Muscle Strength, Motor Units, and Aging

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Master of Science

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This thesis titled

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Abstract

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Muscle Strength, Motor Units, and Aging

Director of Thesis: Sharon R. Rana

Introduction: The interrelationship between muscle strength, motor units, and aging is not well known. Methods: This investigation utilized the motor unit number index technique (MUNIX) to elucidate this relationship. Beforehand, an experiment was conducted to assess the absolute and relative reliability of the MUNIX technique.

Statistical Analysis: The test-retest reliability of the technique was assessed by measures of relative reliability (coefficient of variation) and absolute reliability (limits of agreement and intraclass correlation). The interrelationship between muscle strength, motor units, and aging was determined by an analysis of covariance (ANCOVA) that tested heterogeneity of regression slopes (moderation analysis). Results: The test-retest analysis yielded moderate relative reliability and moderately-high absolute reliability. A positive linear relationship was found between MUNIX and strength in the elderly, but not in the young adults. Discussion: MUNIX is a reliable measure of motor unit number in a laboratory setting. Muscle weakness in the elderly appears to be, in part, moderated by the loss of functional motor units.
Preface

Chapters 3 and 4 contained within the thesis document serve as prepublication manuscripts. These manuscripts have been formatted to meet the guidelines set forth by Thesis and Dissertation Services at Ohio University.
Dedication

For God, my family, and Dani.
Acknowledgments

A special thank you is needed for Dr. Brian Clark for his countless hours of assistance and laboratory expertise. To Drs. Roger Gilders and Sharon Rana, I thank you for feedback and knowledge. To Rich Hoffman and Dr. Masato Nakazawa, for their technical assistance and statistical expertise, respectively.
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Chapter 1: Introduction

Currently, the United States is home to over 57 million older adults, and that number is expected to increase to 92 million by 2030 (U.S. Department of Health and Human Services, 2012b). According to the Administration on Aging (U.S. Department of Health and Human Services, 2012a), as of 2009, 27% of those older adults over 65 years had difficulty performing activities of daily living (e.g., bathing, dressing, eating, walking) which may be attributed to age related strength declines (i.e., dynapenia) (Clark & Manini, 2012). This impairment in voluntary strength production is being extensively studied, and many cellular level muscular and nervous system changes have been cited as contributing factors to this effect (Clark & Taylor, 2011; Delbono, 2011; Deschenes, 2011; Fry & Rasmussen, 2011; Russ, Gregg-Cornell, Conaway, & Clark, 2012; Russ & Lanza, 2011). Regarding the nervous system changes, the loss of functioning motor units with incomplete compensatory reinnervation has been proposed as a potential mechanism to strength loss (Brown, 1972; Brown, Strong, & Snow, 1988; Doherty & Brown, 1993; Galea, 1996; McNeil, Doherty, Stashuk, & Rice, 2005a); however, research in this area is limited or contentious. Recently, McNeil and colleagues (2005) hypothesized motor unit loss was a direct contributor to strength loss in elderly men, yet they found no evidence to support this notion. More recently, Dalton, McNeil, Doherty, & Rice (2008) also observed no relationship between motor unit number and voluntary muscle strength. Accordingly, the purpose of this investigation was to determine the interrelationship between motor unit number, voluntary strength, and aging.
Direct assessment of motor unit number is impossible in living subjects, so a novel motor unit indexing technique, MUNIX, was employed to accomplish the motor unit indexing. Evoked and voluntary electromyographic signals were collected and used in a mathematical model to compute the motor unit index (Nandedkar, Nandedkar, Barkhaus, & Stålberg, 2004).

Since its inception in 2004, the MUNIX technique has not gained widespread popularity; thus, investigations utilizing this technique are limited. As a direct product of the limited number of investigations, studies have failed to produce definitive evidence on the reliability of this technique. For example, Furtula et al. (2012) and Nandedkar, Barkhaus, and Stålberg (2011) reported very low intraclass correlation coefficients (0.38 and 0.43, respectively) of the thenar muscles of healthy and diseased (Amyotrophic Lateral Sclerosis) populations. Conversely, Sandberg, Nandedkar, and Stålberg (2010) reported a very high ICC (0.97), but the retest was performed within minutes of the first test. The poor reproducibility and lack of controlled time between tests prompted the secondary aim of this investigation. The reproducibility of MUNIX was assessed following a 4 week period in a target population whose motor unit values would not be expected to change (i.e., young, healthy individuals).

**Significance of Study**

Identifying age-related declines in neuromuscular function (i.e., muscle strength) that are attributed to alterations of the nervous system may lead to the development of alternative therapeutic interventions to prevent muscle weakness in seniors. As for the
test-retest outcomes, providing sufficient evidence on the reliability may increase its widespread acceptance in clinical and scientific settings.

**Specific Aims and Hypothesis**

**Aim 1.** To examine the reliability of the motor unit number index (MUNIX) technique in young, healthy individuals. Approach: Motor unit number and size were assessed in the abductor pollicis brevis using the MUNIX technique in young, healthy individuals on two occasions separated by 4 weeks. Hypothesis: It was hypothesized that the MUNIX technique would yield reliable indexes of motor unit number and size.

**Aim 2.** To examine the association between voluntary muscle strength and motor unit numbers in both young and older adults. Approach: The MUNIX technique was used to obtain indices of motor unit number and size of the abductor pollicis brevis. Additionally, maximal voluntary pinch grip muscle strength was assessed. An analysis of covariance was conducted with pinch-grip strength and MUNIX serving as the between subjects factors. The model controlled for gender and body mass to limit the extraneous influence on the correlation between pinch-grip strength and MUNIX. Hypothesis: Muscle weakness is associated with a reduced number of motor units in older adults independent of motor unit size.

**Assumptions**

1. All participants refrained from caffeine, exercise, nicotine (4 hours prior), and alcohol (24 hours prior) to all testing sessions.
2. Participants were asked not to undertake strength training or a rehabilitative exercise program during the course of the study, as this may have influenced the reliability measures.

3. Placement of the active recording electrode was in the exact same location in all testing sessions, and was able to detect the maximum physiologic response.

Limitations

1. The abductor pollicis brevis muscle is an intrinsic hand muscle with a small area available for electrode placement. Although the muscle is the most superficial and largest of the thenar muscles, it is possible that electromyographic signals were detected from the other muscles (i.e, flexor pollicis brevis, opponens brevis), and a larger signal amplitude was recorded.

2. The pinch-grip task may not have been the ideal task to fully activate the abductor pollicis brevis muscle.

Delimitation

1. Participants voluntarily engaged in the research, and were not recruited via random sampling.

2. Participants recruited were young, healthy adults between the ages of 18 and 40 years.

Definition of Terms

*Compound muscle action potential.* Artificial stimulation of a motor neuron to elicit an action potential of maximal amplitude.

*In vivo.* Within a living organism.
**Maximal voluntary contraction.** The highest amount of force attained under one’s own volition.

**Motor unit.** An alpha-motor neuron and all the muscle fibers it innervates.

**Surface interference pattern.** Electromyographic signal detected by an electrode positioned over one’s skin during a voluntary contraction.
Chapter 2: Review of Literature

Age-Related Changes in Motor Unit Number and Strength

It is well established that older adults lose voluntary strength (i.e., dynapenia), but the severity of decline and age of occurrence is not fully understood (Clark & Manini, 2012). This concept of age-related strength loss has intrigued scientists, and a whole host of physiological mechanisms are cited as contributors. Of those mechanisms, extensive reviews are provided for the following: motor cortical properties (Clark & Taylor, 2011), excitation-contraction coupling proteins (Delbono, 2011), motor unit and neuromuscular junction remodeling (Deschenes, 2011), protein balance and metabolism (Fry & Rasmussen, 2011), and skeletal muscle energetics (Russ & Lanza, 2011). These concepts are outside the scope of the current research and will not be discussed in depth for the sake of brevity.

One major potential contributor to voluntary strength loss in the elderly could be the reduction in functioning motor units. According to Clark and Manini (2012), this is a logical explanation, especially if collateral reinnervation does not occur or is incomplete, but little or conflicting evidence supports this notion. Forty years ago, Brown (1973) reported reduced number of functioning motor units in persons over the age of 60 years; however, no measureable data were provided regarding strength. Sensing a void in the literature, recent investigations have focused on uncovering the relationship between motor unit number and voluntary muscle strength. The first of those studies conducted by Doherty, Vandervoort, Taylor, and Brown (1993) uncovered a modest correlation between motor unit number estimates and muscle strength ($r = 0.521$). However,
separate investigations conducted on the tibialis anterior of older men reached divergent conclusions. McNeil et al. (2005) divided men into three age categories, average age of 25 years, 65 years, and 80 years. Compared to the younger men, the 65-year-old group showed significant declines in motor unit number, but no reduction in strength. The very old men showed a steep decline in motor unit number as well as voluntary strength, indicating motor unit loss must reach a threshold before strength is lost. Conversely, in a cohort of older men (average age of 75 years) no significant decline in motor unit number of the tibialis anterior was found while maximal voluntary strength was reduced by 39% compared to the young men (average of 27 years) (Dalton et al., 2008).

Additional studies were conducted (Power et al., 2010, 2012) to determine the relationship between motor unit number and aging, all providing equivocal evidence. With the discrepancies in the literature regarding the topic, all studies seeking to explore this relationship have been summarized in Table 1.
Table 1

Motor Unit Number and Muscle Strength: Older Adults Compared to Younger Adults

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Method</th>
<th>Muscle</th>
<th>% Motor unit number</th>
<th>% Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doherty et al.</td>
<td>1993</td>
<td>STA</td>
<td>Biceps brachii</td>
<td>37% ↓</td>
<td>39% ↓</td>
</tr>
<tr>
<td>McNeil et al.</td>
<td>2005</td>
<td>DE-STA</td>
<td>Tibialis anterior</td>
<td>Age 65: 49% ↓</td>
<td>Age 65: N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age 80: 87% ↓</td>
<td>Age 80: 36% ↓</td>
</tr>
<tr>
<td>Dalton et al.</td>
<td>2008</td>
<td>DE-STA</td>
<td>Tibialis anterior</td>
<td>N.S.</td>
<td>39% ↓</td>
</tr>
<tr>
<td>Power et al.</td>
<td>2010</td>
<td>DE-STA</td>
<td>Tibialis anterior</td>
<td>MRs: N.S.</td>
<td>MRs: 29% ↓</td>
</tr>
<tr>
<td>Power et al.</td>
<td>2012</td>
<td>DE-STA</td>
<td>Biceps brachii</td>
<td>MRs: 63% ↓</td>
<td>MRs: 52% ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age 65: 91% ↓</td>
<td>Age 65: 50% ↓</td>
</tr>
</tbody>
</table>

Note. STA = Spike Triggered-Averaging; DE-STA = Decomposition Enhanced Spike Triggered-Averaging; MRs = Master Runners (65 years); N.S. = nonsignificant.

