EVALUATION OF THE LONG-TERM RELIABILITY OF MOTOR UNIT
DISCHARGE RATES OBTAINED BY DECOMPOSITION OF
THE SURFACE ELECTROMYOGRAPHIC SIGNAL

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A thesis submitted in partial completion of the requirements for the degree of Master of
Science in Applied Health Sciences (Kinesiology)

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© September 2018
ABSTRACT

This study evaluated the long-term reliability of motor unit discharge rates (MUDRs) during isometric contractions at 60% maximum voluntary contraction (MVC), obtained by decomposition of the surface electromyographic (sEMG) signal from the flexor carpi radialis (FCR) and tibialis anterior (TA). There were four test sessions: one week between sessions 1 and 2; six weeks between sessions 2, 3, and 4. Participants performed 3 maximal isometric contractions of the wrist flexors and 3-isometric ramp contractions to 60% MVC. A load cell and 5-pin electrode (dEMG System, Delsys, Inc., Boston, MA) were used to monitor force and sEMG, respectively. The MUDRs were obtained using the Precision Decomposition Algorithm III in the dEMG Analysis software, and calculated as the inverse of the smoothed firing intervals. The mean discharge rate was calculated during a one-second window centered at the plateau portion of the 60% MVC ramp contraction. Maximal isometric strength during wrist flexion and dorsiflexion was also monitored. Across the four test sessions, maximal isometric strength of the wrist flexors and dorsiflexors increased 10 and 11.85%, respectively ($p$'s<0.01). The slight lack of stability in means was compensated for a high degree of consistency of strength values within each subject as assess by the intraclass correlation coefficient ($R$’s >0.94). The MUDRs for the FCR (5.2%) and TA (7.8%) also exhibited slight fluctuations across the four test sessions ($p$’s<0.01). The consistency of MUDR values within each subject was still considered good, as the intraclass correlation coefficient for both measures was $R$=0.79. It was concluded that the overall long-term reliability of MUDRs in both the FCR and TA was good.
ACKNOWLEDGEMENTS

I would like to acknowledge my appreciation for my supervisor Dr. David Gabriel for graciously extending me the opportunity to pursue my graduate studies. I must also thank my committee members Dr. Kimberley Gammage and Dr. Allan Adkin for the guidance beyond the words written on the following pages including providing me with a space to work for my tenure. I must also acknowledge my appreciation to colleagues Dr. Lara Green and Greig Inglis for their patience with mentoring me in the laboratory.

It would never seem possible for me to be in this position without the breadth of support and guidance from my sister Johanna and mother, Dr. Susan Brien. My father, Dr. T. Blaine Hoshizaki Sr., your everlasting mentorship and lecturing even when I didn’t think I needed it. It has always allowed me to pursue all of my dreams and yielded the person I am today.

Elizabeth Legace, you have always lent an ear and fostered developing my skills in academia, you have always lead by an example of unwavering determination for which was always encouraging.

To the fine gentleman I have had the absolute pleasure with sharing a workspace with for the past two years; Alex Watson, Holt Messner, Kyle Johnson, and Robert Kumar. Whether it was a morning coffee chat to late evenings in the laboratory, all of your personalities provided a shining light to any kind of situation.

Finally, I would like to extend a thank you to everyone in the Brock community for such a welcoming environment. It was a pleasure to complete my studies at such a vibrant place.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>DEFINITION OF ABBREVIATIONS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xi</td>
</tr>
<tr>
<td><strong>CHAPTER 1 – INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Purpose of the Research</td>
<td>2</td>
</tr>
<tr>
<td>Statement of the Problem</td>
<td>3</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>5</td>
</tr>
<tr>
<td>Assumptions</td>
<td>6</td>
</tr>
<tr>
<td>Delimitations</td>
<td>7</td>
</tr>
<tr>
<td>Limitations</td>
<td>8</td>
</tr>
<tr>
<td><strong>CHAPTER 2 – REVIEW OF LITERATURE</strong></td>
<td>9</td>
</tr>
<tr>
<td>Brief Anatomy of Tibialis Anterior and Flexor Carpi Radialis</td>
<td>9</td>
</tr>
<tr>
<td>Anatomy of Flexor Carpi Radialis</td>
<td>9</td>
</tr>
<tr>
<td>Anatomy of Tibialis Anterior</td>
<td>10</td>
</tr>
<tr>
<td>Electromyographic Signal</td>
<td>12</td>
</tr>
<tr>
<td>Signal Generation</td>
<td>12</td>
</tr>
<tr>
<td>Motor Unit</td>
<td>12</td>
</tr>
<tr>
<td>The Interference Pattern</td>
<td>13</td>
</tr>
<tr>
<td>Motor Unit Behaviour</td>
<td>15</td>
</tr>
<tr>
<td>Surface versus Indwelling Electromyography</td>
<td>17</td>
</tr>
<tr>
<td>Factors affecting the Electromyographic Signal</td>
<td>17</td>
</tr>
<tr>
<td>Interelectrode Distance</td>
<td>18</td>
</tr>
<tr>
<td>Temperature</td>
<td>21</td>
</tr>
<tr>
<td>Fatigue</td>
<td>21</td>
</tr>
<tr>
<td>Skin Preparation</td>
<td>22</td>
</tr>
<tr>
<td>Sweat</td>
<td>22</td>
</tr>
<tr>
<td>Amount of Subcutaneous Tissue</td>
<td>23</td>
</tr>
<tr>
<td>Electrode Location</td>
<td>23</td>
</tr>
<tr>
<td>Electrode Placement and Replacement</td>
<td>24</td>
</tr>
<tr>
<td>sEMG Decomposition</td>
<td>24</td>
</tr>
<tr>
<td>Assessment of Reliability Methods</td>
<td>25</td>
</tr>
<tr>
<td>Consistency</td>
<td>26</td>
</tr>
<tr>
<td>Stability</td>
<td>27</td>
</tr>
<tr>
<td>Reliability studies of force and electromyography</td>
<td>29</td>
</tr>
</tbody>
</table>
# CHAPTER 3 – METHODOLOGY

- Participants 35
- Preliminary Procedures 36
- Measurement Schedule 36
- Experimental Set-up 37
- Force Measurement 37
- Surface EMG 41
- Data Collection Procedure 41
- Criterion Measures 44
- Statistical Analysis 47

# CHAPTER 4 – RESULTS

- Subject Characteristics 50
- Statistical Assumptions 51
- Stability and Consistency 52
- Force 52
- Motor Unit Discharge Rates 53

# CHAPTER 5 – DISCUSSION

- Comparative Values 61
  - Wrist Flexion 61
  - Ankle Dorsiflexion 62
- Motor Unit Discharge Rates 63
  - Flexor Carpi Radialis 63
  - Tibialis Anterior 64
- Reliability Analysis 65
  - Force 65
  - Motor Unit Discharge Rates 68
- Conclusions 74

# REFERENCES

- 75

# APPENDICES

- Appendix A – Research Ethics Board Clearance Certificate 91
- Appendix B – Informed Consent Document 93
- Appendix C – PAR-Q Questionnaire 100
- Appendix D – Participant Questionnaire 105
DEFINITIONS OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD-HDEMG</td>
<td>Double-Differentiated High-Density Electromyography</td>
</tr>
<tr>
<td>dEMG</td>
<td>Delsys Electromyography System</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>FCR</td>
<td>Flexor Carpi Radialis</td>
</tr>
<tr>
<td>ICC</td>
<td>Interclass Correlation Coefficient</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximal Voluntary Contraction</td>
</tr>
<tr>
<td>MU</td>
<td>Motor Unit</td>
</tr>
<tr>
<td>MUDR</td>
<td>Motor Unit Discharge Rates</td>
</tr>
<tr>
<td>MFAP</td>
<td>Muscle Fiber Action Potential</td>
</tr>
<tr>
<td>MFCV</td>
<td>Muscle Fiber Conduction Velocity</td>
</tr>
<tr>
<td>MUFR</td>
<td>Motor Unit Firing Rate</td>
</tr>
<tr>
<td>MUFT</td>
<td>Motor Unit Firing Time</td>
</tr>
<tr>
<td>MN</td>
<td>Mean</td>
</tr>
<tr>
<td>MNF</td>
<td>Mean Frequency</td>
</tr>
<tr>
<td>MUP</td>
<td>Motor Unit Action Potential</td>
</tr>
<tr>
<td>MUPT</td>
<td>Motor Unit Action Potential Trains</td>
</tr>
<tr>
<td>PPS</td>
<td>Pulses Per Second</td>
</tr>
<tr>
<td>RMS</td>
<td>Root Mean Square</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>sEMG</td>
<td>Surface Electromyography</td>
</tr>
<tr>
<td>sMUP</td>
<td>Surface Motor Unit Action Potential</td>
</tr>
</tbody>
</table>
SNR – Signal-to-Noise Ratio

STA – Spike Triggered Averaging

TA – Tibialis Anterior
LIST OF TABLES

TABLE 1  Means (M) and standard deviations (SD) for the physical characteristics of the participants.  Page 50

TABLE 2  Analysis of variance for maximal voluntary contractions.  Page 55

TABLE 3  Intraclass correlation analysis of variance for maximal voluntary contractions  Page 56
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE 1</td>
<td>Illustration showing the anatomical location of the flexor carpi radialis and other flexors of the wrist on the anterior aspect of the forearm (Luttgens &amp; Wells, 1982).</td>
<td>10</td>
</tr>
<tr>
<td>FIGURE 2</td>
<td>Anatomical drawing displaying the orientation and anatomical location of the tibialis anterior and other foot dorsiflexors to show tendon insertion and origin as illustrated in (Luttgens &amp; Wells, 1982).</td>
<td>11</td>
</tr>
<tr>
<td>FIGURE 3</td>
<td>Muscle belly being innervated by the alpha motoneuron where propagation of the muscle fiber action potential will travel bidirectionally from (Griep et al., 1982).</td>
<td>14</td>
</tr>
<tr>
<td>FIGURE 4</td>
<td>Motor unit action potentials trains are composed of the repeated firing of MUAPs. The bottom component is the summation of all of these MUPTs, called the interference pattern (Basmajian, Gopal, &amp; Ghista, 1985).</td>
<td>15</td>
</tr>
<tr>
<td>FIGURE 5</td>
<td>Common mode rejection ratio and the source of how a double differentiated signal is created from bipolar signals (Gabriel and Kamen 2009).</td>
<td>19</td>
</tr>
<tr>
<td>FIGURE 6</td>
<td>Delsys dEMG electrode highlighting surface arrangement as well as the number of recording surfaces</td>
<td>20</td>
</tr>
<tr>
<td>FIGURE</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>FIGURE 7</td>
<td>Itinerary of the data collection sessions and how the time gaps in between of them are displaced from each session.</td>
<td>37</td>
</tr>
<tr>
<td>FIGURE 8</td>
<td>Photo showing the apparatus for assessment of ankle dorsiflexion force.</td>
<td>39</td>
</tr>
<tr>
<td>FIGURE 9</td>
<td>The apparatus used for the collection of the force and surface electromyography (sEMG) of the wrist flexors.</td>
<td>40</td>
</tr>
<tr>
<td>FIGURE 10</td>
<td>Experimental set-up identifying where the monitor was placed for the participant to follow with their progress as they matched the ramp target.</td>
<td>43</td>
</tr>
<tr>
<td>FIGURE 11</td>
<td>Screenshot of identified motor unit potential trains (MUPTs), their firing recordings along with the force trace.</td>
<td>46</td>
</tr>
<tr>
<td>FIGURE 12</td>
<td>Break down of ANOVA model testing and which portion of stability and consistency testing it will be applied to with the testing statistical inferences.</td>
<td>49</td>
</tr>
<tr>
<td>FIGURE 13</td>
<td>The means and standard deviations for maximal isometric wrist flexion force for each subject.</td>
<td>57</td>
</tr>
<tr>
<td>FIGURE 14</td>
<td>The means and standard deviations for maximal isometric ankle dorsiflexion force for each subject.</td>
<td>58</td>
</tr>
<tr>
<td>FIGURE 15</td>
<td>The means and standard deviations for flexor carpi radialis motor unit discharge rate at 60 percent of maximal voluntary contraction for each subject.</td>
<td>59</td>
</tr>
<tr>
<td>FIGURE 16</td>
<td>The means and standard deviations for tibialis anterior motor unit discharge rate at 60 percent of maximal voluntary contraction for each subject.</td>
<td>60</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX A</th>
<th>Research Ethics Board Clearance Certificate</th>
<th>Page 91</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPENDIX B</td>
<td>Informed Consent Document</td>
<td>Page 93</td>
</tr>
<tr>
<td>APPENDIX C</td>
<td>PAR-Q and Participant Screening Questionnaire</td>
<td>Page 100</td>
</tr>
<tr>
<td>APPENDIX D</td>
<td>Questionnaire and Anthropometric Measurements</td>
<td>Page 105</td>
</tr>
</tbody>
</table>
CHAPTER I

