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Reliability of a Modified Motor Unit Number Index (MUNIX) Technique

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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 as 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 et al., 1995; Nandedkar et al., 2004; Rashidipour and Chan, 2008]. The earliest of these techniques was the motor unit number estimation (MUNE) technique introduced in 1971 [McComas et al, 1971], and since this time a number of modifications to the MUNE technique have been developed and implemented [Brown et al, 1988; Daube, 1995; Doherty and Brown, 1994; Doherty and Stashuk, 2003; Kadrie et al., 1976; Shefner et al., 2011; Wang et al., 1995]. The MUNE methods involve estimates of single motor unit action potential size, using either incremental electrical nerve stimulation, spike triggered averaging, or multipoint stimulation 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 and Chan, 2008]. As such, there has been a demand from both scientists and clinicians to develop non-invasive, 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, or M-wave) observed in response to supramaximal electrical stimulation and voluntary surface electromyogram (EMG) recordings associated with a series of submaximal muscle contractions. The MUNIX

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technique is non-invasive, 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 et al., 2010; Nandedkar et al., 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 i) older adults when compared to younger adults [Neuwirth et al., 2011B], and ii) paretic muscle when compared to contralateral muscles of stroke survivors [Li et al., 2011].

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; Neuwith et al., 2011A; Sandberg et al., 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; Sandberg et al., 2011; Neuwith et al., 2011A]. In fact, to our knowledge 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 using a modified version of the original technique. Additionally, we also examined the relative and absolute reliability of the motor unit number size index (MUSIX), which is derived by dividing the maximum CMAP amplitude by the MUNIX. The MUSIX measurement, when interpreted alongside MUNIX, can provide more insight into the spinal motorneuron or motor unit pool. A well-developed simulation designed to parse out the subtleties of the technique noted that reduced MUNIX values without changes in MUSIX may be the result of muscle atrophy or motor unit number loss with incomplete reinnervation. On the contrary, a reduction in MUNIX and increase in MUSIX provides evidence of spinal motor neuron loss (Li et al., 2012).

We should note that our modified MUNIX technique is different than that classically employed as we controlled the contraction intensity by providing individuals visual feedback of their exerted force relative to their target force. Unlike the original technique, where surface interference patterns (SIPs) were obtained from contractions that were not controlled per se, we standardized the technique presented herein in an attempt to better control for the potential error introduced by subjective perception of contraction intensity. As such, these findings should not be interpreted or extrapolated to reflect the reliability of the originally described MUNIX technique, as it is possible that the differences in the two techniques could result in different levels of reliability.

Methods and Materials

Subjects

Nineteen young adults (age = 25±0.9 years; body mass = 71.5±2.9 kg; height = 170.6±2.6 cm) participated in this study approved by. To be eligible for the study, subjects had to have a BMI <30 kg/m^2, 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 et al., 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 four weeks. Participants had their maximal pinch-grip strength assessed, and motor unit number and size indexed via the MUNIX technique. 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). All measurements were carried out and supervised by one individual.

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 non-dominant hand. Specifically, the active recording electrode was placed over the belly of the abductor pollicis brevis muscle halfway between the metacarpo-phalangeal joint and the trapezium of the thumb, 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 3rd–5th 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 1.

Pinch-Grip Strength

Maximal pinch-grip strength was assessed by having subjects perform a minimum of three maximal voluntary isometric contractions (MVCs) (Figure 1). A one to two 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) (Figure 2A) 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) (Figure 2B) were recorded during brief (~ 5 second) pinch-grip submaximal contractions as well as during the MVC's. During the submaximal contractions, study participants performed a series of 12 pinch-grip contractions with the first six increasing in intensity (from 10% MVC to 60% MVC in 10% increments). 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. Data from the first trial at each of the submaximal contraction intensities and the MVC were used in the MUNIX calculation (this resulted in 7 points being used for the curve fitting). If the first trial exhibited artifact or noise, 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; Nandedkar et al., 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: $ICMUC = (CMAP_{Power} * SIP_{Area})/(CMAP_{Area} * SIP_{Power})$. 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 mVms 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 mVms 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; Nandedkar et al., 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: $MUSIX = CMAP_{Amplitude}/MUNIX$.