Motor Unit Defined

A motor unit is defined as an alpha motor neuron and all of the muscle fibers it innervates, and has commonly been referred to as the “final common pathway” (Sherrington, 1925), because it is the last portion of the nervous system that integrates spinal and cortical signals. Skeletal muscles vary in number of motor units owing to their role in the body. It is common for a single motor axon to innervate more than 1,000 muscle fibers in large mass muscles, such as the quadriceps, and as little as 100 fibers in smaller muscles used for fine control, such as the eye (Kandel, Schwartz, & Jessell, 2000). From the above statement, it can be inferred that muscles requiring precise
movements are subject to a low innervation ratio. Innervation ratio can be defined as the number of muscle fibers innervated by a given alpha-motor neuron. Larger muscles requiring gross movements are subject to a much higher innervation ratio.

The neural innervation derived from the motor neuron dictates the fiber type expression of the muscle, thus, all fibers innervated by a single motor neuron are of the same type. Evidence for the motor neuron dictating the identity of the muscle fiber was demonstrated by reinnervation of the cat flexor digitorum longus muscle, normally a fast twitch muscle, with the slow twitch neuron of the soleus muscle (Dum, O’Donovan, Toop, & Burke, 1985). Following reinnervation of the flexor digitorum longus the muscle behaved similarly to the soleus, taking on properties of a slow twitch muscle. Motor unit properties will be covered in detail below.

**Motor Unit Properties**

In humans, motor units give rise to Type I (slow twitch), Type IIa (fast fatigue resistant), and Type IIx (fast fatigable; Type IIb in animals) fibers, and all are concomitantly present within the body. The Type I fibers are best known for their slow contraction velocity and high fatigue resistance, Type IIa fibers for their fast contraction velocity and relatively high fatigue resistance, and Type IIx for their high contraction velocity and low fatigue resistance.

The various motor unit types can also be distinguished by specific anatomical and physiological properties, such as size conduction speed, mitochondrial and myosin ATPase density, and twitch force (McComas, 1996). Type IIx fibers are the largest, followed by the Type IIa fibers, and finally, Type I fibers. A larger diameter axon allows
action potentials to propagate faster, explaining why Type IIx fibers have the highest conduction velocity. The aforementioned order can also be utilized to order the twitch force from highest to lowest. The fast twitch fibers are densely packed with contractile elements and an alternative myosin ATPase isoform than the slow twitch fibers, allowing for greater force generation in a shorter time frame. Conversely, the Type I fibers that do not produce high force are able to sustain low intensity contractions for a substantially longer time than the Type IIx fibers. Type I fibers are surrounded by an extensive capillary network and are dense in mitochondria, which supplies the muscle with adequate glucose and oxygen to support the aerobic energy system. The aerobic system rapidly produces adenosine triphosphate, a substrate necessary for repetitive muscle contractions. Collectively, these properties indicate the variability of each motor unit, and the importance of each to the body (Kandel et al., 2000; McComas, 1996).

Motor neurons follow a specific pattern in which they are recruited, fittingly named orderly recruitment. This concept was first described by Elwood Henneman (1957). He noted that single motor neurons were recruited as action potential intensity increased, and the attenuated stimulation intensity resulted in the motor neurons de-recruiting opposite to their recruitment. Henneman demonstrated that Type I motor units were recruited first, owing to the fact that they have the lowest stimulation threshold on the basis of the neuron having the greatest resistance. This concept can be explained using the common physics’ law, Ohm’s law, which states that a change in voltage can be equated by the product of current and resistance. Consequently, a smaller surface area leads to a faster increase in voltage that initiates the propagation of an action potential
along the axon at a lower threshold; therefore, Type I fibers are activated in the initial stages of a muscular contraction. The greater resistance is counterproductive to conduction velocity resulting in Type I fibers having the slowest rate of conduction, whereas the larger neurons conduct faster as a product of less resistance.

In 1977, the orderly recruitment of motor units was rightfully renamed the “size principle” to encapsulate the notion that smallest motor units are the first to be recruited when movement is initiated (Henneman, 1977). This concept can be better explained by referring to Figure 1 adapted from Monster and Chan (1977). Note that at lower force outputs, two motor unit firing patterns are separated from the majority of the firing patterns (far left of graph). These two patterns represent the Type I motor units which are the first to be recruited. As force increases, the firing of an individual motor unit increases and the graph becomes more concentrated with firing patterns, which denotes the recruitment of Type IIa and Type IIx fibers. Interestingly, all the motor units are initially activated at the same frequency, representing no difference in firing thresholds. They do, however, exhibit a range of discharge that varies greatly with increases in force.
Figure 1. Discharge rate and firing frequency of motor units during an isometric contraction with progressively increasing intensity. Adapted from “Isometric Force Production by Motor Units of Extensor Digitorum Communis Muscle in Man,” by A. W. Monster, & H. Chan, 1977, Journal of Neurophysiology, 40(6), p. 1434. Copyright 1977 by the American Physiological Society. Adapted with permission.

The time period between action potentials firing in an individual motor unit is termed the discharge rate. The different motor unit types, similar to their other properties, vary in their rate of discharge. Referring again to Figure 1, Monster and Chan (1977) demonstrated that Type I fibers continue to discharge over a wide range of forces, but do not discharge as frequently as their faster conducting counterparts. Again, as force increases, the slow twitch (Type I) fibers conduct action potentials over a wider range of lower level force, reinforcing the evidence that Type I fibers maintain low level force for an extended time period. The fast twitch (Type IIa & IIx) fibers fire over a very short
range of higher force outputs, and do not discharge many action potentials before silencing.

Orderly recruitment and discharge rate are imperative to the normal functioning of a muscle contraction. By recruiting the low threshold motor units first, the body ensures that the force being produced is proportional to the force that is needed, because it would not be advantageous to activate high threshold motor units when fine motor control is required (i.e., signing your name). To increase force, greater synaptic input from higher centers is required, and the next higher order motor neuron is stimulated. Concurrently, the discharge of the first order motor neuron increases. The simultaneous execution of the two allows for gradual force production during a given contraction.

**Motor Unit Estimation Techniques**

Quantification of motor units has been performed using a variety of techniques and evolved rapidly throughout the years. The first technique developed quantified the number of motor units based off microscopic counts of cadaver cross sectional cuts (Feinstein, 1955). Following Feinstein’s (1955) work, others have counted motor units by assessing histological cuts, developing reference values for various muscles (Blevins, 1967; Carvalho, 1976; Carvalho, Cintra, & Santo Neto, 1988; English & Blevins, 1969; Santo Neto, Carvalho, & Maraques, 1998; Santo Neto, Carvalho, & Penteado, 1985; Santo Neto, Filho, Passini Jr., & Marques, 2004). Although assessing histological cuts is considered the gold standard, the muscles are from cadavers, making this technique nonapplicable to the clinical setting, or in vivo scientific investigations. Instances when
motor unit count needs to be assessed in vivo (i.e., neuropathies, aging, and injury) a noninvasive, or minimally-invasive method to estimate motor unit numbers is necessary.

In response to the lack of a clinically relevant method for motor unit number quantification, McComas, Fawcett, Campbell, and Sica (1971) developed a technique that measured electromyography (EMG) signals from the extensor digitorum brevis muscle to estimate the number of motor units. This was the first study to calculate motor unit number via a mathematical equation using a motor unit number estimation (MUNE) technique in living subjects. The original technique has been modified into several versions; more specifically, the process of acquiring EMG signals has changed (Doherty & Brown, 1993; Stein & Yang, 1990). The multiple versions of the MUNE and level of difficulty to perform prompted the recent development of the motor unit number index (MUNIX) technique (Nandedkar, et al., 2004). Similar to the MUNE technique, MUNIX is measured by sampling muscle activity with EMG but is less difficult to operate and more time efficient than the MUNE techniques. The development of noninvasive or minimally-invasive techniques to estimate motor unit numbers is important to the early detection of motor unit loss in certain populations. The following sections will provide a procedural overview of each MUNE and MUNIX technique along with advantages and disadvantages of each.

**Motor unit number estimation (MUNE).** In the decades following the introduction of MUNE by McComas et al. (1971), 415 studies (PubMed search) have been performed throughout the world using variations of the MUNE technique. Motor unit number estimation can be performed using the following methods: incremental
stimulation, multipoint stimulation, spike-triggered average, and statistical MUNE
(Daube, 2006).

The motor unit number estimations were developed partially from standard nerve
conduction studies commonly performed in clinical settings (Barkhaus, Kincaid, &
Nandedkar, 2011). Nerve conduction studies are performed by applying an electrical
stimulus to the nerve that innervates the targeted muscle. The electrical current induces a
muscle contraction and the electrical amplitude (mV) is recorded by EMG electrodes.
The nerve is stimulated until maximal amplitude is obtained, generating a compound
muscle action potential, or CMAP (also referred to as M_{max}) (Barkhaus et al., 2011).
Assessing compound muscle action potential amplitude indicates the muscle’s potential
to contract through artificial stimulation. The use of compound muscle action potential
measurement is not the ideal diagnostic, because it lacks the ability to detect motor unit
number. For example, instances where motor unit number is low, compound muscle
action potential may remain elevated due to collateral reinnervation from the surrounding
motor units (i.e., the living neurons generate new nerve branches to innervate the muscle
that lost its neuron) (Nadedkar et al., 2004; Nandedkar, Barkhaus, & Stålberg, 2010).
Studies conducted by Broomberg and Brownell (2008) and Aggarwal and Nicholson
(2002) have shown that CMAP values do not decrease until 50% denervation occurs,
indicating the CMAP measurement lacks the sensitivity to detect motor unit loss in acute
stages. Additionally, the CMAP amplitude is affected by a variety of other physiological
and nonphysiological factors (e.g., subcutaneous adipose tissue), and thus may not
provide an accurate assessment of motor unit numbers (Farina, Merletti, & Enoka, 2004;
Current MUNE techniques measure two items: CMAP amplitude in the same fashion as nerve conduction studies, and surface motor unit action potentials (SMUAP). Each technique, incremental stimulation, multipoint stimulation, and spike-triggered averaging, measures the SMUAPs differently and will be described in detail below.