Introduction

Electromyography (EMG) has been a longstanding tool used by clinicians and scientists to understand the neuromuscular system. Electromyographic investigations involve the use of either surface or indwelling electrodes measure the bioelectrical events associated with muscle action potentials and reflect the status of both the nerve and muscle.

The basic unit of the neuromuscular system is the motor unit (MU), which is a single alpha-motoneuron and all the muscle fibers that it innervates. Repetitive firing of a single MU is measured as the MU action potential train (MUPT). The number of times the MU discharges within a second is referred to as motor unit discharge rate (MUDR) and is given in pulses per second (pps); this is different than a constant frequency given in Hertz, because the inter-pulse interval is irregular (Vander Linden, Kukulka, & Soderberg, 1991). The discharge rate for each MU is derived from its MUPT. Although MUPTs can be identified through surface-based methodologies, they have been traditionally obtained through indwelling recordings (Farina, Holobar, Merletti, & Enoka, 2010; Lee, Adam, & De Luca, 2008; Thornton & Michell, 2012). One commercially-available surface electrode incorporates five recording pins configured in a Laplacian arrangement for spatial filtering, which greatly enhances the selectivity of the recordings (Nawab, Chang, & De Luca, 2010). Signal processing techniques are then used to decompose the surface electromyography (sEMG) into its constituent MUPTs (De Luca, Adam, Wotiz, Gilmore, & Nawab, 2006).
Non-invasive recording of MUPTs are currently being used in kinesiology to investigate the neural control of muscle in response to cold (Mallette, Green, Gabriel, & Cheung, 2018), fatigue (Beck et al., 2005; Bertram, Nishida, Minieka, Janssen, & Levy, 1995; Dideriksen, Farina, & Enoka, 2010; Green, Parro, & Gabriel, 2014), resistance training (Gabriel, Kamen, & Frost, 2006; Vila-Cha, Falla, & Farina, 2010) and stroke rehabilitation (Li et al., 2015). However, the reliability of MUDRs obtained using sEMG has not yet been established. Understanding the reliability of MUDRs is important for pre-experimental planning of sample size estimation, and the number of days and trials required to obtain stable and consistent means within the field of participants, to define a presence of an intervention (Christie, Kamen, Boucher, Greig Inglis, & Gabriel, 2010). The purpose of this thesis is to evaluate reliability of MUDRs obtained using sEMG in the flexor carpi radialis (FCR) and tibialis anterior (TA), over a three-month time period. The length of time was chosen as a common duration for training studies (Gabriel et al., 2006), while the muscles were selected based on those frequently reported in the literature (Beck et al., 2005; Farina, Arendt-Nielsen, Merletti, Indino, & Graven-Nielsen, 2003; Kamen, Sison, Du, & Patten, 1995).

**Purpose of the Research**

The purpose of this research was to evaluate the reliability of MUDRs during isometric actions of the wrist- and ankle dorsi-flexors at 60% of maximum voluntary contraction (MVC). MUDRs were recorded in the FCR and TA muscles during four test sessions. The first two test sessions were one week apart and the remaining two occurred six weeks apart for a total of 13 weeks between the first and last sessions.
Statement of the Problem

There has been an increased trend in electromyographic kinesiology towards non-invasive assessment of motor unit activity patterns using surface electrodes due to the application into clinical environments. One approach has been to use electrode arrays to measure the time it takes the same motor unit action potential to propagate along the array, which allows for the calculation of muscle fiber conduction velocity (MFCV). Increases in MFCV were associated with the recruitment of MUs that require a higher force-threshold to recruit (Farina et al., 2010; Staudenmann, Roeleveld, Stegeman, & van Dieen, 2010; Westad, Westgaard, & De Luca, 2003). Decreases in MFCV indicated a slowing of MUPT propagation due to fatigue (Dideriksen et al., 2010; McManus, Hu, Rymer, Lowery, & Suresh, 2015). It was only after the widespread use of MFCV in the field of kinesiology that the reliability of the measure was assessed and found to be excellent ($R = 0.83-0.98$) during isometric contractions of the dorsiflexors (McIntosh & Gabriel, 2012). It remains to be determined for other commonly used muscles.

The electrode array has been extended to a matrix or grid of detection surfaces, analogous to an electroencephalogram cap, which record electrical activity around the head, associated with the brain. In the case of muscle electrical activity, monopolar recordings taken along the fiber direction by electrode arrays within a matrix can be obtained and used to calculate double-differential high-density EMG (DD-HDEMG, Del Vecchio, Negro, Felici, & Farina, 2017). This type of electrode array allows the identification of unique MUPT shapes associated with different MUs, which can be used to study the relationship between MU activity patterns and changes in measures calculated from the sEMG interference pattern. While the technique has been validated
through simulation and experimental work, the reliability of MUDR data has not been established for use across multiple test sessions to assess the impact of interventions on the control and regulation of muscle force.

Reliability also needs to be established for a commercially available system for recording MU activity (Delsys Inc., Boston, USA). This (Delsys Inc., Boston, USA) system combines a Laplacian five-pin electrode for spatial filtering which increases selectivity, with sophisticated software for MU identification within the sEMG interference pattern (De Luca et al., 2006; De Luca, Chang, Roy, Kline, & Nawab, 2015; De Luca & Nawab, 2011; Holobar, Minetto, & Farina, 2014; Nawab et al., 2010). The extraction of unique MUPs from within the sEMG interference pattern is called “decomposition” because the sEMG signal is decomposed into its constituent MUPs. If each MUP is identified and removed from the sEMG signal, only baseline noise would remain as the residual activity (De Luca, Nawab, & Kline, 2015; Kline & De Luca, 2014). Thus, the commercial device is called the “dEMG system.” The dEMG system (Delsys Inc., Boston, USA) is currently used by a number of investigators. While validity is actively investigated, it has yet to be definitively established (Hu, Jeon, Rymer, Shin, & Suresh, 2014; Hu, Rymer, & Suresh, 2013b). To date, there has been no study of the reliability of MU data obtained using the dEMG system (Delsys Inc., Boston, USA). This issue is critical to understanding the statistical constraints for pre-experimental planning of power, sample size estimation, and the detection of significant differences following the application of an intervention (Gabriel et al., 2006; Holobar et al., 2014; Hu et al., 2014; Inglis, McIntosh, & Gabriel, 2017; Lee et al., 2008; Vila-Cha et al., 2010).
Another method for identifying MUPs from the skin surface, is the spike-triggered-averaging (STA) technique. The STA technique involves inserting a needle into the muscle underneath a surface electrode (Hu et al., 2014; Hu et al., 2013b; Suresh, Kuo, Heckman, & Rymer, 2012; Zhou & Rymer, 2004). A specific MU is identified from the needle electrode and the spike amplitude of that specific MU is used as a trigger. Hundreds of discharges are then used to trigger the averaging of the sEMG signal at a point in time that is specific to that MU. Since the signal-to-noise ratio (SNR) increases with the square of N (the number of averages), the underlying deterministic shape within the sEMG signal is revealed resulting in a surface detected motor unit activity potential (sMUP). The STA technique assumes the shape of sMUP is not changing. The two-source method involving the indwelling and surface signal establishes the validity of the sMUP. Boe, Stashuk, Brown, & Doherty (2005) evaluated the test-retest reliability of sMUP morphology of the thenar muscles, which was found to be excellent ($R > 0.94$). While MUDR was not the focus of the study, the work demonstrated that it is possible to identify the same sMUP across test sessions.

**Hypothesis**

Based on STA technique to identify sMUPs, it is reasonable to expect a high level of reliability ($\approx 0.80$) for MUDR for the FCR and TA, across the four testing sessions within the 13-week period.
Assumptions

1. The dEMG system is valid for identifying MUPs at 60% of maximal voluntary contraction (Hu, Rymer, & Suresh, 2013a).

2. The flexor carpi radialis and the tibialis anterior are the primary agonists during isometric wrist flexion and ankle dorsiflexion, respectively (Maganaris, Baltzopoulos, & Sargeant, 1999; Ramsay, Hunter, & Gonzalez, 2009).

3. Participants will truly contract at 100% of their maximal voluntary effort from which the 60% MVC intensity will be established across test sessions (Chaffin, Lee, & Freivalds, 1980; Sinkjaer, Toft, Larsen, & Andreassen, 1993).

4. Participants will not perform “trick” movements during the required tasks, so that the flexor carpi radialis and the tibialis anterior can be isolated during isometric wrist flexion and ankle dorsiflexion, respectively (Vaughan, 1989).

5. Changes in the neural control of antagonist or synergistic muscles will not affect results (Billot, Simoneau, Van Hoecke, & Martin, 2010; Colacino, Rustighi, & Mace, 2012; Engelhorn, 1983).

6. Participants will not engage in any activity between test sessions that would alter motor unit discharge rates, thereby confounding the reliability of the results (Calder, Hall, Lester, Inglis, & Gabriel, 2005; Kollmitzer, Ebenbichler, & Kopf, 1999).

7. Participants will be motivated to give their best performance during the required tasks (Chaffin et al., 1980; Smidt & Rogers, 1982).