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. For the acceptable SIP signals, a 1-sec epoch was used to calculate the SIP area and power. In order to standardize what epochs were accepted the inclusion criteria previously described by Nandedkar et al. were utilized [Nandedkar et al., 2010]. The inclusion criteria are as follows: 1) SIP area > 20 mVms, 2) 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 average values between testing sessions dependent sample t-tests were 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, and that reliability will be derived from one value.

In addition to the ICC, a 'relative reliability' statistic that assesses the reproducibility of measurement relative to a sample of repeated measurements [Sun et al., 1998], we also chose to assess 'absolute reliability' (the degree in which the repeated measures vary). To fully understand the absolute stability of a measure it is important to understand the contribution of the main components of measurement error. In general, measurement error is broken into two classes: systematic bias and random error. Systematic bias represents the orderly changes in a measure over time, such as a learning effect; whereas random error is the result of biological or mechanical variation [Bland and Altman, 1986]. Since the MUNIX and MUSIX measures require voluntary task performance the potential for systematic bias exists. Therefore, we utilized the LOA method (a measure of absolute reliability), which is a statistical technique that is useful in partitioning out systematic bias vs. random error [Bland and Altman, 1986; Sun et al., 1998]. In doing this, Bland–Altman plots were generated for each variable and analyzed for the presence of heteroscedasticity [Bland and Altman, 1986; Sun et al., 1998]. Heteroscedasticity is when the residuals are not equally distributed throughout the range of scores of the dependent variables, whereby homoscedasticity is when the residuals are approximately equal for all dependent variable scores. This was determined by examining the correlation (R^2) between the absolute differences and the mean values. R^2 values between 0 and 0.1 were considered homoscedastic (no relation between error and the size of the measured variable) and systematic bias and random error were then calculated [Sun et al., 1998]. R^2 values greater than 0.1 were 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 calculated using the following equation: LOA ratio = $[(SD_{diffs}/AVG_{means}) \text{ X } 1.96] \text{ X } 100$. Where SD_{diffs} is the standard deviation of all of the difference scores (visit 2 − visit 1 calculated for each subject), AVG_{means} 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. The LOA ratio is interpreted as "any two tests will differ due to measurement error by no more than X% either in the positive of negative direction" [Sun et al., 1998]. We should caution the interpretation of our LOA findings based on our relatively small sample size impacting the standard deviation of the sample and resulting in a wider LOA.

Results

Descriptive Characteristics

Subjects exhibited a slightly greater pinch grip strength during the first testing session (48.7±4.5 N) when compared to the second testing session (45.3±4.55 N, p=0.05). The CMAP amplitude did not vary across the testing sessions (8.48±0.7 vs 9.21±0.7 mV, p=0.10).

MUNIX Reliability

No mean differences were observed for MUNIX (109.8±33.8 vs. 121.2±41.5) between test one and two (*p*=0.15). A CV of 17.5±2.66% and an ICC of 0.76 (p=0.002) was observed between test one and two. From the Bland-Altman plot, the LOA analysis (Figure 3A) yielded a homoscedastic relationship (R^2=0.06), with follow-up analysis indicating systematic bias and random error on the order of 11.3 and 65.7, respectively.

MUSIX Reliability

No mean difference was observed for MUSIX (79.3±21.6 μV vs. 80.5±21.3μV) between test one and two (*p*=0.79). A CV of 13.5±2.62% and an ICC of 0.73 (p=0.04) was observed between test one and two. From the Bland-Altman plot, the LOA analysis (Figure 3B)

yielded a homoscedastic relationship (R^2=0.0002), with follow-up analysis indicating systematic bias and random error on the order of −1.2 and 38.8, respectively.