**Incremental stimulation.** Incremental stimulation is the original MUNE method pioneered by McComas et al. (1971). Performing the technique requires the tester to stimulate the motor nerve at a frequency of 1 Hz at the same location in a graded fashion. Typically, the first stimulation is delivered at the lowest threshold level, and the intensity is increased slightly until a rise in EMG amplitude above the first stimulation is recorded. The small increase in amplitude represents an additional motor unit reaching threshold and firing. The SMUAPs are considered to be all or none; a rise in amplitude must be reported for that measurement to be taken in to consideration during the motor unit quantification process. According to McComas et al. (1971) 11 SMUAPs are used in the calculation of MUNE.

Once an adequate number of SMUAPs have been obtained, an average value is calculated. The average SMUAP can be calculated by dividing the number of stimulations used into the sum of the SMUAPs. Dividing the average SMUAP into the CMAP will yield a motor unit number estimation (MUNE) via incremental stimulation (McComas et al., 1971).
Advantages. The incremental stimulation model is advantageous to use in certain aspects as described by Rashidipour & Chan (2008). Assessment of motor unit number with incremental stimulation may be obtained with any standard EMG recording device, eliminating the cost of additional software or new equipment. SMUAPs are collected from a single stimulation site. Single site stimulation may decrease the variability between testing sessions as seen in the study by Galea, Bruin, Cavasin, and McComas (1991). They conducted baseline and multiple follow up measures that resulted in an average coefficient of variation of 17% at test one, but that value decreased to 0% at the last testing session demonstrating excellent reliability. Electrical stimulation is performed noninvasively, minimizing patient discomfort and therefore enhancing cooperation on the patient’s behalf. Coinciding with patient comfort, stimulations ranging from 15-20 to acquire both a CMAP and SMUAPs make incremental stimulation an attractive method of estimating motor units.

Disadvantages. Opposing the advantages of this MUNE model, incremental stimulation is not void of downfalls. Artificial stimulation of the motor nerve allows this model to be applicable to distal muscles, yet cannot be applied for proximal muscle assessment (Rashidipour & Chan, 2008). Proximal stimulation sites result in reduced CMAP amplitude, a product of the depth of the nerve and other muscles interfering with the target muscles signal (Barkhaus et al., 2011). Conducting studies employing incremental stimulation also requires a vast amount of expertise as well as time to practice (Rashidipour & Chan, 2008). Application of the graded stimuli requires the tester to accurately identify additional motor units being recruited, therefore creating
tester bias. Defining what constitutes as an increase in SMUAP amplitude varies between testers, and is generally hard to identify. In order to resolve the issue of subjectivity, an automated device was invented to compute the difference in waveforms, eliminating human error (Galea et al., 1991). Although the problem of identifying waveform amplitude change was solved, alternation is an ever present issue associated with the incremental stimulation technique. Alternation has been termed as multiple motor units in the muscle having identical firing thresholds, and with each stimulation, motor units fire in different combinations yielding fictitious motor unit numbers (McComas, 1996). Regardless of what method is used, manual or automated incremental stimulation, alternation cannot be avoided, and ultimately leads to an over estimation of motor unit number (McComas, 1995). Erroneously high motor unit estimation is not an ideal measurement in any setting, especially when assessed clinically. Errors in estimation can lead to misdiagnosis, or even no diagnosis in individuals who are suffering a neurodegenerative disease.

**Multipoint stimulation.** The MUNE technique’s second method of sampling, multipoint stimulation, was developed in attempt to quell the effects of alternation encountered with incremental stimulation. Assessment of SMUAPs with multipoint stimulation requires multiple stimulations of the motor nerve at various locations along its length. At every location the nerve is stimulated until the lowest threshold motor unit is recruited. The nerve is then stimulated multiple times at that exact location with the initial intensity to assure the SMUAP is from the lowest threshold motor unit possible. Differences detected in wave morphology or action potential initiation is the result of
motor units overlapping, indicating that stimulation did not recruit the lowest threshold motor unit. From the multiple location stimulations, the SMUAPs are averaged and divided into the CMAP in the same manner as the incremental stimulation model (Rashidipour & Chan, 2008).

Advantages. Similar to incremental stimulation, electrical stimulations are provided externally, increasing patient comfort levels by using noninvasive electrodes. Patient comfort is further enhanced with multipoint stimulation due to the low intensity stimulations to find the lowest threshold motor neurons (Rashidipour & Chan, 2008). Doherty and Brown (1993) reported that stimulations close to threshold were tolerated very well. Most importantly, alternation is avoided using this method. Doherty & Brown (1993) reported that from 15 sites stimulated, they confidently determined all SMUAPs were derived from single motor units. Stimulating the site multiple times removes tester subjectivity when detecting changes in shape or latency of surface action potentials.

Disadvantages. Multipoint stimulation’s major shortcoming is related to the number of stimulations used to obtain SMUAPs for averaging. It was originally thought that collecting 15 SMUAPs would warrant sufficient reproducibility, as Doherty and Brown (1993) reported a correlation coefficient of 0.88. In recent years, the correlation between number of SMUAPs and reproducibility has been questioned among researchers. Porter, Alvarez, Jones, and Chan (2008) concluded that collecting greater than 15 SMUAPs yielded high percentage error values, Cronbach’s $\alpha = 0.80$ for younger adults and 0.96 for older adults. When only the first 15 sites were considered for percentage error calculation, the Cronbach’s $\alpha = 0.62$ (Porter et al., 2008), indicating the need to
sample from a large number of signals. Multipoint stimulation requires stimulations exceeding 15, but what is the proper amount of stimulations necessary to produce reliable data? Although not statistically significant due to the low sample size, 18 stimulations (6 sites, 3 stimulations each site) indicated the greatest reproducibility in comparison to 9, 12, and 15 stimulations (Goyal, Salameh, Baldassari, & David, 2010). As the research suggests, more stimulations equates to greater reproducibility, but leads to a greater amount of patient compliance and increased time to test. Similar to incremental stimulation, multipoint stimulation requires tester expertise, and is inapplicable to proximal muscles (Rashidipour & Chan, 2008). Tester expertise is required to superficially follow the path of the motor nerve, and to accurately identify the lowest threshold motor neurons. Proximal muscle assessment is difficult in that stimulations do not yield CMAP values that accurately depict the muscles potential, and that various muscles innervated by that nerve root may be stimulated (Barkhaus et al., 2011).

**Spike-triggered averaging method.** The most recent derivative of the original MUNE technique is spike-triggered averaging. The spike-triggered averaging method differs from the previous two methods when sampling the SMUAPs. Rather than electrically stimulating the motor nerve to induce a muscle contraction, spike-triggered averaging utilizes voluntary contractions to gather SMUAPs (Rashidipour & Chan, 2008). A needle electrode is inserted into the patient’s muscle, and the patient contracts at various percentages of their maximal voluntary contraction. Voluntary contraction elicits a spike, displayed on the needle EMG recording, to indicate the initiation of an action potential. The needle EMG data is paired with the surface EMG recording to
allow the SMUAP to be measured (McNeil, Doherty, Stashuk, & Rice, 2005b). The appearance of a spike on the needle EMG recording represents the activation of a motor unit which allows the researcher to measure the SMUAP preceding the spike. The needle electrode is relocated in the muscle a minimum of 10 times, and the series of sub threshold contractions is repeated. SMUAPs recorded at each site are averaged and divided into the CMAP, which is obtained via supra-maximal electrical stimulation (Rashidipour & Chan, 2008).

It is worthy of mention that decomposition-based quantitative EMG (DQEMG) was developed from spike-triggered averaging. The acquisition of SMUAPs and CMAPs is exactly the same as spike-triggered averaging except that DQEMG’s identification of SMUAPs is fully automated. One can infer that errors encountered without automation are avoided much in the same way that automation of the incremental stimulation model quelled alternation and tester subjectivity. This review will not discuss the characteristics of DQEMG, because they closely parallel spike-triggered averaging.

Advantages. Rationale for spike-triggered averaging is that the technique can be applied to both distal and proximal muscles. Unlike the previous two techniques, spike-triggered averaging can be utilized proximally because electrical stimulation is not used to obtain SMUAPs (Rashidipour & Chan, 2008).

Disadvantages. Again, spike-triggered averaging, much like the other techniques, is not void of flaws. Recording waveforms from needle and surface electrodes requires specific software, increasing laboratory costs, and time spent learning a new program (Rashidipour & Chan, 2008). The use of needle electrodes decreases patient comfort
level, especially when the needle is relocated multiple times during the testing session. The most prominent disadvantage is the time consuming nature of recording SMUAPs from different sites along the muscle at each of the various contraction intensities. Over 100 SMUAPs are recorded, which have to be analyzed and calculated offline. It is necessary to collect data from various intensities because evidence is limited to what contraction intensity elicits the best representation of the motor pool. For the tibialis anterior muscle, McNeil et al. (2005) found that 25% of maximal voluntary contraction captured the motor unit number most accurately. Optimal contraction intensities for other muscles and disease populations are not available, leading researchers to sample from a range of contraction intensities and average the MUNE value calculated at each contraction intensity. In regard to the voluntary contractions, patients must hold contractions for 30 seconds to ensure motor units recruited are different, not recurrent firing of a few motor units. Holding contractions for 30 seconds at a high percentage of maximal voluntary contraction becomes difficult and results in an unsteady SMUAP recording, which decreases confidence in the accuracy of the recording (McNeil et al., 2005b).