8. The presence of multiple investigators will not alter the participant’s true performance capabilities (Lamarche, Gammage, & Gabriel, 2011).
**Delimitations**

1. Only individuals who are between the ages of 19-35 years old will be studied.
2. Only isometric contractions will be investigated.
3. The motor unit discharge rates will be recorded only at 60% maximal voluntary contractions.
4. Only two joints will be investigated during flexion (i.e., wrist flexion and ankle dorsiflexion).
5. Only two muscles, the flexor carpi radialis and tibialis anterior, will be studied.
6. One method (dEMG, Delsys Inc., Boston, USA) for detecting motor unit action potentials from the skin surface will be investigated.
Limitations

1. The results will only apply to the individuals between 19 and 35 years old and may not generalize younger or older adults outside this investigated age range.

2. The results will only apply to isometric contractions, and may not extend to isotonic and isokinetic contractions.

3. The results will apply to only one contraction intensity (60% MVC). Higher or lower contraction intensities may not have the same, or similar reliability.

4. The results will only be valid for flexor carpi radialis during wrist flexion and the tibialis anterior during ankle dorsiflexion. Single-joint muscles during simple flexion or extension at any other joints than the muscles being investigated, may not exhibit the same reliability as observed in the present study.

5. Similarly, the results may not apply to two-joint muscles activated during more complex joint actions.
CHAPTER II

Review of Literature

**Brief Anatomy**

**Flexor Carpi Radialis (FCR)**

The flexor carpi radialis is a skeletal muscle made up of pennate fibers on the anterior side of the forearm (Figure 1). It is a prime mover during wrist flexion and is a synergist during radial deviation. The muscle tissue originates on the medial epicondyle of the humerus and inserts into the second and third metacarpal bones on the anterior face of the metacarpals (Boles, Kannam, & Cardwell, 2000). The FCR is innervated by the median nerve, which roots from the brachial plexus being the only nerve to go through the carpal tunnel (Boles et al., 2000). The FCR consists of superficial muscle tissue sharing a deep tendon with pronator teres, flexor digitorum superficialis and palmaris longus (Segal, Wolf, DeCamp, Chopp, & English, 1991). The fibers are arranged longitudinally but they insert at an oblique angle on the tendon (Segal et al., 1991). The muscle length typically measures 59.8 ± 1.5 mm from origin to insertion (Loren et al., 1996), where 45.9% of the muscle is type Ia (Morris, 1969).

The physiological cross-sectional area (PCSA) expresses the amount of contractile tissue in a muscle in terms of its volume divided by muscle fiber length. The FCR has a PCSA of 199 mm² (Gonzalez, Buchanan, & Delp, 1997). Gonzalez and colleagues (1997) measured the moment arm and peak force of FCR. The moment arm ranged from 16 to 17.3 mm with the peak force occurring at 40° of wrist flexion. In two
separate studies, peak force for the FCR was found to range from 51.2 to 60 N in untrained participants (Gonzalez et al., 1997; Loren et al., 1996).

Figure 1. Illustration showing the anatomical location of the flexor carpi radialis and other flexors of the wrist on the anterior aspect of the forearm (Luttgens & Wells, 1982).

**Tibialis Anterior**

The tibialis anterior is primarily involved in dorsiflexion of the foot. The muscle plays a key role during the swing phase of the gait cycle, controlling the forefoot during heel strike (Holmback, Porter, Downham, Andersen, & Lexell, 2003). Similar to the FCR, the superficial nature of the TA allows easy access for surface electromyographic
(sEMG) recordings (McIntosh & Gabriel, 2012; Roy, De Luca, & Schneider, 1986). The length of tibialis anterior has been measured to be $299 \pm 2.6$ mm by using MRI techniques (Fukunaga et al., 1992). The researchers also measured the PCSA of the TA to be $185$ mm$^2$. It is also innervated by the deep peroneal nerve (Saladin, 2008). The fiber-type composition of the TA is 76.7% slow-twitch in females and 77.8% slow-twitch in males, consistent with its role in gait (Holmback et al., 2003; Johnson, Polgar, Weightman, & Appleton, 1973). The moment arm of the tibialis anterior can range from $42 \pm 4.0$ mm at 15 degrees plantar flexion, to $49 \pm 4.0$ mm when in anatomical position (Maganaris, 2001).

Figure 2. Anatomical drawing displaying the orientation and anatomical location of the tibialis anterior and other foot dorsiflexors to show tendon insertion and origin as illustrated in (Luttgens & Wells, 1982).
The Electromyographic Signal

Signal Generation

In skeletal muscle, unlike its smooth and cardiac counterparts, when not agitated by reflex, contraction initiates with propagation stemming from the motor cortex of the brain. This propagation travels by way of the spinal cord to the correct branch in the target limb and subsequent neural endpoint that innervates the specific muscle Fibers. This is where the site of muscle contractions’ biochemical processes occurs. Muscle fibers have a resting membrane potential of $-90\, \text{mV}$ (De Luca & Forrest, 1973). This measurement is based on the electrochemical gradient that potassium ($\text{K}^+$), sodium ($\text{Na}^+$) and calcium ($\text{Ca}^{2+}$) ions create from residing on separate sides of the cellular membrane.

It is important to note that the resting membrane potential can be somewhat influenced by exercise training, but generally will stay at the same level while at rest (Moss, Wedding, & Sanders, 1983).

Motor Unit

Depolarization of the alpha motoneuron will result in the propagation of action potentials down the nerve branches, each associated with its own muscle fiber, to cause excitation-contraction coupling (Lateva, McGill, & Johanson, 2002). All the muscle fibers will contract at the same time (Westad et al., 2003). A single alpha motoneuron and all the fibers that it innervates is termed a motor unit, and it is considered the smallest functional unit of the neuromuscular system (Sherrington, 1906). The average number of muscle fibers per motor unit within a muscle, termed the innervation ratio, depends whether it is used for fine motor control or gross motor behavior (Lateva et al., 2002).
Muscles used for fine motor control will have a lower innervation ratio than gross motor movements (De Luca & Contessa, 2011). For example, the TA has an innervation ratio of 329 muscle fibers per motor unit (Gath & Stalberg, 1981). There is no data on the innervation ratio for the FCR. However, there is published data for a comparable muscle, the extensor digitorum longus, with an average of 165 muscle fibers per motor unit (Clark, 1931).

**The Interference Pattern**

An action potential traveling down a single muscle fiber is termed a muscle fiber action potential (MFP). Muscle fiber action potentials originate at the neuromuscular junction more centrally, and travel bi-directionally along the muscle fiber towards the distal ends (Figure 3) Since the motor unit is comprised of individual muscle fibers innervated by the same nerve, the MFPs summate, resulting in a motor unit action potential (MUP). Muscle fibers belonging to the same motor unit are not located adjacent to each other but spatially distributed (spatial dispersion) within a region of the muscle, close to each other. Muscle fibers of a given motor unit are therefore, interdigitated with the muscle fibers of other motor units. Spatial dispersion therefore results in slight differences in the time that action potentials reach the motor endplates of fibers within the same motor unit. As a result, the summation of MFAPs at the electrode produces an irregularly shaped MUP, not a smooth biphasic or tri-phasic potential as would occur if all the fibers were activated synchronously.

Once activated during a muscle contraction, a motor unit will discharge repetitively, resulting in a motor unit action potential train (MUPT). The top portion of
Figure 4 shows a group of motor units firing asynchronously during a brief period of time (200 ms), as occurs during a voluntary contraction. The bottom portion of figure 4 shows how all the MUPTs summate at the electrode to generate an interference pattern. The interference pattern is an indirect measure of neural drive to muscle, as the product of discharge rates and motor unit recruitment (Farina et al., 2010). The complexity of the interference pattern increases with an increase in motor unit discharge rate and the recruitment of additional motor units, because both factors increase the probability of temporal overlap between numerous positive and negative waveforms. Decomposition of the resulting interference pattern is the process of extracting the individual MUPTs that created it, termed motor unit decomposition. Reliable motor unit decomposition therefore begins with having a quality, low-noise interference pattern (Stashuk, 2001).

Figure 3. Muscle belly being innervated by the alpha motoneuron where propagation of the muscle fiber action potential will travel bi-directionally from (Griep et al., 1982).
Figure 4. Motor unit action potentials trains are composed of the repeated firing of MUAPs. The bottom component is the summation of all of these MUPTs, called the interference pattern (Basmajian et al., 1985).

**Motor unit behavior**

Motor unit behavior is based on the gradation of muscle force. As the strength of the muscle contraction slowly increases motor units are recruited based on their size: from low threshold smaller motor units with few muscle fibers to higher threshold larger motor units that have thousands of muscle fibers. Henneman (1957) initially described the orderly recruitment of motor units and termed it, “the size principle” (Henneman, 1957). The principle also works in reverse sequence during deactivation. When the force threshold for recruitment for a motor unit has been reached, it will initially discharge at approximately 8 PPS, and the discharge rate will increase if more force is required, this phenomenon has been termed “rate-coding” (Calancie & Bawa, 1985; Erim, De Luca, Mineo, & Aoki, 1996). As the force requirement of the task increases, additional higher threshold motor units will be recruited, but will have the same initial firing rate as the
earlier recruited motor units. While these higher threshold motor units are associated with faster twitch (Type II), larger motor units, they have lower firing rates during constant force contractions than the earlier recruited motor units (De Luca & Hostage, 2010). The overall relationship between the threshold of motor unit recruitment and rate-coding was first observed by DeLuca and colleagues in 1982 and was termed, the “onion skin phenomenon” (De Luca, LeFever, McCue, & Xenakis, 1982). DeLuca and Erim (1996) later showed that, if the duration of a near maximal isometric contraction is long enough, all the observable motor units will converge to a final maximal discharge rate (De Luca, Foley, & Erim, 1996).

There are two other force gradation strategies used by the nervous system that can increase the complexity of the interference pattern. The rapid succession of two consecutive firings of a motor unit with an interpulse interval of less than 20 ms, is termed a doublet (Burke, Rudomin, & Zajac, 1970, 1976). Doublets occur most often at the initiation of a muscle contraction to accelerate the rate of increased force output, and it is thought to take advantage of the catch-like properties of skeletal muscle (Binder-Macleod & Barrish, 1992). Finally, motor units have been observed to synchronize their activity, presumably to summate their twitch forces (Semmler, Kornatz, Dinengo, Zhou, & Enoka, 2002). When groups of motor units are synchronously active, the superposition of many MUPTs makes it more difficult to identify the individual MUPs that constitute the more complex waveform (Semmler et al., 2002). Finally, synchronization is sometimes confused with common drive. De Luca and Mambrito (1987) observed that, even though motor units exhibit the “onion skin phenomenon” during a force tracking task, their discharge rates change in unison. The phrase onion skin alludes to a plot of
low- and high-threshold motor units, where their instantaneous firing rates have layers with respect to each other, similar to that of an onion skin. The same was true for motor unit discharge rates between agonist and antagonist muscle. Common drive is not believed to increase the complexity of the interference pattern, but is a control scheme to simplify motor commands (De Luca & Mambrito, 1987).

