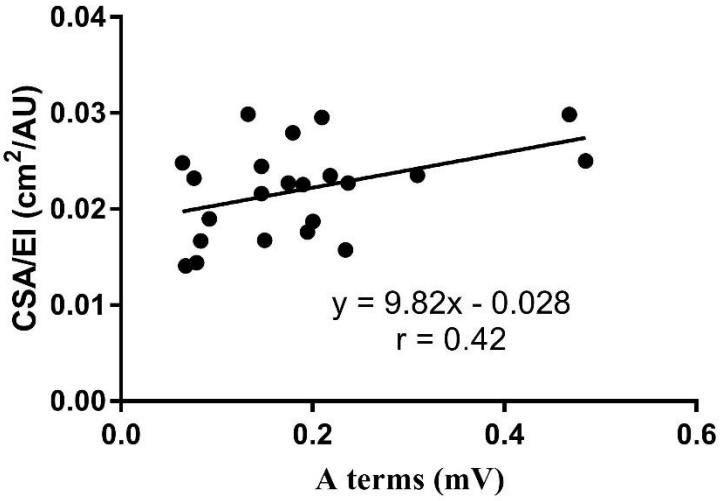

A
B
C
D
E
F
NW
OW
Mean firing rate (pps)
MUAP$_{SIZE}$ (mV)
0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0
0.5
1.0
1.5
2.0
2.5

Figure 5


0.04
0.03
0.02
0.01
0.00
CSA/EI (cm2/AU)
y = 9.82x - 0.028
r = 0.42
0.0
0.2
0.4
0.6
A terms (mV)

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APPENDIX

Informed Consent

Neuromuscular performance in children ages 8-10.

INTRODUCTION

The Department of Health, Sport and Exercise Sciences at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish for your child to participate in the present study. You may refuse to sign this form and not consent to your child's participation in this study. You should be aware that even if you agree to allow your child to participate, you are free to withdraw them at any time. If you do withdraw them from this study, it will not affect you or your child's relationship with this unit, the services it may provide to you or your child, or the University of Kansas.

We are recruiting 50 total participants to complete the first two visits of this study. Of those 50 participants, we are going to ask 28 participants to come back to the laboratory for a third visit. Therefore, your child will be recruited to participate in the first two visits and during the second visit, we will indicate if you are eligible for the third visit. If asked, you and your child can decide whether you want to participate in the third visit.

PURPOSE OF THE STUDY

Muscle strength has been reported to be related to body composition in adults. However, it remains unclear if body composition and muscle strength are similarly related in young children. In addition, materials measured from blood may provide insight on the differences in strength in children. Ultrasound images will be taken, and electrodes will be placed on the muscles of your child's index finger and leg muscles to monitor muscle strength. In addition, skinfolds and ultrasound images of your child's lower and upper body (seven sites) will be taken to assess body composition (muscle vs fat). For example, skinfolds and ultrasound images will be taken from the back of the arm, stomach next to the belly button, back of shoulder, etc. In addition, on the third visit, your child will have a blood sample taken after not eating food for 8 hours. This visit can take place in the morning. Therefore, the purpose of this study is to measure muscle strength from your child's index finger and right leg and to understand the influence of materials in the blood and body composition on these measures of muscle strength.

PROCEDURES

A time-line of the testing procedures and an overview of the testing sequence are below. You and your child will be asked to come to the Neuromechanics Laboratory (Robinson Center, Rm 101BE) for two or three separate visits. The first visit will include the body composition measures and your child performing the strength tests of the leg and practicing the strength testing of the hand that will occur during the second visit. Visit 2 will include the strength testing of the index finger muscles. The first and second visit will last approximately 90 minutes. If eligible and willing, visit 3 will include the fasting blood draw. We can only draw blood from 28 children and, therefore, some of the 50 children will not be asked to complete the third visit.

Visit 1: (90 minutes): A seven-site skinfold thickness test will be administered (with an additional site on the right hand), and ultrasound images will be taken at those seven-sites and of your child's muscles that control their pointer finger and their upper leg. Your child will perform the power and fatigue tests of the right leg which includes a warm-up, followed by a total of 30-35 maximal isokinetic knee extensions, with several rest periods. Your child will practice the strength tests of their right pointer (index) finger that will be performed in visit 2. In addition, your child will have the option to experience a very small electrical stimulation of the pointer finger. Your child will then get to choose to perform the study with or without the small electrical stimulation. Following familiarization of the tests,

Visit 2: (90 minutes): The sites where your child will have sensors placed over their skin will be prepared for sensor placement. Your child's right hand and elbow will be shaved, and will be cleaned with rubbing alcohol and adhesive tape in order to prepare the sites where the sensors will be placed. Your child will have sensors placed on the muscles that control their pointer finger. Next, they will perform warm-up strength tests followed by two to three high intensity strength tests of their pointer finger. If your child is ok with the electrical stimulation, electrical stimulation will be given to your child a few times at rest, and before and following five to eight low and moderate intensity strength tests of their pointer finger. Following the low and moderate strength tests, your child will again perform one or two high intensity strength tests. You will also be asked to complete a few questionnaires regarding your child's health and activity levels.