**Motor unit number index (MUNIX).** Developed in 2004 by Sanjeev Nandedkar, Ph.D., the MUNIX technique is essentially a MUNE technique. While similar to the classical MUNE techniques in many aspects, the MUNIX technique is probably the quickest and most feasible technique currently available. Compound muscle action potential amplitude is acquired identically to the other techniques, and gathering surface interference patterns (SIP) is conducted in the same fashion as collecting SMUAPs with
the spike-triggered averaging model. The difference is that the recording electrodes are located on the surface of the skin. Following collection of these variables, analysis of the data is performed offline with custom written software. Here, the area and power are calculated for both CMAP and SIPs (at each percentage of maximal contraction). Once the area and power are calculated, an ideal condition motor unit count (ICMUC) is calculated for each contraction intensity. The ICMUC at each intensity is plotted over the SIP area and a power regression curve is created. To obtain a MUNIX value, which represents an index of motor units in the muscle, the power curve is extrapolated backwards to 20mV on the SIP area axis (x-axis). A contraction intensity corresponding to 20mV assumes that all motor units are activated, as this represents a low level of force output. In doing so, the number of motor units are not estimated, but rather are indexed for future reference when documenting a patient’s absolute change in motor units over time. In addition to indexing the motor units, this technique offers insight into the size of the motor unit by calculating MUSIX to indicate if collateral reinnervation has occurred. MUSIX can be calculated by dividing the CMAP amplitude by the MUNIX value. For a complete description of the MUNIX technique methodology, see chapter three.

**Advantages.** Utilizing MUNIX for motor unit indexing is appealing due to the low amount of electrical stimulations required and the short time frame needed to sample one muscle (Sandberg et al., 2011). Additionally, the technical expertise required to perform the MUNIX technique is relatively low. MUNIX is also applicable to large, proximal muscles; the biceps brachii and tibialis anterior have shown reliable values (Neuwirth, Nandedkar, Stålberg, & Weber, 2011; Sandberg et al., 2011). Lastly, for most
muscles, the intra- and inter-rater reliability is above the clinically accepted value when sampling from healthy and diseased populations (Ahn et al., 2010; Nandedkar, Barkhaus, & Stålberg, 2011; Neuwirth et al., 2011). Although reproducibility was high overall, particular muscles are found to have poor reproducibility.

**Disadvantages.** The disadvantages of the MUNIX technique will be discussed in this section. Similar to the STA technique, contraction intensity is not commonly monitored by the tester. The regression curve created to calculate MUNIX is dependent on a wide array of data points. Failure to represent a spread of contraction intensities will yield an erroneous MUNIX. Also, the time for isometric contraction has not been defined; only a 300-millisecond sample is used to obtain data for a SIP (Nandedkar et al., 2004). The short time frame may not capture all motor units firing at that given intensity (McNeil et al., 2005b). As mentioned above, certain muscles display low reliability. Specifically the abductor pollicis brevis yielded a low inter- and intra-rater reliability when measured by independent researchers (Neuwirth et al., 2011). On small muscles such as the abductor pollicis brevis and others of similar size, accurate electrode placement is imperative. There is no standard placement of electrodes for each muscle, leading to the variable placement among researchers, thereby reducing the reproducibility of the MUNIX value.

**Rationale for MUNIX**

As stated above, MUNIX, technically speaking, is not a motor unit number estimation, but an index used to compare absolute changes in motor unit quantity over time. A previous investigation showed that MUNIX was able to detect motor unit
decrements over a 3-15 month period in Amyotrophic Lateral Sclerosis patients, a disease known to degenerate motor neurons (Neuwith, Nandedkar, Stålberg, & Weber, 2010). The MUNIX technique has also proven to be noninvasive and minimally painful, and to require a shorter time to perform with respect to the other techniques.
Chapter 3: Reliability of the Motor Unit Number Index (MUNIX)\(^1\)

Abstract

**Introduction:** The purpose of this study was to examine the relative and absolute between-day reliability of the motor unit number index (MUNIX). **Methods:** Young, healthy adults (n = 19) attended two testing sessions separated by 4 weeks where their maximal pinch-grip strength, MUNIX, and motor unit size index (MUSIX) were assessed. Reliability was assessed by intraclass correlation coefficients (ICC), coefficient of variation (CV), and limits of agreement (LOA). **Results:** No mean differences were observed for MUNIX or MUSIX. The CV for the MUNIX and MUSIX measures were between 14-16%. The ICC for MUNIX and MUSIX values were moderate to moderately-high (0.68-0.75). The LOA for both indicated a homoscedastic relationship. **Discussion:** My findings indicate moderate to moderately-high reliability for both MUNIX and MUSIX. Future work is needed to ensure both measures are reliable in other muscle groups and cohorts, and further investigations are required to examine the validity of MUNIX.

**KEYWORDS:** Motor unit, EMG, Muscle, MUNIX, MUSIX

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\(^1\) This chapter represents a prepublication manuscript submitted to Journal of Electromyography and Kinesiology (March 16, 2013), which has been adapted slightly to conform to Ohio University’s thesis format. Authors are Ryan D. Kaya (School of Applied Health Sciences and Wellness, College of Health Sciences and Professions, Ohio University, Athens, OH), with Richard L. Hoffman and Brian C. Clark (Ohio Neurological and Musculoskeletal Institute, Heritage College of Medicine, Ohio University, Athens, OH).
Introduction

A motor unit is defined as an alpha motor neuron and all of the muscle fibers it innervates (Sherrington, 1925). Quantification of motor unit number has long been of clinical and scientific significance, because it relates to monitoring disease progression and/or assessing the effects of pharmacologic and behavioral interventions on motor unit numbers (Broomberg, 2013). Over the past four decades, a number of techniques have been developed to estimate motor unit number in vivo (Doherty, 1995; Nandedkar, Nandedkar, Barkhaus, & Stålberg, 2004; Rashidipour & Chan, 2008). The earliest of these techniques was the motor unit number estimation (MUNE) technique introduced in 1971 (McComas, Fawcett, Campbell, & Sica, 1971), and since that time a number of modifications to the MUNE technique have been developed and implemented (Brown, Strong, & Snow, 1988; Daube, 1995; Doherty & Brown, 1994; Doherty & Stashuk, 2003; Kadrie, Yates, Milner-Brown, & Brown, 1976; Shefner, 2011; Wang & Delwaide, 1995). The MUNE methods involve estimates of single motor unit action potential size using either incremental electrical nerve stimulation or spike triggered averaging techniques. While these methods are generally considered the standard for in vivo motor unit number quantification, they are not without shortcomings. For instance, the methods can be time consuming for both patient and examiner and physically uncomfortable for the patient, e.g., the high number of electrical stimuli and/or insertion of a needle electrode into a muscle can be painful (Rashidipour & Chan, 2008). As such, there has been a demand from both scientists and clinicians to develop noninvasive, easy to implement, and highly
tolerable alternative techniques to obtain an in vivo estimate of motor unit number (Broomberg, 2013).

In 2004, Nandedkar and colleagues proposed a novel neurophysiological technique (the motor unit number index, or MUNIX) to derive an index associated with the number of motor units in a muscle (Nandedkar et al., 2004). The MUNIX is derived from the maximum compound muscle fiber action potential (CMAP) observed in response to supramaximal electrical stimulation and voluntary surface electromyogram (EMG) recordings associated with a series of submaximal muscle contractions. The MUNIX technique is noninvasive, quick (i.e., it requires ~10 minutes to derive a MUNIX value per muscle), and easy to implement (i.e., the instrumentation and technical expertise required are readily available in most clinical and research settings). Additionally, it is considered quite tolerable to most (e.g., only a few electrical stimuli and muscle contractions are required). In general, the MUNIX is considered a value that is proportional to the motor unit numbers in a muscle, as opposed to representing an absolute number of motor units in a muscle (Nandedkar et al., 2004; Nandedkar, Barkhaus, & Stålberg, 2010; Nandedkar, Barkhaus, & Stålberg, 2011). In recent years, MUNIX has been used to quantify motoneuron loss in Amyotrophic Lateral Sclerosis (ALS), and it has been observed to be lower in a) older adults when compared to younger adults (Neuwirth et al., 2011b), and b) paretic muscle when compared to contralateral muscles of stroke survivors (Li, Rymer, & Zhou, 2012).

Only a few studies have examined the reliability of the MUNIX in healthy individuals where no changes in motor unit numbers are expected to occur over time.
(Ahn et al., 2010; Furtula et al., 2013; Nandedkar et al., 2011; Sandberg, Nandedkar, & Stålberg, 2011). The majority of these studies were poorly controlled in that within- and between-day test-retest data were pooled to derive measures of MUNIX stability (Ahn et al., 2010; Furtula et al., 2013; Neuwirth et al., 2011a; Sandberg et al., 2011). In fact, only one study has reported the between-day reliability of the MUNIX, and this study simply noted that the mean MUNIX values were similar across testing sessions occurring up to one-year apart in a small number of healthy subjects (n = 6-8; range: 154 to 162) (Nandedkar et al., 2011). Accordingly, the purpose of the present study was to comprehensively examine the relative and absolute reliability of the MUNIX in young, healthy individuals when assessed on two occasions separated by 4-weeks. Additionally, relative and absolute reliability of the motor unit number size index (MUSIX) was examined, which is derived by dividing the maximum CMAP amplitude by the MUNIX.

**Methods and Materials**

**Subjects.** Nineteen young adults (age range 18-33 years) participated in this study. To be eligible for the study, subjects had to have a BMI < 30 kg/m², and subjects were excluded if they were taking any medications or supplements, or had any known neurological or orthopedic conditions. Regular resistance exercise (> 1/week), or a score of “very low active” or “high active” on the Lipids Research Physical Activity Questionnaire (Ainsworth, Jacobs, & Leon, 1993) were also grounds for exclusion. The Ohio University Institutional Review Board approved this study and all subjects provided informed consent prior to participation.
Overview of the experimental design. Following an initial orientation, qualified individuals attended two testing sessions separated by 4 weeks. Participants had their maximal pinch-grip strength assessed, motor unit number indexed by means of MUNIX, and the motor unit size indexed by means of MUSIX. Subjects were asked to refrain from alcohol (24 hours) and caffeine, nicotine, and exercise (4 hours) before the testing sessions. In the period between test one and test two, participants were asked to maintain their normal lifestyle and diet. Relative reliability was assessed for the MUNIX and MUSIX variables using the calculation of intraclass correlation coefficients (ICC), and absolute reliability was assessed via coefficient of variation (CV), standard error of the mean (SEM) and limits of agreement (LOA).

Electrical and mechanical recordings. EMG signals were recorded using surface electrodes (Kendall Soft-E H69PSurface Electrodes, Kendall Ltp, Mansfield, MA) arranged in a monopolar fashion on the palmar surface of the nondominant hand. Specifically, the active recording electrode was placed over the belly of the abductor pollicis brevis muscle, the reference electrode was placed on the ipsilateral wrist flexor tendon, and the ground electrode was placed on the bony portion of the dorsal aspect of the contralateral hand. The EMG signals were amplified (500-1,000x), bandpass-filtered (10-500 Hz), and sampled at 10,000 Hz (MP150, Biopac Systems, Goleta, CA).