**Surface versus the indwelling signal**

Until this point, this literature review has focused more broadly on the electromyographic signal, as the aforementioned factors outlined above are common to both indwelling and surface recordings. Indwelling recordings involve the insertion of the electrode by way of a needle or wire directly into the muscle, while surface recordings apply an electrode to the skin surface, secured by two-sided tape or by other means. Surface recordings are a more indirect measure of muscle electrical activity because the signal must pass several layers of tissue, fat, fascia and skin and is altered at each step. The effect is compared to low-pass filtering, which reduces the amplitude and frequency content of the signal compared to indwelling recordings (Beck et al., 2005).

**Factors that affect the surface EMG signal**

The scope of this thesis is focused on motor unit discharge rates obtained from the decomposed surface electromyographic signal. Any factor that affects the shape, amplitude and duration of the surface electromyographic signal will affect the measurement of motor unit firing rates.
**Interelectrode Distance and Configuration**

Surface electrodes are inherently susceptible to electromagnetic noise and crosstalk from biological sources, extended configurations are designed to take advantage of this common mode noise rejection by differential amplifiers (Roeleveld, Stegeman, Vingerhoets, & Van Oosterom, 1997). Double-differential electrodes have four detection surfaces (e). The first differentiation involves e1-e2, e2-e3, and e3-e4 to yield three sets of bipolar recordings (see Figure 5). Most, if not all of the common mode noise due to power-line interference is minimized by the first difference. The second differentiation takes the first and second bipolar signals (e1-e2 and e2-e3) and the second and third bipolar signal (e2-e3 and e3-e4) to yield two signals that have been differentiated twice. Both differences provide a spatially filtered result, such that more of the electrical energy comes from a small volume of tissue under the electrodes. The reduction common mode noise and spatial filtering allows MUPs to be identified from the skin surface and track their propagation velocity (Roeleveld et al., 1997). Common mode noise reduction increases with the number of detection surfaces being utilized.

Figure 6 illustrates a dEMG electrode (Delsys Inc. Boston, USA). The dEMG electrode (Delsys Inc. Boston, USA) has five detection surfaces spaced 5 mm apart, geometrically arranged in a Laplacian configuration, which allows for an enhancement of the differentiation process (Hogrel, 2003). Four bipolar channels are recorded by this surface quadrifillar configuration, which records the MUP from four spatially different locations, analogous to a 4-dimensional picture. Potentials from different MUPs can have similar shapes across one or two channels, but it is progressively less likely to be the case across three and four channels. Since different MUPs rarely have the same shape
across all four channels, it is easier to identify the different motor units that are active during the contraction, based on their unique four channel shape.

Figure 5. The double differentiated electrode. Two bipolar signals being E₁ and E₂ are components of the double differentiated signal S₁. In the case where S₁ and S₂, being two double differentiated signals would be created from the prior bipolar signals of E₁ E₂ and E₃, this is derived from S₁ = [E₁ + (-E₂)], and S₂ = [E₂ + (-E₃)] (Gabriel and Kamen, 2010, page 88).
Figure 6. Trigno surface sensor employed by the dEMG system with multiple recording surfaces, for recording motor unit action potentials from the skin surface (Delsys Inc.,).

Interelectrode distance affects both the volume of muscle tissue that is recorded and the frequency content of the signal (Hogan & Mann, 1980, Smith et al., 2017). First, interelectrode distance (IED) affects the recording volume of tissue, making the recording more or less selective. A very small inter-electrode distance, as occurs with the dEMG electrode (IED = 5 mm) allows for more selective recordings from a limited volume of tissue. Recording from a limited volume of tissue enhances the ability to detect MUPs directly beneath the electrode. Low-level activity from distant MUPs which would interfere with its identification process is then minimized (De Luca, Kuznetsov, Gilmore, & Roy, 2012). The small inter-electrode distance is also specific to the wavelength of the MUP, to record it with minimal distortion (De Luca & Forrest, 1972). As a result, inter-electrode distance also increases the frequency content of the signal.
**Temperature**

Muscle temperature affects both the amplitude and frequency content of the surface electromyographic signal and is related to ion channel kinetics across the muscle membrane. An increase in muscle temperature increases sodium channel kinetics which reduces the total current flux. There is a concomitant increase in propagation velocity of potentials along the muscle membrane. The result is a sEMG signal that is lower in amplitude and higher in frequency (Bigland-Ritchie, Thomas, Rice, Howarth, & Woods, 1992). The converse is true with muscle cooling. There is a reduction in sodium channel kinetics allowing for a greater current flux but there is a decrease in propagation velocity. An increase in amplitude and a decrease in frequency content of the surface electromyographic signal is the result (Engelhorn, 1983). Temperature is therefore a critical methodological control. More recently, it has been shown that muscle temperature also affects motor unit variables recorded from the surface electromyographic signal. Mallette and colleagues (2018) showed that muscle cooling resulted in an increase in motor unit recruitment during ramp isometric contractions to 50% MVC (Mallette et al., 2018).

**Fatigue**

The metabolic by-products of muscle fatigue result in the accumulation of hydrogen ions that increase the potential difference across the muscle membrane, making it more difficult to reach threshold (Nocella, Cecchi, & Colombini, 2017). The relationship with the onset of fatigue, there is a concomitant decrease in MFP conduction velocity. The slowly propagating potentials then spend more time underneath the
electrodes, recording larger amplitude signals. Muscle fatigue therefore results in an increase in the amplitude and a decrease in the frequency content of the sEMG signal (Dideriksen et al., 2010; McManus et al., 2015)

Changes in the sEMG signal are also moderated by the specific task used to induce fatigue. Sustained submaximal constant force contractions elicit compensatory motor unit behavior to maintain force level. There is a gradual increase in signal amplitude due to an increase in motor unit discharge rate (Beck et al., 2005; McManus et al., 2015). However, when completing a maximal contraction until exhaustion, there is a decrease in signal amplitude due to a reduction in motor unit discharge rate (Dideriksen et al., 2010; Kroll, 1967). The additional impact of motor unit recruitment and/or drop-out on the amplitude of the surface electromyographic signal is still the subject of debate (Vollestad, 1997).

Skin preparation

It is important to reduce the skin-electrode impedance to at least 10 kΩ to maximize the common mode rejection ratio of the differential amplifier (Green, McGuire, & Gabriel, 2015; Hu et al., 2013b). Skin preparation involves cleaning the skin of dirt and oils, and the removal of superficial layers (De Luca, Nawab, et al., 2015; Gabriel, 2000; Thornton & Michell, 2012).

Sweat

Perspiration through the skin surface is a parasympathetic response. Whether the response is to temperature, physical activity, or psychological stress, perspiration
influences the amplitude and frequency content of the surface electromyographic signal (Abdoli-Eramaki, Damecour, Christenson, & Stevenson, 2012). Although there is a “dampening effect” on the amplitude of the signal, perspiration does not compromise the resolution of the signal. Skin perspiration is only detrimental if participants sweat enough to cause the recording surface to displace relative to the skin, resulting in movement artifacts, or cause a short between electrode surfaces (Roy et al., 1986). Skin temperature levels must be monitored and significant changes that would induce a perspiration response should be reported. Additionally, there should be sufficient rest between trials and environmental control of the data collection space.

**The amount of subcutaneous tissue**

The surface electromyographic signal is affected by the amount of subcutaneous tissue (Nordander et al., 2003). Body mass index (BMI) has been used as an indication of how much subcutaneous tissue an individual may have, which can influence the amplitude and frequency characteristics of the surface electromyographic signal (Nordander et al., 2003). The strength of the signal is inversely related to electrode-source distance, which decreases with increases in the thickness of subcutaneous tissue (Bartuzi, Tokarski, & Roman-Liu, 2010; McManus et al., 2015).

**Electrode location**

Bipolar electrodes may accidently straddle the motor point, and differential amplification of bidirectional propagation of action potentials would result in an unstable signal amplitude (Beck et al., 2005). Standard practice for electrode placement has therefore been to avoid the motor point by placing both electrodes at least 1 centimeter
away (De Luca et al., 2012). Routine practice in the Electromyographic Kinesiology Laboratory of Brock University is to first electrically identify the motor point, then place the electrode at the specified location more than 1 cm away from the respective point (Green et al., 2015).

**Electrode placement and replacement**

Electrode placement and replacement is one of the most significant factors affecting the reliability of the surface electromyographic signal over multiple test sessions (Green et al., 2015). Typical training studies last about 6 to 12 weeks (Gabriel et al., 2006). Marked electrode locations with indelible ink over a long duration of time can be successfully maintained by participants, but should only be used as a secondary reference (Calder, Agnew, Stashuk, & McLean, 2008). Highly reliable surface electromyographic data is most easily obtained by electrically locating the motor point, as its location does not change over time. Measuring a specific distance (i.e., 1-2 cm) away from the motor point and placing the electrode on that location can be reproduced across multiple test sessions over several months (Calder et al., 2008). This method of electrode placement and replacement has been shown to be successful for both the FCR (Green et al., 2015; Green et al., 2014; Mallette et al., 2018; McIntosh & Gabriel, 2012) and the tibialis anterior (McIntosh & Gabriel, 2012).

**Surface EMG Decomposition**

Surface EMG decomposition involves several steps to obtain the MUDRs from the recorded MUPs. For dEMG, each motor unit has a unique MUP shape that is defined across four sEMG channels. The shapes across the four channels are used to create a
“template” for that motor unit. For each channel, the template represents the mean of all the individual MUPs within the interference pattern. The four channels of sEMG signals are then searched for the MUPs that fit each template. The process is called template matching. Matching occurs when individual MUPs for each channel falls within the 95% confidence interval of the template, which is assumed to be constant. Other statistical criteria may be used. A key feature of template matching is that it can be updated and refined with each newly identified MUP. The discharge times for a MU correspond to each instance that the MUPs are identified by the template. The MUPT is then the series of discharge times that occur during the contraction. Motor unit discharge rate is calculated as the reciprocal of the discharge times (Fang et al., 1999; Florestal et al., 2009; DeLuca et al., 2006; Hamid et al., 2008; Holobar et al., 2009; Zennaro et al., 2002; Martinez-Valdes et al., 2016; Merletti et al., 2008).

**Assessment of Reliability and Statistical Models**

The reliability of a measure depends on the consistency of scores within a subject and the stability of the group means across testing sessions (MacIntosh & Gabriel, 2012). The stability of the group mean is a statistical term that reflects whether or not there is significant difference in the group mean across test sessions (Kroll, 1967). The stability of means across test sessions is evaluated using the $F$-ratio from a similar ANOVA model, explained below. The consistency of a measure is evaluated by the use of the intraclass correlation coefficient, which is a ratio calculated from the different sources of variability that reflect how well a subject can reproduce their score (Safrit, 1976). The magnitude of the different sources of variability is given by the sum of squares (SS) from a unique application of the fully nested ANOVA (Safrit, 1976; Kirk, 1968).
Consistency

Consistency is one-half the assessment of reliability. It is possible for the means across days to be stable because subjects change their ranks across days, resulting in a non-significant $F$-ratio for the days main effect. For example, if high strength individuals decrease their MVC scores, and lower strength individuals increase their MVC scores, the means are stable but the subjects are inconsistent (MacIntosh & Gabriel, 2012).