Discussion

The current 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 four weeks in healthy adults. We observed moderate to moderately-high relative test-retest reliability (i.e., ICC's of 0.73 to 0.76) for the MUSIX and MUNIX values. We observed moderate absolute test-retest reliability (CV's of 13.5–17.5%) for MUSIX and MUNIX, with the majority of the measurement error 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 that we observed for the modified 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, our observed MUNIX CV finding (17.5%) is lower than that previously reported for the hypothenar, biceps brachii, abductor digiti minimi, abductor poliicis brevis, tibialis anterior, extensor digitorum brevis, and abductor hallucis muscles [Ahn et al., 2010; Nandedkar et al., 2004; Nandedkar et al., 2010; Neuwirth et al., 2011A; Sandberg et al., 2011], and the first to be less than 20%. Similarly, our ICC (0.76) was comparable to that found in the APB muscle of patients with ALS (0.74) when assessed on two occasions separated by two weeks [Boekestein et al., 2012], and considerably higher than that reported in healthy individuals by others [Furtula et al., 2013; Neuwirth et al., 2011A; Neuwirth et al., 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 performed the retest within minutes of the first and/or did not define or control the actual amount of time between tests [Ahn et al., 2010; Furtula et al., 2013; Neuwirth et al., 2011A; Sandberg et al., 2011]. Accordingly, our findings suggest that moderate to moderately-high test-retest reliability measures of MUNIX can be obtained under well-controlled circumstances while monitoring the contraction intensities at percentages of the subjects' maximal voluntary contraction. To our 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.

We should note that the average MUNIX values in our study (~ 115) are slightly lower than previous MUNIX investigations that assessed the APB muscle (~ 177–190 depending on the study) [Neuwirth et al., 2010; Neuwirth et al., 2011B], and 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, as it has been reported that the MUNIX value varies depending on subtle differences in contraction tasks [Zhou et al., 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 et al., 2005]. Much like other MUNIX investigations of the APB muscle [Neuwirth et al., 2010; Neuwirth et al., 2011B], the average values in our study indexed below what the various MUNE techniques have reported [Doherty et al., 1993; Galea et al., 1991; Wang et al., 1995]. MUNIX, as mentioned earlier is generally considered to represent an 'index' of motor unit numbers in a muscle, as opposed to representing an 'estimate' of the number of motor units in a muscle [Nandedkar et al., 2004; Nandedkar et al, 2010]. For instance, the typical approach for

calculating MUNIX involves determining the ICMUC value that corresponds to a SIP area of 20 mVms. This SIP area of 20 mVms 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], with these studies finding 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 [Boekestein et al., 2012; Furtula et al., 2013]. 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 muscle [Li et al., 2012; Neuwirth et al., 2010]. Additionally, the sensitivity of the MUNIX technique to changes in motor neuron and muscle properties was recently explored by Li and colleagues (2012) using a simulation approach utilizing variations on published motor neuron pool and surface EMG models (Li et al., 2012). Their results indicated that, when keeping motor neuron pool and muscle parameters unchanged and varying the input motor unit numbers to the model, that MUNIX estimates can appropriately characterize changes in motor unit numbers (Li et al., 2012). As such, there is growing data providing some basic construct validity of the novel technique. One major limitation to addressing this question is that the MUNE techniques also simply represent an *estimate* of motor unit number ‘function’. 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 as this study was conducted in a well-controlled laboratory setting where the same investigator tested all subjects to ensure precise consistency of testing procedures. The variant of the technique that we chose to use (i.e., controlling the contraction intensity) differs from the originally developed technique, and may have contributed to the high reliability we observed in this investigation. It is unclear, however, what impact the precise force matching may cause for the reliability. At this point we can only offer an anecdotal explanation that would far from suffice as an acceptable hypothesis to the problem. Another limitation is that our investigation only assessed one muscle, and thus our 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. There are also broader limitations of the MUNIX technique that, while not directly related to this study per se, are worth noting. Namely, caution has been suggested when interpreting MUNIX values in populations with atrophied muscle, particularly in cases where it is unclear whether the atrophy is accompanied by loss of motor units or loss of muscle fiber size (Li et al., 2012).