Pinch-grip strength was quantified using a force transducer (TSD121C, Biopac Systems), and the signals were smoothed over a 5-msec running average (AcqKnowledge 4.2, Biopac Systems). Participants’ forearm and wrist were positioned in an anatomically neutral position at the level of the xiphoid process while seated at a table. During the
pinch-grip task, participants pinched a 3.7 cm wide transducer with the pads of the thumb (1st finger) and index finger (2nd finger). The third through fifth fingers were flexed and secured to the hand using an elastic band (Fabrifoam, Exton, Pennsylvania). Subjects were given real-time visual feedback on a computer monitor located 0.5 meters in front of them (AcqKnowledge 4.2, Biopac Systems). An illustration of the experimental set-up is shown in Figure 2.
Figure 2. Experimental setup of subject performing graded contractions at percentage(s) of their maximal voluntary contraction force. Inset: Enlarged view of the force transducer and grip task protocol.

**Pinch-grip strength.** Maximal pinch-grip strength was assessed by having subjects perform a minimum of three maximal voluntary isometric contractions (MVCs) (see Figure 2). A 1- to 2-minute rest period was given between trials. The MVC force was considered as the highest value recorded. If the participants’ highest values were not within 5% of each other, additional trials were permitted. Verbal encouragement was given with each attempt and subjects were provided visual feedback of their force output on the computer monitor.

**Compound muscle action potentials and surface interference patterns.** The maximal compound muscle action potential (CMAP) (see Figure 3) was evoked by
stimulation of the median nerve with a constant current stimulator (200-microsecond pulse; Model DS7AH, Digitmer Ltd., England) in the area two centimeters proximal of the wrist crease. The maximum CMAP was determined by failure of the M-wave to increase in amplitude despite an increase in stimulator output.

Surface interference patterns (SIPs) (see Figure 3) were recorded during brief (~ 5 second) submaximal contractions. Here, the study participants performed a series of 12 submaximal isometric contractions. The first six increased in intensity, starting at a contraction intensity of 10% and increased in 10% increments until a contraction intensity of 60% of MVC was achieved. Subsequently, participants performed the same series of submaximal contractions in reverse order (i.e., a brief contraction at 60% MVC followed by decreasing intensity contractions in 10% increments). During each of these contractions, a target line representing each specified contraction level was displayed on the computer monitor and subjects were asked to match this target line. At least 20-seconds rest was allowed between each contraction.
Figure 3. Representative example of an evoked compound muscle action potential (left trace), and the surface electrographic interference patterns (top right trace) during brief pinch-grip isometric force contraction tasks (bottom right trace).

A total of 12 voluntary contractions were performed, but only six of the corresponding signals were used in the subsequent analyses. This was done to ensure that at least one of the two signals at each of the intensities was usable in the MUNIX calculation. Specifically, the first trial was used in the MUNIX calculation unless this trial exhibited artifact or noise, and in these instances the second trial was used for analysis (see below for further details).

**MUNIX calculation.** The MUNIX value is based on a mathematical model that relies on evoked CMAP’s and surface EMG data from the voluntary contractions (i.e., the SIP’s) (Ahn et al., 2010; Nandedkar et al., 2004, 2010). Specifically, the CMAP and SIP
area and power were calculated, and applied to equation 1 to yield an ideal case motor unit count (ICMUC) for every contraction intensity.

\[
\text{ICMUC} = \frac{\text{CMAP}_{\text{Power}} \times \text{SIP}_{\text{Area}}}{\text{CMAP}_{\text{Area}} \times \text{SIP}_{\text{Power}}} \quad (1)
\]

The ICMUC assumes that all motor unit action potentials are the same and no phase cancellation occurs, resulting in an index of the number of motor units in the muscle. Each ICMUC value was plotted over the SIP area, and the points were fit with a power regression. Where the line crosses 20-mV on the x-axis, the ICMUC value is reported as the MUNIX value, representing an estimate of the number of motor units in the muscle. The SIP area of 20-mV is based on the premise that it reflects a low level of force output that elicits the firing of all low threshold motor units (Nandedkar et al., 2004, 2010).

To provide further insight about the reliability of motor unit properties, the motor unit size index (MUSIX) was also calculated. Once MUNIX is calculated, MUSIX was computed based on equation 2.

\[
\text{MUSIX} = \frac{\text{CMAP}_{\text{Amplitude}}}{\text{MUNIX}} \quad (2)
\]

Prior to the analyses, the SIP signals were visually scrutinized to identify any artifact in the signal. If substantial noise was detected, the SIP epoch was rejected for analysis. In order to standardize what epochs were accepted, the inclusion criteria previously described by Nandedkar et al. (2010) were utilized. The inclusion criteria were as follows: a) SIP area > 20 mV/ms, b) ICMUC < 100, 3) SIP area/CMAP area > 1.

**Statistical Analysis.** Test-retest reliability for MUNIX and MUSIX values were determined by calculating the CV, ICC (two way random effects model with a single
measure of reliability) and 95% LOA. To compare the means between testing sessions, a dependent sample t-test was used. The CVs, a measure of intrasubject variability, were calculated by computing CVs individually for each subject then averaging the values for each respective outcome variable. The ICC (2,1) was a two-way random effects model with a single measure of reliability in which variance over repeated sessions was considered. A (2,1) model was chosen in that systematic bias can be determined from it.

In addition to the ICC measure (a “relative reliability” statistic), “absolute reliability” was assessed independently of the CV. The absolute stability of a measure is comprised of random error and systematic bias, and it is critical to understanding the stability of the measure to know how these components contribute to it. Systematic bias is the orderly change in a measure over time (e.g., a learning effect). Random error is the result of biological or mechanical variation (Bland & Altman, 1986). The LOA method that partitions out systematic bias vs. random error was used because both MUNIX and MUSIX require voluntary task contractions, indicating that the potential for systematic bias exists (Atkinson & Nevill, 1998; Bland & Altman, 1986). Bland–Altman plots were generated for MUNIX and MUSIX, respectively, and analyzed for the presence of heteroscedasticity (residuals are not equally distributed throughout the range of scores of the dependent variables) (Atkinson & Nevill, 1998; Bland & Altman, 1986). This was determined by examining the explained variation ($R^2$) between the differences (Test 1–Test 2) and the mean values. $R^2$ values between 0 and 0.1 were considered homoscedastic (no relation between residuals and the magnitude of the measured variable) and systematic bias and random error were then calculated (Atkinson & Nevill,
1998). $R^2 > 0.1$ was considered heteroscedastic (amount of random error increases as the measured values increases) and the ratio LOA were then calculated (Sun et al., 1998).

The LOA ratio is as follows: $\text{LOA ratio} = \left[ \frac{\text{SDdiffs}}{\text{AVGmeans}} \right] \times 1.96 \times 100$. Where SDdiffs is the standard deviation of all of the difference scores (Test 1 – Test 2 calculated for each subject), AVGmeans is the average of all of the mean scores (mean of visits 1 and 2 for each subject), and the factor of 1.96 represents the inclusion of 95% of observations of the difference score (Atkinson & Nevill, 1998).

**Results**

**Descriptive characteristics.** The mean age of the subjects was $22.5 \pm 0.9$ years with an average body mass and height of $71.5 \pm 2.9$ kg and $170.6 \pm 2.6$ cm, respectively. Subjects exhibited a slightly greater pinch grip strength during the first testing session ($48.7 \pm 4.5$ N) when compared to the second testing session ($45.3 \pm 4.55$ N, $p = 0.05$). The CMAP amplitude did not vary across the testing sessions ($8.48 \pm 0.7$ vs. $9.21 \pm 0.7$ mV, $p = 0.10$).

**MUNIX reliability.** No mean differences were observed for MUNIX ($111.4 \pm 8.4$ vs. $121.7 \pm 10.1$) between Test 1 and 2 ($p = 0.20$). A CV of $16.8 \pm 2.7\%$ and an ICC of $0.75$ ($p = 0.02$) was observed between Test 1 and 2. From the Bland-Altman plot, the LOA analysis (see Figure 4A) yielded a homoscedastic relationship ($R^2 = 0.06$), with follow-up analysis indicating systematic bias and random error on the order of 10.3 and 56.1, respectively.

**MUSIX reliability.** No mean difference was observed for MUSIX ($78.2 \pm 4.7$ vs. $80.8 \pm 5.1$) between Test 1 and 2 ($p = 0.60$). A CV of $14.8 \pm 2.9\%$ and an ICC of $0.68$ ($p$
= 0.01) was observed between Test 1 and 2. From the Bland-Altman plot, the LOA analysis (see Figure 4B) yielded a homoscedastic relationship ($R^2 = 0.01$), with follow-up analysis indicating systematic bias and random error on the order of 2.6 and 38.7, respectively.
Figure 4. (A) Bland-Altman plot for the motor unit number index (MUNIX) values. The random error bars represent the confidence interval that 95% of all cases will have a test-retest difference of ± 56.1. (B) Bland-Altman plot for the motor unit size index (MUSIX) values. The random error bars represent the confidence interval that 95% of all cases will have a test-retest difference of ± 38.7.
Discussion

The extant literature surrounding the reliability of the MUNIX technique in a healthy adult population where no changes in motor unit numbers are expected to occur over time is limited. As such, the primary objective of this investigation was to comprehensively examine the relative and absolute reliability of the aforementioned technique over a consistent time period of 4 weeks in healthy adults. Moderate to moderately-high relative test-retest reliability (i.e., ICC’s of 0.68 to 0.75) for the MUSIX and MUNIX values was observed. Moderate absolute test-retest reliability was observed (CV’s of 14-16%) for MUNIX and MUSIX, with the majority of the variation being attributed to random error. Understanding the reliability of the MUNIX and MUSIX measures over time is critical to the future development and implementation of these techniques in both the clinical and scientific settings.