To determine the consistency across multiple days and trials, the "fully-nested" model nests days within subjects, days (subjects). Trials are then further nested within days. The trials within days is the within-cell error term, within-cell (W.Cell) variance. Since the experimental conditions are the same within a single measurement session, differences between repeated measurements within the same days is theoretically due to measurement error (Sokal & Rohlf, 1995). Reliability studies are supposed to be conducted in the absence of any experimental treatments so that the variability across days reflects biological variability and measurement error (Safrit, 1976). The term biological variability is limited and will be expanded upon later. The trials nested within days, which are also nested within subjects, provides an indication of the total variability of the scores within subjects and the type of variability that dominates.

A good test or measure is one that can differentiate between subjects. If subjects are good at reproducing their own score, because the measurement error is small and biological variability is minimal, the scores will group tightly around the subjects’ own mean. The score of one subject will not overlap significantly with those of another who is “truly” different on that particular measure. The result would then be a high “between
subjects” variance, which is also called the “true score” variance. Therefore, the fully nested model accommodates and assigns all scores within each subject and evaluates the impact of the different sources of variability (Trials and Days) on the ability to detect differences between subjects. That is, the ability to detect differences between subjects when they “truly” exist (Feldt & McGee, 1958; MacIntosh & Gabriel, 2012).

It is important to emphasize that the fully-nested ANOVA table is not used for hypothesis testing. It is used to determine the magnitude of the sources of variation in the experiment: subjects, days (subjects), and W.Cell (trials). We calculate the total variance then determine what percentage of the total variance each component contributes to the experiment. If the measure is reliable, then the subjects’ sum of squares (SS) should be the greatest proportion of the total variance. Ideally, the days (subjects) should be greater than our W.Cell variance (repeated measurement within days, i.e., trials). Biological variation across days within subjects should be small but at least greater than the measurement error (Feldt & McGee, 1958; Christie et al., 2010; MacIntosh & Gabriel, 2012). There is a potential problem when there is little difference between subjects, and the variability is sufficient to cause the scores to overlap. As a result, reliability assessment on a homogeneous group can artificially deflate the intraclass correlation coefficient, or when there is a physiological parameter that has a very narrow range of values (MacIntosh & Gabriel, 2012).

**Stability**

Stability is the second part of reliability. Subjects can be consistent at reproducing their own scores, which group tightly around their own mean, but undergo a similar
magnitude of change across days, as would occur with an increase in strength due to maximal isometric strength testing, alone (Kroll, 1963). If subjects all experience similar strength gains, and they are all consistent at reproducing their own score, they will maintain their relative ranks within the sample, across test days. The between subjects SS will still be high but the means are not stable. A hypothesis test is needed to evaluate stability of means across days (MacIntosh & Gabriel, 2012).

Hypothesis testing using the fully nested model is parallel to the completely randomized factorial model (Kirk, 1968). The completely randomized factorial (CRF) model has traditionally been used to determine if there is a significant difference in means across test days. The model asks the question: is the variance between days within subjects greater than our within-cell variance (measurement error)? This model is used only because it is linked with how the sum of squares are obtained to calculate the intraclass correlation coefficient through the fully nested model (Feld & McGee, 1957; Kroll, 1962; 1963). It should be used with caution in comparison to a more appropriate repeated measures models (Kirk, 1995, page 461-464; 482).

The data format for the fully-nested model is used to obtain $F$-ratios for subjects, days, and the days $\times$ subjects interaction terms in the CRF model. The error term for days involves the Within-Cell sum of squares, which is typically small. Further, the degrees of freedom for this $F$-ratio is very large. The result is an overly sensitive $F$-test and type I error (Kirk, 1995, page 461-464; 482). For the CRF model, the error term is taken from the within-cell SS and assumed to only result from measurement error, completely ignoring the fact that different subjects may have different response magnitudes to the treatment (i.e., biological response variability). Using the appropriate
statistical model involves evaluating significant differences in days and trials directly, using the correct error terms. The correct error term is the subjects’ interaction for that particular main effect, not the Within-Cell estimate of measurement error. The correct model also acknowledges that the repeated measures are “correlated,” which have additional assumptions (i.e., sphericity) to which they must adhere. Thus, a second test of stability using a two-factor repeated measures (day × trials) analysis of variance should be completed, with evaluation of the statistical assumptions (Kirk, 1995, page 461-464; 482).

**Reliability Studies of Force and Electromyographic Activity**

The majority of studies in the literature only assess the consistency portion of reliability without acknowledging stability. Another difficulty is that there are few studies on muscles relevant to the present study. Furthermore, there is only one reliability study on surface electromyographic decomposition (Hu et al., 2014). Although, it is reasonable to argue that any factor (intrinsic or extrinsic) that affects the reliability of the root-mean-square (RMS) amplitude, mean power frequency (MNF), and the peak-to-peak (P-P) amplitude of the M-wave, would also affect motor unit discharge rates obtained by surface EMG decomposition (DeLuca, 1997). The literature review below will therefore include other surface EMG measures.

Calder et al. (2005) studied the reliability of peak-to-peak (P-P) amplitude and shape of the M-wave in the biceps brachii in the absence of any intervention. The change in mean P-P amplitude was no greater than 4.3% indicating a high degree of stability, while an intraclass correlation coefficient of $R = 0.96$ suggests excellent consistency.
within subjects. The study demonstrated that with careful skin preparation, measurement and marking of electrode location for accurate replacement across test sessions, there can be excellent reliability in surface EMG measures.

A predecessor of surface EMG signal decomposition is the spike triggered averaging (STA) technique. The technique involves inserting a needle electrode into the muscle and a surface electrode on the skin surface. The amplitude of a specific motor unit action potential (MUP) is recorded from the needle electrode and is then used to trigger the sampling of the surface EMG signal (Hu et al., 2013). Thousands of averages of the surface EMG signal encompass an epoch around the discharge time, then reveal a surface recording of that particular MUP. Boe et al. (2005) demonstrated systematic changes in surface MUPs across different force levels that included 10, 20, 30, 40, and 50% of maximal voluntary contraction of the first dorsal interosseus (FDI) muscle. Maintaining the same force level across test session should therefore be an important methodological control for obtaining reproducible surface MUP variables.

In 2008, Calder et al. (2008) examined the test-retest reliability of P-P amplitude of surface MUPs in the extensor carpi radialis muscle (ECR), obtained by STA. Wrist extension force was $8.54 \pm 1.73\%$ of MVC on the first day of testing and increased to $10.71 \pm 1.73\%$ of MVC on the second test day. Despite a 20.26% difference in force between the test and re-test session, the consistency of the P-P amplitude of the ECR surface MUPs was $R = 0.90$, which is excellent. There was no significant difference in means between the two days, suggesting that data were stable. Although the researchers
evaluated a very small sample size (N=6), the reliability of P-P amplitude of ECR surface MUPs was deemed to be good enough for electrodiagnostic purposes.

In the upper limb, the most directly comparable study was conducted by Green et al. (2015), who evaluated the reliability of maximal isometric strength of the wrist flexors and flexor carpi radialis (FCR) root-mean-square (RMS) surface EMG amplitude, mean power frequency (MNF), and P-P amplitude of the M-wave. There were four sessions with at least 48 hours in between each test. The study showed with careful methodological controls, particularly with respect to skin preparation and electrode location, all three measures can exhibit excellent reliability. Across test session, the measures changed by less than 10%, indicating a high degree of stability in means. The measures also exhibited a high degree of consistency within subjects, as the intraclass correlation coefficients range from $R = 0.84$ to 0.90.

The findings of Green et al. (2015) are in stark contrast to the earlier work of Barr et al. (2001) who evaluated the reliability of FCR RMS amplitude during maximal isometric wrist flexion. The FCR RMS amplitude was highly stable and changed only 1.5% across three test sessions, but the consistency of FCR RMS amplitude was extremely poor with an intraclass correlation coefficient of $R = 0.34$. It is interesting to note that the surface EMG was normalized, which can have a dramatic effect on the intraclass correlation coefficient (Chapman et al., 2010). Green et al. (2015) showed that normalization decreases the variance of the data. The subjects’ scores become more homogeneous, which artificially deflates the true score variance and the overall intraclass correlation coefficient.
The reliability of surface EMG measures are more established in the TA than in the FCR. MacIntosh and Gabriel (2012) studied the reliability of muscle fiber conduction velocity, RMS amplitude, and MNF of the surface EMG signal during isometric dorsiflexion at 30 and 100% of MVC. Since it takes a degree of skill to perform submaximal contractions to a target force (Salonikidis et al., 2009; 2011; Orizio et al., 2010), the inclusion of a submaximal condition is particularly relevant to this thesis.

None of the means across sessions for either force or surface EMG measures changed by more than 5%, demonstrating remarkable stability. The intraclass correlation coefficients ranged from $R = 0.83$ to 0.98, which indicated a high level of consistency with subjects, similar to the FCR. The results show that, with careful skin preparation and electrode placement and replacement, highly reliable surface EMG measures can also be obtained in the lower limb. The findings of MacIntosh and Gabriel (2012) are slightly different from the results of Souron et al. (2016). Souron et al. (2016) reported excellent stability in maximal isometric dorsiflexion strength, as it changed by less than 1% across the three test sessions. The consistency of scores within each participant resulted in an intraclass correlation coefficient of $R = 0.98$. It is somewhat surprising, however, that the overall reliability of TA RMS amplitude surface EMG activity was merely adequate. Changes in TA RMS amplitude across test sessions were no more than 8.3%, which is relatively stable, but the intraclass correlation coefficient was $R = 0.68$. Once again, the high stability but low consistency may reflect the fact that the surface EMG amplitude was normalized to 100% MVC.

Martinez-Valdes et al. (2016) evaluated the reliability of motor unit variables obtained by surface EMG decomposition of signals recorded by a high-density electrode
grid. The method of decomposition differs from that proposed by De Luca et al (2006) for use by dEMG system (Delsys Inc., Boston, USA., De Luca et al., 2006). The study does however demonstrate the feasibility of recording motor unit variables from the skin surface over multiple test sessions. Participants performed isometric contractions of the knee extensors at 10, 30, 50, and 70% MVC on each of three test sessions, each spaced seven days apart. Unfortunately, the authors did not report on the reliability of the submaximal forces, however, there was no significant difference in 100% MVC across the three test sessions.

Assuming the validity of the surface EMG decomposition, the study by Martinez-Valdes et al. (2016) supports the original contention expressed at the beginning of this section. That is, those factors that affect the reliability of the surface EMG signal will also affect the reliability of the motor unit variables. Consequently, the reliability of motor unit variables obtained by surface EMG decomposition should be comparable to that of other measures obtained from the surface EMG signal, such as muscle fiber conduction velocity. Raw data were not presented in a table, but lack of significant difference in means across test sessions shows MFCV was stable.