Visit 3: (30 minutes): Fasted blood draw samples will be collected by an experience phlebotomist using standard techniques. No more than 20 cubic centimeters of blood will be drawn, which is about how much a nurse would take for standard blood work in a clinic. Following the blood draw, children will be provided a snack.

MEASUREMENTS:

Finger strength tests - The muscle strength tests will be performed on your child's right pointer finger. These tests will involve your child pushing their finger against a small plate. The child will trace a line on a computer screen using the strength from his/her finger. They will be given two to five minutes rest between strength tests.

Electrical stimulation - Electrical stimulation will be applied to your child's right pointer finger at the very beginning of the experimental testing visit and then again prior and after each low or moderate intensitystrength test to assess muscle performance. The electrical stimulation will last 1 millisecond. The electrical stimulation will only be given during the familiarization and the experimental visits if the child is comfortable with this technique.

Leg strength tests – The leg strength tests will be performed on your child's right leg. These tests will involve your child extending their leg at the knee against a padded lever arm. The child will extend their leg as hard as they can while the padded lever arm moves at a constant speed. They will be given two to five minutes rest between power and fatigue tests.

Skinfold measurements - Skinfold thickness, as a measurement of body composition, will be performed using the seven-site caliper method. Skinfold calipers will be used to measure skinfold thickness at each

of the following sites: 1. pectoral (chest), 2. abdomen (belly), 3. quadriceps (thigh), 4. triceps (back of upper arm), 5. subscapula (lower tip of shoulder blade), 6. suprailiac (front of hip), and 7. midaxillary (side of torso at 5th rib). In addition, an 8th site will be measured in order to validate the findings of the strength tests of the index finger.

Ultrasound imaging - The index finger will be scanned while the participants hand rests on foam pads upon a table. The quadriceps scans will be completed with the subject laying supine on a padded bench with their right leg elevated. In addition, images will be taken at each skinfold site mentioned above with your child in a standing position. Before the measure, a generous amount of water-soluble transmission gel will be applied to the skin to reduce possible near-field artifacts and enhance acoustic coupling.

Blood Sample - Fasted blood draw samples will be collected by an experience phlebotomist using standard techniques. No more than 20 cubic centimeters of blood will be drawn, which is about how much a nurse would take for standard blood work in a clinic.

RISKS

There is the potential for participants to experience some physical stress during and muscle soreness following the maximal and submaximal strength and power tests. Your child may have skin abrasions due to shaving and cleansing the skin with alcohol prior to electrode placement. In addition, if your child is qualified for, and completes the third visit (blood draw) they may experience some physical or psychological stress during the blood draw and some bruising of the skin following the blood draw.

BENEFITS

You and your child will not directly benefit from participating in this study. However, you and your child will gain an increased understanding of how your muscles work. Specifically, how body composition is important for muscle strength. A copy of all personal data from the tests will be provided to you, and your child's data will be completely explained to you and your child by a member of the investigation team.

PAYMENT TO PARTICIPANTS

Subjects will be compensated for their participation in this study. Upon your child's completion of the first two visits of the study a stipend of $20 will be provided. If your child is qualified for, and completes the third visit, and additional $15 will be provided. Investigators may ask for your social security number in order to comply with federal and state tax and accounting regulations.

PARTICIPANT CONFIDENTIALITY

Your child's name will not be associated in any publication or presentation with the information collected about your child or with the research findings from this study. Instead, the researcher(s) will use a study number or a pseudonym rather than your child's name. Your child's identifiable information will not be shared unless (a) it is required by law or university policy, or (b) you give written permission.

Permission granted on this date to use and disclose your child's information remains in effect indefinitely. By signing this form you give permission for the use and disclosure of your child's information for purposes of this study at any time in the future.

INSTITUTIONAL DISCLAIMER STATEMENT

In the event of injury, the Kansas Tort Claims Act provides for compensation if it can be demonstrated that the injury was caused by the negligent or wrongful act or omission of a state employee acting within the scope of his/her employment.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION

You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, your child cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION

You may withdraw your consent for your child to participate in this study at any time. You also have the right to cancel your permission to use and disclose further information collected about your child, in writing, at any time, by sending your written request to: Trent J. Herda, 1301 Sunnyside Avenue 101BE, Robinson Center, Lawrence, KS 66045.