Overall, the degree of reliability observed for the MUNIX technique is higher than the majority of other studies that have examined the stability of MUNIX in both healthy and diseased individuals. For instance, the observed MUNIX CV finding (16%) is lower than that previously reported (Ahn et al., 2010; Nandedkar et al., 2004, 2010; Neuwirth et al., 2011a; Sandberg et al, 2011), and is the first to be less than 20%. Similarly, my ICC (0.75) was comparable to that found in the APB muscle of patients with ALS (0.74) when assessed on two occasions separated by 2 weeks (Boekestein et al., 2012), and considerably higher than that reported in healthy individuals by others (Furtula et al., 2013; Neuwirth et al., 2011a, 2011b). However, it should be noted that these comparison studies were largely conducted in clinical settings (as opposed to a
controlled laboratory setting) and in many instances the investigators did not control for the time between testing sessions (Furtula et al., 2013; Li et al., 2012; Neuwirth et al., 2011a; Sandberg et al., 2011). Accordingly, these findings suggest that moderate to moderately-high test-retest reliability measures of MUNIX can be obtained under well-controlled circumstances. To my knowledge, no prior work has examined the reliability of MUSIX, so, making comparisons to the literature for this measure is not possible at this time.

It should be noted that the average MUNIX values in this study (~ 115) are slightly lower than previous MUNIX investigations that assessed the APB muscle (~ 177-190 depending on the study) (Neuwirth et al., 2011b, 2010) and are higher than those reported in patients with ALS (~ 80) (Nandedkar et al., 2011). It is likely that subtle differences in the mechanical setup and tasks could explain these differences, because it has been reported that the MUNIX value varies depending on subtle differences in contraction tasks (Zhou, Li, & Rymer, 2012). Perhaps the larger question surrounding the MUNIX technique relates to the validity of the technique when compared to other more commonly accepted in vivo techniques to assess motor unit numbers (e.g., selected MUNE techniques) (Major & Jones, 2005). Much like other MUNIX investigations of the APB muscle (Neuwirth et al., 2011b, 2010), the average values in this study indexed below what the various MUNE techniques have reported (Doherty, Vandervoort, Taylor, & Brown, 1993; Galea, de Bruin, Cavasin, & McComas, 1991; Stein & Yang, 1990; Wand & Delwaide, 1995). MUNIX, as mentioned earlier (Nandedkar et al., 2004, 2010) is generally considered to represent an “index” of motor unit numbers in a muscle, as
opposed to representing an absolute “estimate” of the number of motor units in a muscle (Nandedkar et al., 2004, 2010). For instance, the typical approach for calculating MUNIX involves determining the ICMUC value that corresponds to a SIP area of 20-mV. This SIP area value of 20mV appears to have been set arbitrarily, so perhaps other approaches could be developed that result in values that are closer to those observed with MUNE techniques. To date, only a couple of studies have directly compared MUNIX values to those observed with some MUNE methods (Boekestein et al., 2012; Furtula et al., 2013), but these studies have reported discrepant results. For instance, Furtula et al. (2013) reported no significant correlations between the MUNIX and the incremental stimulation MUNE values in both healthy and diseased (ALS) individuals. Conversely, Boekestein and colleagues (2012) reported a significant positive correlation between MUNIX and high-density MUNE values in ALS patients (r values ranging 0.49-0.56 depending on the time point of assessment); however, no correlation was observed in healthy individuals (Boekestein et al., 2012). As such, the question of the validity of the MUNIX technique needs to be further addressed. It should be noted that the MUNIX technique is able to track motoneuron loss in ALS and detects lower MUNIX values in the elderly and in paretic muscles; as such, these data provide some basic construct validity of the novel technique (Li et al., 2012; Neuwirth et al., 2010). One major limitation to addressing this question is that the MUNE techniques only represent an estimate of motor unit number “function.” Cadaveric counts, which certainly represent a “gold standard” measure of anatomical number, cannot account for the possibility of a motor neuron being functionally unresponsive and cannot be used as an in vivo measure.
Thus, validating in vivo measures of motor unit number is a challenging and difficult field.

There are several limitations to the current work that should be noted. First, the findings of this work may not be generalizable to the clinical testing environment, because this study was conducted in a well-controlled laboratory setting where the same investigator tested all subjects to ensure precise consistency of testing procedures. This may have contributed to the higher reliability measures that we observed in comparison to those reported in the literature. Another limitation is that this investigation only assessed one muscle, and thus the findings may not be transferable to other muscles. Lastly, it should be noted that the subjects used in this study, were young, physically active individuals with a low body mass index. Therefore, it may be inappropriate to extrapolate these findings to other populations where the physical characteristics may directly influence many of these variables. For example, it is well known that subcutaneous adipose tissue acts as a low-pass filter on the recorded surface EMG signal (Bilodeau et al., 1995), thus in populations where this may vary (i.e., obesity), the reliability may be different.

Conclusions

The purpose of the present study was to comprehensively examine the relative and absolute reliability of measures of motor unit number and size using this technique in young, healthy individuals when assessed on two occasions separated by 4 weeks. The findings indicated moderately-high relative reliability for MUNIX (ICC = 0.75), with slightly lower relative reliability for MUSIX (ICC = 0.68). Absolute reliability is likely
within the acceptable range for many neuromuscular outcomes (CV = 14-16%), and the LOA analysis for both MUNIX and MUSIX indicated a homoscedastic relationship with the majority of the variability across testing sessions being attributed to random error and a lesser contribution from systematic bias. Future work is needed to ensure MUNIX and MUSIX measures are reliable in other muscles and cohorts of individuals, and further investigations are required to examine the validity of MUNIX-based measures.

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Chapter 4: Is Age-Related Weakness a Result of Functional Motor Unit Loss?  

Abstract

**Background:** The interrelationship between muscle strength, motor unit (MU) numbers, and age is currently unknown. The purpose of this pilot experiment was to determine whether age-related differences in muscle strength are moderated by estimates of functioning MU number and size. **Methods:** Eighteen older adults (OA; 67.3 ± 5.3 years) and 24 young adults (YA; 22.1 ± 3.6 years) participated in this study. Maximum voluntary pinch grip strength of the nondominant hand was determined and estimates of MU number were obtained from the abductor pollicis brevis muscle using the noninvasive motor unit number index (MUNIX) technique. An Analysis of Covariance (Age Group X Strength) was used to test heterogeneity of regression slopes, with body mass and gender serving as covariates. **Results:** The slope of pinch grip strength on the estimated number of MUs between YA and OA differed, indicated by an Age Group X Strength interaction (p < 0.01). Specifically, after controlling for the effect of body mass and gender, the slope in OA was significantly positive (2.22 ± 0.72 MUs/N), whereas no such relationship was found in YA (0.08 ± 0.51 MUs/N). **Conclusions:** These preliminary findings suggest an interrelationship between muscle strength, MU numbers, and aging. However, we caution over interpretation of our findings based on concerns over comparing MU numbers between groups calculated via the MUNIX technique.

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2 This chapter represents a prepublication manuscript, which has been adapted slightly to conform to Ohio University’s thesis format. Authors are Ryan D. Kaya (School of Applied Health Sciences and Wellness, College of Health Sciences and Professions, Ohio University, Athens, OH), with Richard L. Hoffman and Brian C. Clark (Ohio Neurological and Musculoskeletal Institute, Heritage College of Medicine, Ohio University, Athens, OH), and Masato Nakazawa (Office of Research & Grants, College of Osteopathic Medicine, Ohio University, Athens, Ohio).
Introduction

In 2011, the U.S. Census estimated there were 41.4 million persons aged 65 and older, or 13% of the population. By 2030, the number of older persons is expected to increase to more than 72 million (20%) and continue to increase thru 2050. Persons over 85 years of age and older represent the fastest growing age cohort in the U.S. (U.S. Department of Health and Human Services, 2012). Aging is associated with a myriad of physiological and functional changes, with one dramatic change being a reduction in neuromuscular function (e.g., decreased muscle strength) (Manini & Clark, 2011). A number of cellular and systems level neuromuscular changes have been suggested to occur with advancing age (Clark & Taylor, 2011; Delbono, 2011; Deschenes, 2011; Fry & Rasmussen, 2011; Russ, Gregg-Cornell, Conaway, & Clark, 2012; Russ & Lanza, 2011), with one of the most commonly cited changes being a reduction in the number of functioning motor units (MUs) with incomplete compensatory reinnervation (Brown 1972; Brown, Strong, & Snow, 1988; Doherty, Vandervoort, Taylor, & Brown, 1993; Galea, 1996; McNeil, Doherty, Stashuk, & Rice, 2005). The loss of motor units with advancing age has previously been hypothesized to be functionally significant by directly contributing to muscle weakness commonly observed in elders (McNeil et al., 2005). A positive linear correlation was found when motor unit number was regressed over strength in a sample of physically active older adults (Doherty et al., 1993), indicating the role motor units play in modulating strength production in elders. However, other studies have reported that muscle weakness in aging is not necessarily related to reduced motor unit numbers (Power et al., 2010).
The purpose of this pilot experiment was to determine whether age-related differences in muscle strength are moderated by estimates of functioning motor unit number via the motor unit number index (MUNIX) technique (Nandedkar, Nandedkar, Barkhaus, & Stålberg, 2004). Additionally, we also calculated an index of motor unit size (motor unit size index, or MUSIX) to examine the interactive effects of age and muscle strength on this outcome. It was hypothesized that there is an interrelationship between muscle strength, motor unit numbers, and aging, such that weaker older adults exhibit a reduced number of motor units in the absence of differences in motor unit size.

**Methods**

**General overview of the study.** Eighteen older adults and 24 young adults participated in this study. Following an orientation visit to the laboratory, subjects underwent maximum voluntary pinch grip strength testing of the nondominant hand and estimates of motor unit number were obtained from the abductor pollicis brevis muscle using the noninvasive motor unit number index (MUNIX) technique. The MUNIX technique is a recently developed noninvasive neurophysiological technique that has been suggested to provide an index proportional to the number of MUs in a muscle (Nandedkar et al., 2004, 2010). The MUNIX is derived from mathematical derivations based on the area and power of the maximum M-wave and voluntary surface electromyogram (EMG) recordings. An Analysis of Covariance (Age Group X Strength) was used to test heterogeneity of regression slopes (moderation analysis), with body mass and gender serving as a covariate.
Subjects. Community dwelling older (67.3 ± 5.3 years; 12 females and 6 males) and younger adults (22.1 ± 3.6 years; 10 females and 14 males) participated in this study. To be eligible for the study, subjects had to have a BMI <30 kg/m² were recruited to participate in this investigation. Individuals were excluded if they were regularly taking medications or supplements, or had any known neurological or orthopedic conditions. Regular resistance exercise (> 1/week), or a score of “very low active” or “high active” on the Lipids Research Physical Activity Questionnaire (Ainsworth, Jacobs, & Leon, 1993) were grounds for exclusion. Following an initial orientation (physical tests and questionnaires), qualified individuals attended one testing session. Participants had their maximal pinch-grip strength assessed, motor unit number indexed by means of MUNIX, and the motor unit size index by means of MUSIX. Subjects were asked to refrain from alcohol (24 hours) and caffeine, nicotine, and exercise (4 hours) before the testing sessions.