Muscle fiber conduction velocity further showed excellent consistency within subjects, with intraclass correlation coefficients across force level ranging from $R = 0.91$ to 0.95. The reliability coefficients are similar to those observed by McIntosh and Gabriel (2012). No significant differences in motor unit discharge rates (MUDRs) were observed in the means across test sessions, for all force levels. The MUDR for all force levels also exhibited excellent consistency within participants, with intraclass correlations coefficients ranging from $R = 0.81$ to 0.92. More recently, in a companion study,
Martinez-Valdes et al. (2017) demonstrated that up to 40% of the same motor units could be tracked from one session to the next. While the intersession interval for the Martinez-Valdes et al. (2016; 2017) studies were seven days, the thesis endeavoured to increase the interval to one that is more common to interventions studies.

Placement and replacement of the electrode in the same position on the skin surface is an important methodological control, because location of the detection surface relative to the propagating source affects the morphology of the potentials (Farina et al., 2002; 2014). The change in morphology could mask any alteration due to neural control (Arabadzhiev et al., 2014). It is well-known that indwelling EMG variables exhibit poor test re-test reliability, because inserting a needle or wire electrode into the exact same position is impossible (Viitasalo & Komi, 1975). Inserting the electrode at the same “relative” position, but to the exact same depth within the muscle is the only possible methodological control (Watanabe at al., 2011). Kamen et al. (1995) are the only investigators to report on the test re-test reliability of MUDRs in the first dorsal interosseous (FDI) muscle during maximal isometric adduction of the index finger. The stability of the MVC means was not reported, but the consistency within subjects was excellent as evidenced by an intraclass correlation coefficient of $R = 0.93$, consistent with other joint actions. The consistency of MUDRs was similarly high with an intraclass correlation coefficient of $R = 0.90$, but the mean MUDRs across test days were not reported nor was stability evaluated. Thus, whether recording MUDRs from the skin surface or indwelling electrodes, the studies outlined above lead to the expectation that the measure can be highly consistent and stable.
CHAPTER III

Methodology

The data presented in this thesis are part of an intervention study on cross-
education in the upper and lower limbs, conducted by Lara Green (Green, 2018) and
published in the Journal of Neurophysiology (Green & Gabriel, 2018). There were two
groups: an upper limb training group and a lower limb training group. The upper limb
training group was assessed for bilateral maximal isometric wrist flexion strength and
MUDR of the FCR at 60% MVC. Bilateral maximal isometric dorsiflexion strength and
MUDR of the TA at 60% MVC were also measured in the untrained lower limbs as a
sham condition. The lower leg training group went through the opposite testing pattern.
Reliability analysis was conducted on maximal isometric wrist flexion strength and
MUDR of the FCR at 60% MVC of the untrained dominant upper limb. Likewise,
reliability of maximal isometric dorsiflexion strength and MUDR of the TA at 60% MVC
of the untrained dominant leg was also evaluated. Finally, the names used for each test
session presented in this thesis refer to their specific purpose within the overall
measurement schedule utilized by Green (2018).

Participants

Forty subjects (20 males and 20 females) participated in the study, divided equally
between the upper and lower limb training groups. Subjects with neurological or
musculoskeletal disorders of the upper or lower dominant limbs, and/or who are currently
engaged in any resistance training as well those who were outside the age range of 18-35,
were excluded from the study. This study was cleared by Brock University Research Ethics Board (REB: #16-313, Appendix A).

**Preliminary procedures**

Participants were invited to the laboratory prior to the first testing session, to become familiarized with the nature of the experiment and the equipment. Following they were asked to read and sign an informed consent document (Appendix B), which outlined the requirements of participation, including the inherent risks, possible benefits, and the right to discontinue at any point in time without prejudice. Next, the PAR-Q questionnaire was completed (Appendix C). Anthropometric measurements of the forearm and leg were also completed (Appendix D).

**Measurement Schedule**

A total of four test sessions were required in the training study (Figure 7). The first session was familiarization so that a stable baseline was established on the second test session (Carolan & Cafarelli, 1992; Knight & Kamen, 2001). The time interval between the first two test sessions ranged between 48-72 hours. The third test session was completed six weeks after the second, and with an additional six-week interval between the third and fourth test sessions. These time intervals were selected because they are common in neuromuscular training studies (Carolan & Cafarelli, 1992; Knight & Kamen, 2001).
Figure 7. Itinerary of the data collection sessions and how the time gaps in between them were displaced from each session.

**Experimental Set-Up**

**Force Measurement**

All testing was completed in the Electromyographic Kinesiology Laboratory at Brock University within a grounded Faraday Cage. There was an apparatus to isolate the wrist flexors (Figure 9) and the dorsiflexors (Figure 8) during isometric contractions. Each device incorporated a load cell (MB-100 and SSMH, Interface Inc., Scottsdale, AZ) for recording forces. Wrist flexion force was assessed with the elbow 160° relative to the humerus, with the hand placed in a half-supinated position. The axis of rotation of the wrist was aligned with the axis of rotation of the lever arm on the load cell. The wrist was kept in a neutral position. Wrist extension force output is relatively constant throughout the joint range of motion. While a neutral position is in the middle of the wrist flexion force-output curve (Hallbeck, 1994), it minimizes the tendency to use shoulder adduction during testing.
Dorsiflexion force was assessed with the knee and hip joints at a 90° angle. The ankle joint was placed in slight plantar flexion at a 110° angle relative to the tibia. Slight plantarflexion is an optimal muscle length dorsiflexion force output. Further, the shorter muscle length for the triceps surae (plantar flexors) placed the muscle group in passive insufficiency to minimize their contribution as antagonists during dorsiflexion (Billot, Simoneau, Ballay, Van Hoecke & Martin, 2011, Miaki, Someya & Tachino, 1999, Marsh, Sale, McComas & Quinlan, 1981). The footplate consisted of a cushioned metal bar covering the fifth metatarsal to secure the foot, with the load cell immediately beneath.
Figure 8. Photo showing the apparatus for assessment of ankle dorsiflexion force, including surface electrographic (sEMG) electrode on the tibialis anterior. The training study used a modified bipolar electrode configuration, with one of the recording surfaces on the motor point (Green & Gabriel, 2018). The dEMG sensor is then placed approximately 1 cm distal to the sEMG electrode on the motor point.
Figure 9. The apparatus used for the collection of the force and surface electromyography (sEMG) of the wrist flexors. The training study used a modified bipolar electrode configuration, with one of the recording surfaces on the motor point (Green & Gabriel, 2018). The dEMG sensor was placed approximately 1 cm distal to the sEMG electrode on the motor point. The adjustable bars on either side of the wrist to ensure restraint, with the load cell on the palmar side of the hand. Foam padding under the forearm leading to a felt wedge beneath the upper arm maintains the elbow at a comfortable angle (160°).
Surface Electromyography

The skin was first prepared to reduce skin-electrode input impedance. This involved alcohol wipes to first clean initial surface debris, followed by using an exfoliate (NuPrep®, Colorado, USA) to remove dead skin, with a final alcohol wipe to rid the exfoliate. After skin preparation, the motor point was located so that the dEMG sensor (Delsys Inc., Bagnoli, Boston, USA) was placed in line with the muscle fibers, 1 cm distal from the motor point. Location of the motor point consisted of using a stimulator (Grass Stimulation Isolation Unit, Warwick, USA, Model: SIU8TC) set to 1.5 pps, at the lowest possible current. The anode was placed on the agonist muscle, while a small cathode probe (2 mm) was used to locate the surface area of the skin that elicited a barely visible contraction with the least amount of stimulation; this area was the identified motor point.

The dEMG sensor consists of 5 pins embedded in a 5 × 5 mm rectangular platform; it was secured to the skin surface with tape, one-centimeter away from the motor point. The sEMG signals were band-passed between 20 and 450 Hz, and amplified to maximize the resolution of the 16-bit analogue-to-digital converter. Finally, the force and sEMG signals were digitized at 20 kHz using the Bagnoli-16 and EMGworks 4.2 (Delsys, Inc., Boston, MA).

Data Collection Procedure

Participants completed the following series of contractions after the electrodes were secured to the skin surface. There were three maximal voluntary contractions, each lasting 4-seconds in duration with 2-minute inter-trial rest periods. The instructions to
the participants were to contract ‘hard-and-fast’ and hold their maximum steady (Gabriel, Lester, Lenhardt, & Cambridge, 2007). Feedback was provided to the participants through the use of a computer monitor displaying a force trace which was positioned in such a way that it was in comfortable viewing of the participants from either of the apparatus (Mallette et al., 2018). After 5 minutes of rest, participants completed a 6-second ramp contraction to 20% MVC. The sEMG signal decomposition software requires this test contraction to evaluate signal quality from the dEMG electrode prior to continuing the protocol. Three isometric ramp contractions at 60% MVC force were completed with 2-minutes between each trial. The increase and decrease in force occurred at 10% MVC per second, with a 6-second plateau at 60%. A trapezoidal “target” and real-time force output from the load cell was presented on a computer screen for participants to follow during the ramp contractions (Figure 10). These procedures were followed for both wrist flexion and dorsiflexion, presented in balanced order across participants.
Figure 10. Experimental set-up showing where the monitor was placed for the participant to follow with their progress as they try to match the ramp target. The monitor displayed the force-time graph with 60% MVC-force level to match the force signal transferred from the load cell. There was no quantitative feedback to ensure that participants were not trying to replicate score but rather the entire ramp pattern.
**Criterion Measures**

Both force and motor unit discharge rates (MUDR) were obtained from a one-second window in the middle of the 100% and 60% MVC contractions. Mean maximal force was calculated as average maximal force over a one-second window. Motor unit discharge rates were extracted from the sEMG signal using the Precision Decomposition Algorithm III in the dEMG analysis software (version 1.1, Delsys, Inc., Boston, MA), which identifies each motor unit and the firing instances (Chang, Liu, Lin, Tsaih, & Hsu, 2008; De Luca et al., 2006; Nawab et al., 2010). Figure 11 shows a representative ramp isometric contraction. Note that the force trace is trapezoidal in shape, and the vertical bars show the firing instances for each motor unit potential train.

Motor unit potential trains used for analysis had to meet the following criteria assigned by the dEMG analysis software (version 1.1, Delsys, Inc., Boston, MA): (1) the motor unit firing instances must meet a minimum decomposition accuracy of 90%; (2) the trial must have a minimum of 5 motor units with greater than 90% decomposition accuracy; and (3) the MUDR must have a coefficient of variation no greater than 20%. The dEMG analysis software (version 1.1, Delsys, Inc., Boston, MA) then calculated the instantaneous MUDR for each MU, as the inverse of the Hanning window (0.95 seconds), smoothed inter-pulse interval.