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. Our findings indicated

moderately-high relative reliability for MUNIX (ICC=0.75), with slightly lower relative reliability for MUSIX (ICC=0.73). Absolute reliability is likely within the acceptable range for many neuromuscular outcomes (CV 13.5–17.5%), 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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Biographies

Ryan Kaya is a research scientist at the Ohio Musculoskeletal and Neurological Institute (OMNI) at Ohio University. OMNI's overarching mission is to improve the diagnosis, treatment, and prevention of musculoskeletal and neurological disorders. Mr. Kaya holds a M.S. degree in exercise physiology from Ohio University. His research interests include i) identifying the neuromuscular mechanisms of poor physical function in the elderly, and ii) developing therapeutic modalities (i.e., exercise and cognitive tasks) to slow the onset of Alzheimer's disease.

Rich Hoffman is a research scientist at the Ohio Musculoskeletal and Neurological Institute (OMNI) at Ohio University. OMNI's overarching mission is to improve the diagnosis, treatment, and prevention of musculoskeletal and neurological disorders. Mr. Hoffman's primary scientific efforts are focused on supporting experiments in OMNI's two research divisions pertaining to i) musculoskeletal and neurological pain disorders, and ii) healthy aging. He holds a M.S. degree in exercise physiology from Miami University, and has technical expertise in electromyography, evoked potentials, and study coordination.

Brian Clark is Professor of Physiology and Neuroscience in the Department of Biomedical Sciences at Ohio University where he also serves as the Executive Director of the Ohio Musculoskeletal and Neurological Institute (OMNI). He received a B.S. in Biology from Western Carolina, and M.S. and Ph.D. degrees in Exercise Physiology from Syracuse University. The overarching aim of Dr. Clark's research is to determine the neuromuscular mechanisms that mediate acute adjustments and chronic adaptations in response to changes in physical activity and under pathological conditions. The goal is to develop effective and implementable interventions that increase muscle function (e.g., muscle strength, motor control, fatigue-resistance) and physical performance in older adults or patients of any age who have orthopedic and neurologic disabilities for preventative and rehabilitation medicine. He has published more than 70 articles and chapters in the past 10-years, and has

received and served as principal investigator or project director on federal, foundation, and industry grants totaling > $37 million USD.

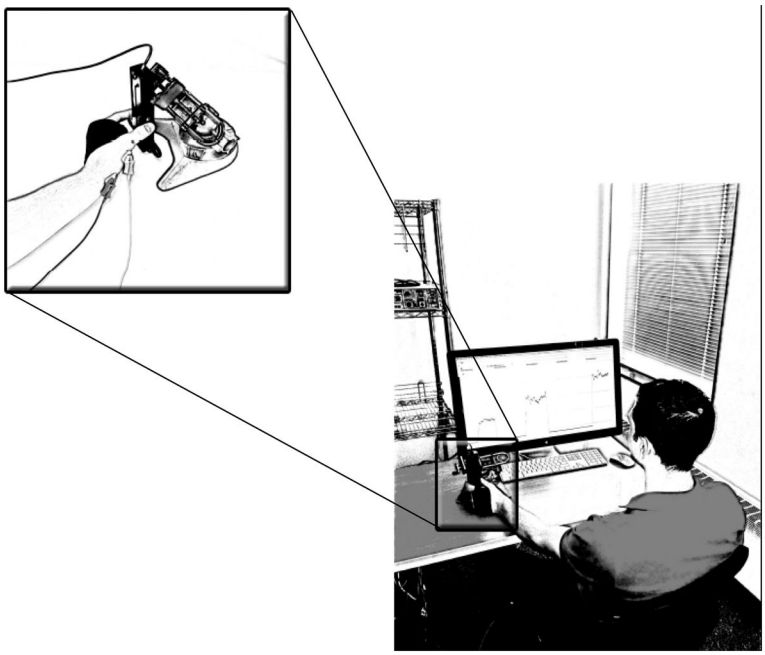

Figure 1.
Experimental setup of subject performing graded contractions at percentage(s) of their maximal voluntary contraction force. Inset: Enlarged view of the force transducer and grip task protocol.