Electrical and mechanical recordings. Electromyographic (EMG) signals were recorded using surface electrodes (Kendall Soft-E H69PSurface Electrodes, Kendall Ltp, Mansfield, MA) arranged in a monopolar fashion on the palmar surface of the nondominant hand. Specifically, the active recording electrode was placed over the belly of the abductor pollicis brevis muscle, the reference electrode was placed on the wrist flexor tendon, and the ground electrode was placed on the bony portion of the dorsal aspect of the contralateral hand. The EMG signals were amplified (500-1,000x), bandpass-filtered (10-500 Hz), and sampled at 10,000 Hz (MP150, Biopac Systems, Goleta, CA).
Pinch-grip force was quantified using a force transducer (TSD121C, Biopac Systems), and the signals were smoothed over a 5-msec running average (AcqKnowledge 4.2, Biopac Systems). Participants forearm and wrist were positioned in an anatomically neutral position at the level of the xiphoid process while seated at a table. During the pinch grip task, participants pinched a 3.7 cm wide transducer with the pads of the thumb (1st finger) and index finger (2nd finger). The third through fifth fingers were flexed and secured to the hand using an elastic band (Fabrifoam, Exton, Pennsylvania). Subjects were given real-time visual feedback on a computer monitor located 0.5 meters in front of them (AcqKnowledge 4.2, Biopac Systems).

**Pinch-grip strength.** Maximal pinch-grip strength was assessed by having subjects perform a minimum of three maximal voluntary isometric contractions (MVC). A 1- to 2-minute rest period was given between trials. The MVC force was considered as the highest value recorded. If the participants’ highest values were not within 5% of each other additional trials were permitted. Verbal encouragement was given with each attempt and subjects were provided visual feedback of their force output on the computer monitor.

**Compound muscle action potentials and surface interference patterns.** The maximal compound muscle fiber action potential (CMAP or Mmax) was evoked by stimulation of the median nerve with a constant current stimulator (200-microsecond pulse; Model DS7AH, Digitimer Ltd., England) in the area 2 centimeters proximal of the wrist crease. The maximum CMAP was determined by failure of the M-wave to increase in amplitude despite an increase in stimulator output.
Surface interference patterns (SIP) were recorded during brief (~ 5 second) submaximal contractions. Here, the study participants performed a series of 12 submaximal isometric contractions. The first six increased in intensity, starting at a contraction intensity of 10% and increased in 10% increments until a contraction intensity of 60% of MVC was achieved. Subsequently, participants performed the same series of submaximal contractions in reverse order (i.e., a brief contraction at 60% MVC followed by decreasing intensity contractions in 10% increments). During each of these contractions, a target line representing each specified contraction level was displayed on the computer monitor and subjects were asked to match this target line. At least 20-seconds rest was allowed between each contraction.

**MUNIX calculations.** The MUNIX value is based on a mathematical model that relies on evoked CMAP’s and surface EMG data from the voluntary contractions (i.e., the SIP’s); for excellent schematics and analytical review refer to Ahn et al. (2012); Nandedkar et al. (2004, 2010). Respectively, the CMAP and SIP area and power were calculated, and applied to equation 1 below to yield an ideal case motor unit count (ICMUC) for every contraction intensity.

\[
ICMUC = \frac{CMAP_{\text{Power}} \times SIP_{\text{Area}}}{CMAP_{\text{Area}} \times SIP_{\text{Power}}} \tag{1}
\]

The ICMUC assumes that all motor unit action potentials are the same and no phase cancellation occurs, giving an indication of the number of motor units in the muscle. Each ICMUC value was plotted over the SIP area, and the points were fit with a power regression. Where the line crosses 20-mV on the x-axis the ICMUC value is reported as the MUNIX value, representing an estimate of the number of motor units in the muscle.
The SIP area of 20-mV is based on the premise that it reflects a low level of force output that elicits the firing of all low threshold motor units (Nandedkar et al., 2004, 2010).

To provide further insight about motor unit losses, the motor unit size index (MUSIX) was also calculated and used as an index of collateral reinnervation. Once MUNIX is calculated, MUSIX was computer based on equation 2:

\[
\text{MUSIX} = \frac{\text{CMAP}_{\text{Amplitude}}}{\text{MUNIX}} \tag{2}
\]

Prior to the analyses, the SIP signals were visually scrutinized to identify any artifact in the signal. If substantial noise was detected the SIP epoch was rejected for analysis. In order to standardize what epochs were accepted the inclusion criteria previously described by Nandedkar et al. (2010) were utilized. The inclusion criteria are as follows: a) SIP area > 20 mV/ms, b) ICMUC < 100, c) SIP area/CMAP area > 1.

**Statistical Analysis**

Descriptive statistics between groups were compared using independent t-tests. All values were reported in mean ± standard deviation. Analysis of Covariance procedures (Age Group X Strength) were used to test heterogeneity of regression slopes (moderation analysis), with body mass and biological gender serving as a covariates for both the MUNIX and MUSIX estimates. For all analyses, a p-value ≤ 0.05 was required for statistical significance.
Results

Descriptive characteristics. The mean age of the older adults was 67.3 ± 5.3 years, and the young adults were 22.1 ± 3.6 years (p = 0.00). The older adults had a similar body weight and height as the young adults (69.7 ± 11.8 vs. 72.6 ± 11.7 kg, p = 0.44; 167.7 ± 8.4 inches vs. 173.2 ± 11.25 cm, p = 0.09). The older adults pinch grip strength was weaker than the young adults (34.1 ± 15.4 vs. 46.2 ± 18.5 N, p = 0.03). The older adults CMAP amplitude was smaller than the young adults (5.53 ± 2.4 vs. 7.9 ± 2.9 mV, p < 0.01). See Table 2 for descriptive characteristics.

Motor unit number and size index. The slope of pinch grip strength on the abductor pollicis brevis MUNIX between the older adults and the young adults differed, as indicated by a Age Group X Strength interaction (p = 0.01). Specifically, the model implied that after controlling for the effect of body mass and gender, the slope in older adults was significantly positive (2.22 ± 0.72 MUs/N of strength, p = 0.00; see Figure 5A), whereas no such relationship was found in YA (0.08 ± 0.51 MUs/N of strength, p = 0.88; see Figure 5B). No age group main effect was observed indicating that the older adults, on average, exhibited a similar MUNIX when compared to the young adults (124.7 ± 69.2 vs. 114.0 ± 55.9, p = 0.65). A significant Age Group X Strength interaction was also observed for MUSIX (p = 0.03; see Figure 5C). In contrast to MUNIX, the slope in younger adults was significantly positive (0.73 ± 0.21, p = 0.001; see Figure 5C), whereas no such relationship was found in older adults (-0.03 ± 0.29, p = 0.20; see Figure 5D). In addition, an age group main effect was observed indicating that the older adults exhibited a 27.0% smaller MUSIX when
compared to the young adults (54.0 ± 38.8 vs. 74.0 ± 31.1, p < 0.01). Neither gender nor body weight were correlated with either MUNIX or MUSIX (p > 0.10).

Table 2

Subject Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Young n=24</th>
<th>Old n=18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.1 ± 3.6</td>
<td>67.3 ± 5.3</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>72.6 ± 11.7</td>
<td>69.7 ± 11.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.2 ± 11.25</td>
<td>167.7 ± 8.4</td>
</tr>
<tr>
<td>Strength (N)</td>
<td>46.2 ± 18.5</td>
<td>34.1 ± 15.4</td>
</tr>
<tr>
<td>CMAP (mV)</td>
<td>7.9 ± 2.9</td>
<td>5.53 ± 2.4</td>
</tr>
<tr>
<td>MUNIX</td>
<td>114.0 ± 55.9</td>
<td>124.7 ± 69</td>
</tr>
<tr>
<td>MUSIX (µV)</td>
<td>74.0 ± 31.1</td>
<td>54.0 ± 38.8</td>
</tr>
</tbody>
</table>
Figure 5. The relationship between motor unit number and pinch-grip strength in both cohorts. (A) No relationship between strength and motor unit number (MUNIX) was found in the young. (B) A positive linear relationship between strength and motor unit number (MUNIX) was found in the elderly. (C) A positive linear relationship between strength and motor unit size (MUSIX) was found in the young. (D) No relationship between strength and motor unit size (MUSIX) was found in the elderly.
Discussion

This investigation utilized the MUNIX technique to obtain an index of functioning motor unit number and size in younger and older adults. It was hypothesized that there would be an interrelationship between muscle strength, motor unit numbers, and aging, such that weaker older adults would exhibit a reduced number of motor units in the absence of differences in motor unit size. Consistent with the hypothesis, the relationship between muscle strength and MUNIX amongst the older adult cohort indicated that the weaker individuals exhibited a smaller MUNIX value. No such relationship was observed for the older adult cohort as it related to MUSIX. Also consistent with the hypothesis, these relationships differed between the younger and older adult groups, indicating the age-dependent nature of these relationships. To my knowledge this is one of the only studies to report an interrelationship between muscle strength, motor unit, and aging. Below these findings are discussed in the context of the aging literature, limitations of the current work, clinical relevance, and future directions.