The steps involved calculating the instantaneous MUDR were as follows. A data vector consisting of zeros was created that was the same length as the contraction. A unit pulse was then inserted at each of the recorded discharge times. The data vector of ones and zeros was then convolved with the Hanning window. The width of the Hanning
window was specified in terms of time (i.e., 0.95 seconds), but the actual number of data points of the window was based on the sampling frequency (20kHz). The mean MUDR of all the active MUs within the one-second plateau force of the force trace was used for analysis.
Figure 11. Representative trial of participant in the study displaying identified motor unit potential trains (MUPTs), firing instances along with the force trace. The circles on the force trace indicate the recruitment threshold for each motor unit. The program also displays an internal “accuracy rate” next to each identified motor unit potential.
**Statistical Analysis**

Assessment of reliability involved determining the stability of means across test sessions. The statistical model used to examine the stability of the means across test sessions is a two factor (Days × Subjects) analysis of variance (ANOVA). The repeated measurements (trials) on each subject in each day constitute a “within-cells” replication of measures, and assessment of measurement error (Kirk, 1995). However, group means can remain unchanged because changes in the scores of one subject may be compensated for by complementary changes in the scores of another. A robust assessment of reliability therefore involves the determination of the consistency of scores within participants. Consistency was evaluated through the use of the intraclass correlation coefficient (Christie et al., 2010; Feldt & Mckee, 1958).

The ANOVA model used for this component of reliability estimation as originally conceived by Feldt and McKee (1958) requires a fully nested ANOVA model, also with two dimensions (days × subjects). The repeated-measurements (trials) on each subject within each test day constitute a “Within-Cells” replication of measures that are nested within each Day, wherein each Day is nested within each Subject. In this way, all repeated measures were nested within each subject, so that the consistency of scores within subjects could be evaluated as the percentage of total variance attributable to each factor-component.

The intraclass correlation coefficient (R) is simply the ratio constructed from the different components of variance, which are calculated from the mean squares from the fully nested model ANOVA model in the following way:
\[ R = \frac{\sigma^2_{\text{true}}}{\sigma^2_{\text{true}} + \frac{\sigma^2_{\hat{e}_1}}{a \cdot n} + \frac{\sigma^2_{\hat{e}_2}}{a}} \]

\[ \sigma^2_{\hat{e}_1} = MS_{\text{trials}} \]

\[ \sigma^2_{\hat{e}_2} = \frac{MS_{\text{days}} - MS_{\text{trials}}}{n} \]

\[ \sigma^2_{\text{true}} = \frac{MS_{\text{subjects}} - MS_{\text{days}}}{a \cdot n} \]

In equations above, \( a \) is number of days, \( n \) is number of trials, \( \sigma^2_{\hat{e}_2} \) is error variance due to days, \( \sigma^2_{\hat{e}_1} \) is error variance due to trials, and \( \sigma^2_{\text{true}} \) is the true score variance. The total variance, \( \sigma^2_{\text{total}} \) was then calculated as the sum of the variances \( \left( \sigma^2_{\text{true}} + \sigma^2_{\hat{e}_1} + \sigma^2_{\hat{e}_2} \right) \). The portion of total variance attributable to each of the source of error was defined as the day-to-day variance \( \left( \sigma^2_{\hat{e}_2}/\sigma^2_{\text{total}} \right) \), the trial-to-trial variance \( \left( \sigma^2_{\hat{e}_1}/\sigma^2_{\text{total}} \right) \), and the between subjects variance \( \left( \sigma^2_{\text{subjects}}/\sigma^2_{\text{total}} \right) \).

It is common to adopt the convention delineated by Fleiss (1986), where an intraclass correlation coefficient (R) below 0.40 indicates poor reliability, between 0.40 and 0.75 is fair reliability, while values greater than 0.75 represent excellent reliability. However, the intraclass correlation coefficient must also be evaluated against other diagnostic criteria, such as the homogeneity of scores which can artificially deflate its
magnitude. Kurtosis was calculated consistent with McIntosh and Gabriel (2012) who illustrated that it can be used to evaluate homogeneity of scores. The magnitude of the intraclass correlation coefficient was also evaluated using the standard error of measurement (SEM) within an individual (Green et al., 2015). The SEM was calculated as the square-root of the total mean square error from the ANOVA table (Weir, 2005). The intrasubject coefficient of variation was also calculated as the grand mean across the four test sessions divided by the SEM (McIntosh & Gabriel, 2012).

Figure 12. Break down of the ANOVA model in relation to the calculation of stability and consistency.
CHAPTER IV

Results

The primary objective of this study was to examine the reliability of motor unit discharge rates in the flexor carpi radialis and tibialis anterior during isometric contractions at 60% of maximal voluntary contraction. The reliability of maximal voluntary contractions was also assessed (Martinez-Valdes et al., 2017)

Subject Characteristics

The means and standard deviations for the physical characteristics for the participants in the arm and leg training groups for which the untrained limb was used for reliability analysis are presented in Table 1.

Table 1 – Means (M) and standard deviations (SD) for the physical characteristics of the participants.

<table>
<thead>
<tr>
<th>Physical Characteristic</th>
<th>Arm Group (N = 20)</th>
<th>Leg Group (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.15 ± 1.81</td>
<td>24.83 ± 2.48</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.6 ± 10.7</td>
<td>173.7 ± 10.0</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>69.3 ± 10.2</td>
<td>71.3 ± 9.0</td>
</tr>
<tr>
<td>Forearm Length (cm)</td>
<td>25.6 ± 1.9</td>
<td>Reliability Analysis</td>
</tr>
<tr>
<td>Leg Length (cm)</td>
<td>Reliability Analysis</td>
<td>37.3 ± 2.6</td>
</tr>
</tbody>
</table>
Statistical Assumptions

The first step was to determine if the data conformed to the standard univariate assumptions that underlie the use of repeated measures analysis of variance. Any violation of the assumptions can lower the estimate of reliability (Kroll, 1962). The basic assumption that errors, $e_{k(ij)}$, are independent, normally distributed, mean of zero, and variance equal to population value $\sigma^2$ is tested by evaluating the raw scores (Kirk, 1995). For the fully-nested completely randomized models, none of the measures had a ‘within cell’ (trials) skewness of greater than 1. The same was true for kurtosis, except one cell had a value of 2.59. Glass and colleagues (1972) have demonstrated that the analysis of variance is robust to mild departures from normality for balanced designs with moderate sample sizes. Robust refers to the fact that the probability of type I and type II errors for the $F$-test remain relatively unchanged (Glass, Peckham, & Sanders, 1972).

Repeated measures ANOVA cannot meet the assumption of independence of error, because participants produce multiple scores. The errors are by necessity correlated. The assumption of the independence of errors is replaced by the assumption of sphericity (Tabachnick & Fidell, 2007). Sphericity means that the variance of the difference scores between any two levels of a within-subjects factor should remain constant. The assumption has also been called the “homogeneity-of-variances-of-differences” assumption. There were four levels of the independent variable in this thesis, test days one through four. The difference scores were calculated between each of the four test days for each measure. The sphericity assumption assumes that the variance of these difference scores is not significantly different. The Mauchly’s sphericity test
supported that the assumption was upheld for each of the criterion measures evaluated in this study, with \( p \)-values between 0.77 and 0.81.

**Stability and consistency**

**Force**

Force is presented first because it represents a nearly ideal example of what constitutes a highly reliable measure. Table 2 reports that there was a significant increase in maximal isometric wrist flexion force from \( 86.62 \pm 47.22 \) N on test day one to \( 95.83 \pm 54.96 \) N test day four \( (p < 0.01) \). Tukey’s post-hoc testing further showed that there was a significant 6.3% increase between sessions one and two \( (p < 0.01) \). The remaining 3.9% increase from test days two through four was non-significant, for a total of 10%. The differences between means across test days accounted for only 3.93% of the total variance (Table 2). The grand mean was 92.80 N with a standard error of measurement (SEM) of 20.14 N.

Figure 13 depicts the mean (circle) and standard deviation (vertical bars) of the wrist flexion force values for each subject, respectively. The spread of force scores for each subject was generally grouped tightly around its own mean. Equally important, the vertical bars show that spread of scores within each subject was low enough so that there is little overlap of the force scores between different subjects. Thus, the between subjects variance (true score variance) was high \( (\approx 93\%) \). If the true score variance accounts for the greatest proportion of the variance, the intraclass correlation coefficient will be high. The overall result was an intraclass correlation coefficient of 0.99 (Table 2).
Table 2 shows that maximal isometric dorsiflexion force rose from 260.36 ± 93.85 N on test session one to 295.35 ± 95.23 N on test session four, amounting a significant increase of 11.85% ($p < 0.01$). Tukey’s post-hoc testing further showed that, after the first test session, the increase in dorsiflexion strength was significantly greater from one session to the next until test session four ($p$’s < 0.01). The slight lack of stability in means was offset by a high degree of consistency of scores within subjects. Figure 14 shows that the spread of scores within each subject was grouped tightly around its own mean, so that there is little overlap between the vertical bars for each subject. The grand mean was 281.00 N with a SEM of 79.80 N. The resulting true score variance, which is calculated from the between subjects sum of squares (which is high when the scores between subjects have minimal overlap), comprised the greatest percentage of the total variance (~80%).

**Motor unit discharge rate**

The means and standard deviations for motor unit discharge rate (MUDR) for the flexor carpi radialis and the tibialis anterior are presented in Table 2. The greatest difference between means across test sessions was 0.85 pps, which was only 5.2% ($p < 0.01$). The grand mean was 15.69 pps with a SEM of 3.57 pps. The small but statistically significant difference for the main effect for days, is a classic example of why a traditional repeated measures ANOVA must be conducted in conjunction with the completely randomized factorial model. When the appropriate subjects × days interaction term is used as the denominator for the $F$-ratio, the resulting $p$-value was 0.22.
Nevertheless, the day-to-day error was ≈34%, greater than that of maximal isometric strength measures.

The trial-to-trial error (≈26%) was also greater than maximal isometric strength measures. In fact, the true score variance still occupied the greatest proportion of the total variance (≈41%). The consistency of the flexor carpi radialis MUDR was 0.79, which is still considered quite good. Figure 15 and 16 showed that the total day-to-day and trial-to-trial variance increased the spread of MUDR scores so that there was overlap between the scores of each subject. The increased overlap decreased the between subjects sum of squares (true score variance), so the intraclass correlation coefficient was lower than that observed for maximal isometric strength.

The results for the tibialis anterior were similar to those of the flexor carpi radialis. That is, MUDR was 14.82 ± 2.52 pps on the first test session and 19.10 ± 2.66 pps on the third test session, so that the greatest difference in means was nearly 8% (p<0.01). By convention, a 10% difference between means has been used for sample size estimation, where a statistically significant difference is also of practical importance (Lagasse, 1974). Interestingly, when the Day main effect was evaluated with the appropriate error term, the p-value of the F-ratio was 0.0623. The grand mean was 15.42 with a SEM of 4.12 pps. For the tibialis anterior, the day-to-day error MUDR was greater (≈ 40%) and the trial-to-trial error was much less (≈ 18%) than that observed for the FCR. Since the true score variance for the tibialis anterior was comparable (≈43%) to that of the FCR, the differences in the day-to-day and trial-to-trial error variances compensated for each other, so that the intraclass correlation coefficients were identical at R = 0.79.
Table 2 – Analysis of variance for maximal voluntary contractions. The units for force are in Newtons (N) and the units for motor unit discharge rate (MUDR) are pulses per second (pps). Force was obtained during maximal isometric wrist flexion and dorsiflexion. Motor unit discharge rate was obtained at 60% of maximal voluntary contraction.