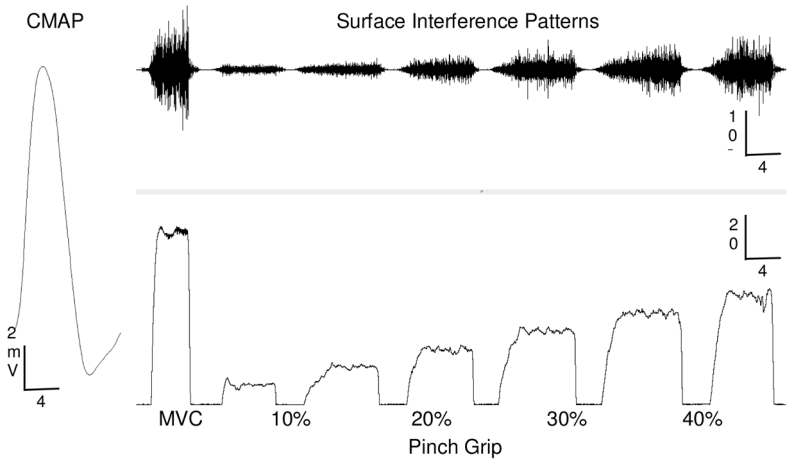

Figure 2.
Representative example of an evoked compound muscle action potential (left trace), and the surface electrographic interference patterns (top right trace) during brief pinch-grip isometric force contraction tasks (bottom right trace).

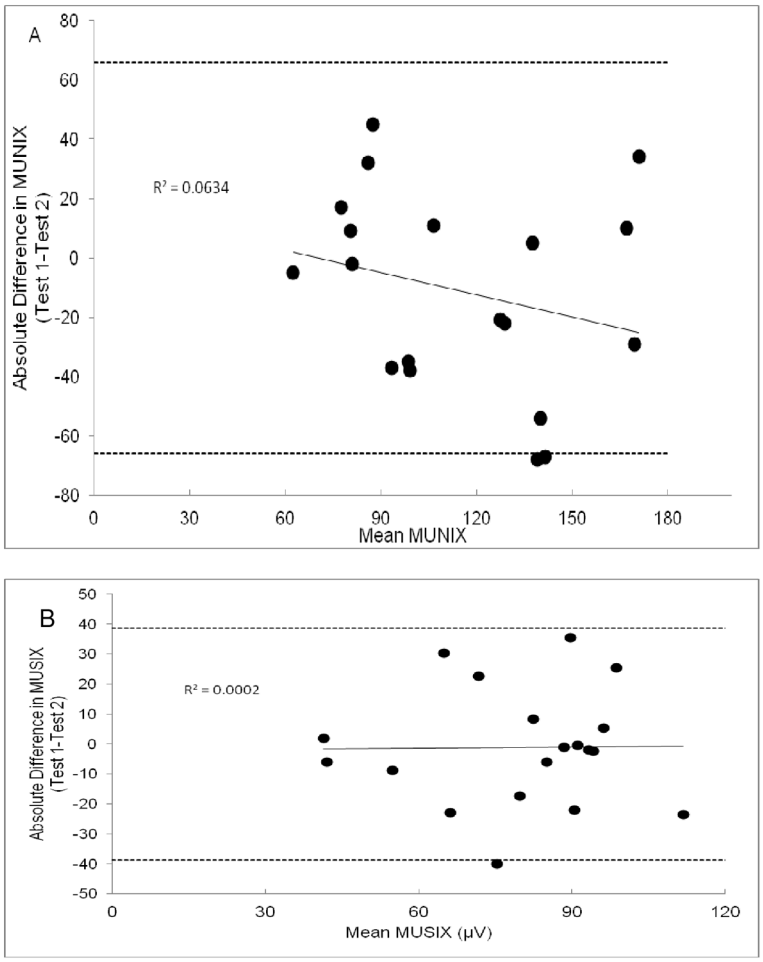

Figure 3.

(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 ±65.7. (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 ±38.8.