**Muscle strength, motor units, and aging.** The finding that voluntary strength loss was mediated by estimates of functioning motor unit in an aged population is in line with earlier findings by Doherty and colleagues (1993) when the spike-triggered averaging MUNE technique was used to estimate motor unit number. Here, a modest positive linear correlation ($r = 0.52$) was found when estimates of motor unit number were regressed over maximal voluntary strength of the biceps brachii in healthy, physically active older adults ($> 60$ years). Conversely, although there is generally a marked reduction in motor unit number in the elderly, a concomitant reduction in
voluntary strength is not always observed (McNeil et al., 2005); as such, this finding is somewhat in disagreement with those of others. For instance, McNeil et al. (2005) reported that motor unit numbers of the tibialis anterior muscle were reduced in older men (60-69 years; 91 ± 22 MUs) and very old men (80-89 years; 59 ± 15 MU’s) when compared to young adults (23-32 years; 150 ± 43 MUs); however, dorsiflexion muscle strength was only significantly reduced in the very old cohort. Similarly, Power and colleagues (2010) observed that older runners (64 ± 3 years) exhibited 25% less dorsiflexion muscle strength when compared to young, recreationally active adults, but no differences in motor unit numbers were observed. Most recently, Drey, Grosch, Neuwirth, Bauer, and Sieber (2013) used the MUNIX technique to estimate motor unit number and size of the hypothenar muscle in 27 sarcopenic patients (i.e., individuals with low gender-specific skeletal muscle mass index and exhibiting impairments in physical function). While muscle strength was not assessed, they did observe that the sarcopenic patients MUNIX values, on average, were lower than those observed in healthy, young adults, and the authors even suggested that a subset of the patients’ sarcopenia could be directly attributed to the loss of motor neurons.

This investigation’s findings further contribute to the understanding of the relationship between aging and motor units. At first glance, the results suggest that older and younger adults do not necessarily exhibit differences in motor unit numbers (as evidenced by no mean difference between the cohorts); however, closer inspection using a moderation analysis indicates that an interrelationship between muscle strength, motor unit numbers, and aging exists with the weaker elders demonstrating fewer motor units.
Interestingly, an interrelationship was also observed as it relates to estimates of motor unit size; however, this relationship was primarily driven by the young adult group, because the older adult group did not demonstrate a significant relationship between MUSIX and muscle strength. The older adults’ lack of relationship between MUSIX and strength conceptually could be indicative of the weaker older adults experiencing a loss of motor units without collateral reinnervation. Conversely, it is possible that the findings of stronger young adults exhibiting larger motor units may be indicative of them having larger threshold motor units with higher innervation ratios, which have been show to result in higher evoked muscle force (Kwa, Korfage, & Weijs, 1995).

**Motor unit number estimation techniques and limitations of the current work.** There are several limitations of the current work that should be noted. First, while moderate to moderately-high reliability of the MUNIX and MUSIX measures have been demonstrated, it is critical to recognize that these techniques are still in their relative infancy and there are still questions surrounding the validity of the MUNIX technique to quantify motor unit number and size. The major concern previous investigations have cited is the inability of MUNIX to accurately reflect the true number of motor units in the studied muscle. MUNIX was designed as an “index,” a measurement that is not designed to be used as an “estimate,” therefore investigators should not interpret the MUNIX and MUSIX values as true reflections of anatomic motor unit number and size. When compared to the more widely accepted MUNE technique, studies have shown no correlation between the MUNE and MUNIX techniques in healthy individuals (Boekestein et al., 2013). The only significant correlation observed was in the diseased
(ALS) population in which a positive relationship was discovered. It should be noted that the MUNE values are “estimates” of functioning motor unit number; as such, MUNE’s ability to predict cadaveric motor unit number is also limited. The lack of validity surrounding the two techniques in terms of cadaveric motor unit number raises the question whether it is necessary that the two techniques correlate with one and other. More research is needed to address the validity of these electrophysiological techniques to anatomic studies. A previous investigation by Li, Rymer, and Zhou (2012) proposed that a primary limitation to the MUNIX technique may be its inability to be applied to atrophied muscles. This investigation studied elders who potentially had muscular atrophy; however, recent evidence by Drey et al. (2013) indicated that the MUNIX technique is sensitive enough to detect differences in motor unit number in patients clinically diagnosed with sarcopenia.

The limitations related to the construct of the present study are as follows: the small sample size, muscle studied, and the heterogeneity of the sample of older adults. First, further study with a larger sample size will be needed to replicate and substantiate these findings. Second, examination of other muscles in the body will be needed. The APB is estimated to be comprised of ~63% Type I fibers (Johnson, Polgar, Weightman, & Appleton, 1973), so it is of interest to know whether muscles with varying proportions of fiber type produce similar findings. Lastly, the age range of older adults that comprised our sample may have been too wide (60-73 years), possibly explaining the heterogeneity of physiological outcomes. The individuals in their early 60’s may not truly represent the “aged” individual.
Clinical relevance and future directions. With 20% of the U.S. population expected to be over the age of 65 by the year 2030, it is imperative we elucidate the mechanisms underlying age-related strength declines (U.S. Department of Health and Human Services, Administration on Aging, 2012). This study demonstrates that functioning motor unit number, independent of motor unit size, mediates voluntary muscle strength as determined by MUNIX. Strategies aimed at targeting the motor neuron and muscle fiber (i.e., therapeutic or pharmaceutical) need to be developed. But first, additional work is needed to determine the validity of this relationship as well as studying other muscle groups.

Conclusion

The role of the motor units in age-related decline in strength was poorly understood. This study determined that functioning motor unit number is a mediator of voluntary muscle strength in the elderly. This finding is important to further the quality of life of the ever aging population.

References


Chapter 5: Conclusions

The overarching aim of this investigation was to identify one of the mechanisms underlying age-related muscular weakness. To do so, the motor unit number index (MUNIX) technique was utilized to determine this relationship.

Prior to determining the interrelationship between muscle strength, motor units, and aging, it was important to exhibit the relative and absolute reliability of the MUNIX technique. The results from this study show that the technique had moderate to moderately-high relative and absolute reliability, respectively, but also demonstrated my effectiveness in performing this electrophysiological technique. The consistent results obtained from the reliability analysis made the MUNIX technique an appropriate choice of measure to determine the relationship between motor unit number and strength in the aging study.

The interrelationship between muscle strength, motor units, and aging has been unclear for a number of years, and it was the primary aim of this investigation to elucidate these relationships. Consistent with the hypothesis, it was concluded that weaker older adults exhibited fewer motor units than stronger older adults, and this relationship occurred independently of motor unit size. From a practical standpoint, this relationship in the older adult provides evidence for the need for targeted interventions for the older adult to quell this reduction in voluntary strength.

Although we have gained insight into the relationship between muscle strength and motor unit number and size from this investigation, the underlying physiology is still unclear. In older adults, the relationship between muscle strength and MUNIX
independent of MUSIX indicates that weak individuals may be experiencing muscle
denervation without collateral reinnervation. Without reinnervation from surviving
motor neurons, it is possible that whole muscle atrophy can be occurring to further
diminish force-producing capabilities. However, it is difficult to conclude definitively,
because this investigation only sampled 18 older adults. Identifying if this relationship in
older adults is true will require more participants or a longitudinal investigation to
monitor the changes in motor unit number and size over an extended period of time.

The investigation was limited in a number of ways. First, the technique itself has
been under scrutiny since its inception, because it has downfalls inherent to the
methodology. However, the strength of the opposition is undermined by the fact that all
in vivo investigation techniques of motor unit number fail to accurately predict
anatomical motor unit number. As for the construct of both studies, results from the
muscle examined may not be transferable to other skeletal muscles. In regard to age
effects, investigation the heterogeneity of the older group may have tempered the
significance of the relationship found. The sample in the study consisted of individuals
between the ages of 60 and 73, so it is plausible that the age of our cohort did not
accurately reflect the true aged population. Lastly, it is unclear if the origin of reduced
motor unit number in the weak elders is a result of muscular atrophy or declines in
nervous system function.
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Appendix: MUNIX Calculation

CMAP Extraction from Acqknowledge to Excel

1. Highlight CMAP deflection (screenshot)
2. On Toolbar, click Edit→Clipboard→Copy Wave Data Points
3. Open Microsoft Excel and Paste into Tab1; label it CMAP
4. Number of data points will adjust according to sampling epoch and frequency

Calculating CMAP AREA

1. Area needs to be calculated for each data point
2. Use formula,

\[ \text{Area} = \text{Abs}(\text{Data Point} - 0.065) \times \text{data points per unit time} \]

If frequency is 10,000 Hz use 0.0001 for data point per unit time, as 1 data point is measured every 0.0001 sec
3. Once each data point is converted to absolute value summate the points to obtain AREA

Calculating CMAP POWER

1. Power needs to be calculated for each data point
2. Use formula,

\[ \text{Power} = (\text{Data Point} - 0.065) \times (\text{Data Point} - 0.065) \times 0.0001 \]
3. Once each data point is converted to absolute value summate the points to obtain POWER

SIP Extraction from Acqknowledge to Excel

1. Highlight one second epoch of muscle’s electrical signal starting at lowest contraction intensity, e.g., 10% (screenshot)
2. Take this measure when force output is most steady
3. On Toolbar click Edit→Clipboard→Copy Wave Data Points
4. Open Microsoft Excel and Paste into Tab 2; label it 10% SIP
5. Number of data points will equal sampling frequency, i.e., 10,000 Hz=10,000 data points
6. Repeat these steps for all contraction intensities
Calculating SIP AREA

1. Summate all data points
2. Divide that number by sampling frequency to obtain an average
3. Subtract the average from each data point to obtain an “offset removed” value for each point
4. Area needs to be calculated for each data point
5. Use formula,

\[ Area = \text{Absolute Value} \times (\text{Data Point} \times 0.0001) \]

6. Once each data point is converted to absolute value summate the points to obtain AREA
7. Repeat these steps for all contraction intensities

Calculating SIP POWER

1. Power needs to be calculated for each data point
2. Use formula,

\[ Power = \text{Data Point} \times (\text{Data Point}) \times 0.0001 \]

3. Once each data point is converted to absolute value summate the points to obtain POWER
4. Repeat these steps for all contraction intensities

Calculating ICMUC

1. Obtain an Ideal Case Motor Unit Count for each contraction
2. Use formula,

\[ ICMUC = \frac{CMAPPower \times SIPArea}{CMAPArea \times SIPPower} \]

3. Repeat this formula for all contraction intensities

Creating the Graph

1. Set up the values in Excel so that SIP AREA represents the x-axis and its corresponding ICMUC value represents the y-axis (screenshot)
2. Plot points in a scatter plot
3. Click on graph points and right click→ Add Trendline→ Power→ Forecast: Backward 1.0 periods
4. Set the x-axis minimum value to 20mV (in the case of this study 0.02 mV is equal to 20mV as the gain was set to 1000)
5. When the trend crosses the 20mV mark, the MUNIX value is reported