If you cancel permission to use your information, the researchers will stop collecting additional information about your child. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

QUESTIONS ABOUT PARTICIPATION

Questions about procedures should be directed to the researcher(s) listed at the end of this consent form.

PARTICIPANT CERTIFICATION:

I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study. I understand that if I have any additional questions about my rights as a research participant, I may call (785) 864-7429 or (785) 864-7385, write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7568, or email irb@ku.edu.

I agree to my child's participation in this study as a research participant. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

_____ _____

Type/Print Guardian's Name Date

_____

Guardian's Signature

Researcher Contact Information

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Assent Form

Neuromuscular performance in children ages 8-10.

My name is Jonathan Miller. I am interested learning about how your muscles work and how they may work better if you exercise and eat healthy. If you would like, you can be in my study, and we will meet two or three times in my laboratory.

If you decide you want to be in my study, you will perform following tests:

- You will do surveys that ask you about the foods you eat.
- We will take pictures of some of your muscles using an ultrasound machine.
- We will measure how thick your skin is on a few places around your body.
- You will do strength tests with your right pointer finger and right leg. During some of these strength tests you will get to play a computer game where you trace a line on a screen.
- We will place stickers on your wrist to cause your muscles to move. You can let us know if you don't want to do this part.
- We may have you come back for the third visit and have a nurse draw some of your blood, about as much as they might take at a hospital, and we will give you a snack after you are done.

The benefits to the study are the following:

- You will learn about the strength of your finger and leg.
- You will learn how exercise and eating healthy can make you stronger.

The risks to the study are the following:

- Your finger or leg may get sore.
- You may get a bruise from the blood draw.
- Your skin may get sore from the stickers.

Other people will not know if you are in my study. If you want to be in the study now and change your mind later, that's OK. You can stop at any time.

Health History Questionnaire

Participant ID: _____ Date_____

Legal Guardian Contact Info: Email: _____ Phone: _____

Home Address_____

Person to contact in case of emergency _____

Emergency Contact Phone _____ Birthday (mm/dd/yy)____/_____/_____

Personal Physician _____ Physician's Phone_____

Gender _____ Age _____ (yrs) Height _____(ft)_____(in) Weight_____(lbs)

A. JOINT-MUSCLE STATUS (✓Check areas where you currently have problems)

Joint Areas
( ) Wrists
( ) Elbows
( ) Shoulders
( ) Upper Spine & Neck
( ) Lower Spine
( ) Hips
( ) Knees
( ) Ankles
( ) Feet
( ) Other_____

Muscle Areas
( ) Arms
( ) Shoulders
( ) Chest
( ) Upper Back & Neck
( ) Abdominal Regions
( ) Lower Back
( ) Buttocks
( ) Thighs
( ) Lower Leg
( ) Feet
( ) Other_____

B. HEALTH STATUS (✓Check if you currently have any of the following conditions)

( ) High Blood Pressure
( ) Heart Disease or Dysfunction
( ) Allergic reactions to rubbing alcohol
( ) Peripheral Circulatory Disorder
( ) Lung Disease or Dysfunction
( ) Arthritis or Gout
( ) Edema
( ) Epilepsy
( ) Multiply Sclerosis
( ) High Blood Cholesterol or
( ) Triglyceride Levels
( ) Acute Infection
( ) Diabetes or Blood Sugar Level Abnormality
( ) Anemia
( ) Hernias
( ) Thyroid Dysfunction
( ) Pancreas Dysfunction
( ) Liver Dysfunction
( ) Kidney Dysfunction
( ) Phenylketonuria (PKU)
( ) Loss of Consciousness

C. PHYSICAL EXAMINATION HISTORY

Approximate date of your last physical examination_____

Physical problems noted at that time_____

Has a physician ever made any recommendations relative to limiting your level of physical exertion? _____YES _____NO
If YES, what limitations were recommended?_____
_____

D. CURRENT MEDICATION USAGE (List the drug name and the condition being managed)

MEDICATION	CONDITION
_____	_____
_____	_____
_____	_____

E. PHYSICAL PERCEPTIONS (Indicate any unusual sensations or perceptions. ✓Check if you have recently experienced any of the following during or soon after *physical activity* (PA); or during *sedentary periods* (SED))

PA	SED		PA	SED	
( )	( )	Chest Pain	( )	( )	Nausea
( )	( )	Heart Palpitations	( )	( )	Light Headedness
( )	( )	Unusually Rapid Breathing	( )	( )	Loss of Consciousness
( )	( )	Overheating	( )	( )	Loss of Balance
( )	( )	Muscle Cramping	( )	( )	Loss of Coordination
( )	( )	Muscle Pain	( )	( )	Extreme Weakness
( )	( )	Joint Pain	( )	( )	Numbness
( )	( )	Other_____	( )	( )	Mental Confusion