<table>
<thead>
<tr>
<th>Test Day</th>
<th>Wrist Flexion</th>
<th>Dorsiflexion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Force (Newton)</td>
<td>MUDR (pps)</td>
</tr>
<tr>
<td>1</td>
<td>86.62 ± 47.22</td>
<td>15.42 ± 2.57</td>
</tr>
<tr>
<td>2</td>
<td>92.48 ± 49.06</td>
<td>15.47 ± 2.15</td>
</tr>
<tr>
<td>3</td>
<td>96.28 ± 49.37</td>
<td>16.27 ± 1.86</td>
</tr>
<tr>
<td>4</td>
<td>95.83 ± 54.96</td>
<td>15.59 ± 2.14</td>
</tr>
<tr>
<td></td>
<td>Greatest Percent Difference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.66 (10.0%)</td>
<td>0.85 (5.2%)</td>
</tr>
</tbody>
</table>

ANOVA F-Ratios

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>3</td>
<td>1192.99*</td>
<td>9.32*</td>
<td>13611.01*</td>
</tr>
<tr>
<td>Subjects</td>
<td>19</td>
<td>29704.61*</td>
<td>30.53*</td>
<td>89882.04*</td>
</tr>
<tr>
<td>Days × Subjects</td>
<td>57</td>
<td>339.42*</td>
<td>6.17*</td>
<td>4847.49*</td>
</tr>
<tr>
<td>Within Cells</td>
<td>160</td>
<td>73.9568</td>
<td>1.29</td>
<td>202.43</td>
</tr>
</tbody>
</table>

* Significant at the 0.01 probability level; Percent Change = ((Minimum/Maximum) –1) × 100
Table 3 – Intraclass correlation analysis of variance for maximal voluntary contractions. Below are the mean squares (MS), variance components, the grand mean, standard error of measurement (SEM), the resultant intraclass correlation coefficients (R) for force and motor unit discharge rate (MUDR) during wrist flexion and dorsiflexion at 60% of maximal voluntary contraction.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Wrist Flexion</th>
<th>Dorsiflexion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Force</td>
<td>MUDR</td>
</tr>
<tr>
<td>Subjects</td>
<td>19</td>
<td>29704.61</td>
<td>30.53</td>
</tr>
<tr>
<td>Day (Subjects)</td>
<td>60</td>
<td>382.10</td>
<td>6.33</td>
</tr>
<tr>
<td>Within Cell</td>
<td>160</td>
<td></td>
<td>(2.82%)</td>
</tr>
<tr>
<td>(σ²_e1 – Trials)</td>
<td></td>
<td>73.96 (2.82%)</td>
<td>1.29 (25.79%)</td>
</tr>
<tr>
<td>σ²_e2 – Days</td>
<td></td>
<td>102.71 (3.92%)</td>
<td>1.68 (33.73%)</td>
</tr>
<tr>
<td>σ²_t – True</td>
<td></td>
<td>2443.5 (93.26%)</td>
<td>2.02 (40.48%)</td>
</tr>
<tr>
<td>Grand Mean</td>
<td></td>
<td>92.80 N</td>
<td>15.69 pps</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>20.1402 N</td>
<td>3.57 pps</td>
</tr>
<tr>
<td>R</td>
<td></td>
<td>0.99</td>
<td>0.79</td>
</tr>
</tbody>
</table>
Figure 13. The means (circles) and standard deviations (vertical bar) for maximal isometric wrist flexion force for each subject. The number below the vertical bar is the coefficient of variation for the individual subject.
Figure 14. The means (circles) and standard deviations (vertical bar) for maximal isometric dorsiflexion force for each subject. The number below the vertical bar is the coefficient of variation for the individual subject.
Figure 15. The means (circles) and standard deviations (vertical bar) for flexor carpi radialis motor unit discharge rate at 60 percent of maximal voluntary contraction (MVC) for each subject. The number below the vertical bar is the coefficient of variation for the individual subject.
Figure 16. The means (circles) and standard deviations (vertical bar) for tibialis anterior motor unit discharge rate at 60 percent of maximal voluntary contraction (MVC) for each subject. The number below the vertical bar is the coefficient of variation for the individual subjects.
CHAPTER V

Discussion

The purpose of this thesis was to evaluate the reliability of the motor unit discharge rate obtained by decomposition of the sEMG signal, over a longer period of time as would occur with an intervention study involving resistance training. The FCR and TA were studied during isometric contractions at 60% MVC. Maximal isometric strength during wrist flexion and dorsiflexion strength were assessed because they exhibit the classic characteristics of reliable measures and can serve as a gold-standard comparison. As expected, maximal isometric strength for both joint actions was relatively stable and highly consistent, and were found to have excellent reliability. The motor unit discharge rates for both muscles were found to be highly stable and consistent, but the intraclass correlation coefficients were moderate. The theoretical and practical applications of these findings will be discussed below.

Comparative Values

Force

Wrist Flexion

The wrist flexion force values were recorded in Newtons but are converted to Newton-meters using anthropometric measures obtained on the subjects, for the purpose of comparison to other studies. Conversion to Newton-meters (Nm) based on anthropometric measurements was not done originally, so as to not introduce any
additional measurement error. The grand mean maximal isometric wrist flexion torque in the present work was 10.20 ± 2.2 Nm, which is well within the range of values reported in the literature. Previous values reported include: 11.3 ± 3.0 Nm (Al-Eisawi, Kerk, & Congleton, 1998), 14.81 ± 5.2 Nm (Vanswearingen, 1983), 13.7 ± 3.5 Nm (Seo & Armstrong, 2008), and 15.9 ± 5.4 Nm (Green et al., 2015). Distinctly greater values have been reported by other investigators: 25 ± 6 Nm (Harbo, Brincks, & Andersen, 2012), 24.9 ± 5.9 Nm (Salonikidis et al., 2009), and 25.5 ± 6.1 Nm (Salonikidis, Amiridis, Oxyzoglou, Giagazoglou, & Akrivopoulou, 2011).

Wrist flexion strength is greatly affected by the biomechanics of the task. For example, higher values can be obtained when a handle is gripped versus placing an open hand against a flat surface and flexing the wrist, as occurred in this study. Performing wrist flexion with an open hand against a flat surface minimizes the additional involvement of the fingers (Hallbeck, 1994; Leger & Milner, 2000; Sanes, 1986). Other factors that can affect maximum isometric wrist flexion strength include static forearm position in pronation/supination and in flexion/extension, which can affect muscle length, posture, and moment arms resulting in large differences in wrist joint torque (Buchanan, Moniz, Dewald, & Zev Rymer, 1993; Gonzalez et al., 1997).

**Ankle Dorsiflexion**

Dorsiflexion force values do not require conversion to torque as the literature generally reports maximal isometric strength in Newtons. The grand mean maximal isometric dorsiflexion strength was 281.0 ± 78.8 N. Maximal isometric dorsiflexion observed in the present work is slightly greater than the following values reported in the
literature: 238.0 ± 53.0 N (Green et al., 2014), 225.9 N ± 43.54 N (Lenhardt, McIntosh, & Gabriel, 2009), 262.0 ± 19.0 N (Kent-Braun & Ng, 1999), and 251.0 ± 8.0 N (Patten & Kamen, 2000). The differences in maximum strength could be attributed to the apparatus or test position, but participants in the current study used the same apparatus and ankle position as Green et al. (2014) and Lenhardt et al. (2009). Thus, it is reasonable to conclude the current sample may be slightly stronger than in previous studies.

**Motor Unit Discharge Rate**

**Flexor Carpi Radialis**

To the best of our knowledge, there are no comparable studies on the FCR to evaluate motor unit discharge rate. Other forearm muscles will therefore be reviewed to offer a basis of comparison. The FCR had a motor unit discharge rate of 15.69 ± 2.21 pps at 60% MVC, which is slighter higher than previously reported for the extensor muscles during clinical assessment. Testing protocol for clinical (indwelling) EMG involves generating a 5 to 20% MVC for 20 to 30 seconds, to obtain stable motor unit discharge statistics. Using this protocol, Calder et al. (2008) reported that the extensor carpi radialis had a motor unit discharge rate of 14.98 ± 2.97 pps. Birch et al. (2000) observed a motor unit discharge rate of 13.2 ± 1.98 pps for the extensor carpi ulnaris for contractions between 6.7 and 10% MVC (Birch, Christensen, Arendt-Nielsen, Graven-Nielsen, & Sogaard, 2000). Considering the fact that the current study recorded motor unit discharge rate during a 60% MVC, a slightly higher value can be expected.
The tibialis anterior motor unit discharge rate at 60% MVC was 15.42 ± 2.53 pps, nearly identical to the FCR. Fortunately, motor unit variables in the tibialis anterior have been well studied using indwelling recordings. McNeil et al. (2005) recorded indwelling MU activity from the tibialis anterior at 40% MVC, and observed a motor unit discharge rate of 14.9 ± 1.1 pps. The difference in magnitude follows differences in MVC (McNeil, Doherty, Stashuk, & Rice, 2005). In contrast, Inglis et al. (2011) reported a markedly higher tibialis anterior motor unit discharge rate of 20.3 ± 0.8 pps at 50% MVC (Inglis, Howard, McIntosh, Gabriel, & Vandenboom, 2011). While no standard deviation was reported, Connelly et al. (1999) reported an even greater mean motor unit discharge rate of 23 pps, also at 50% MVC (Connelly, Rice, Roos, & Vandervoort, 1999). Patten and Kamen (2000) observed a mean motor unit discharge rate of 20.1 ± 0.45 (SEM) pps similar to Inglis et al. (2011) but it was during a higher contraction intensity; the same as was employed in the present work (60% MVC).

The tibialis anterior motor unit discharge rate observed in this study is within the same order of magnitude as indwelling studies, and easily explained by the method of calculation. Inglis et al. (2011), for example, used the instantaneous discharge rate, calculated as the inverse of the five shortest, consecutive interpulse intervals (IPIs) as described in Kamen and Du (1999). This method is referred to as the rectangular, or boxcar method and the number of consecutive IPIs can vary between two and five (Kamen & Du 1999; Liu, Bonato, & Clancy, 2016). Instantaneous motor unit discharge rates calculated directly from a few consecutive IPIs, can result in slightly higher values than those calculated from the Hanning window method, as done by the dEMG analysis.
software (version 1.1, Delsys, Inc., Boston, MA). The longer the Hanning window, the smoother the curve is for instantaneous motor unit discharge rate over time (De Luca & Mambrito, 1987). The smoother curve decreases the variability and can slightly lower values.

**Reliability Analysis**

**Force**

Walter Kroll (1962) was the first investigator to demonstrate that the measurement schedule, itself, could result in an increase in maximal isometric strength, in the absence of any resistive exercise training (Kroll, 1962). It was theorized that the measurement schedule resulted in motor learning, where participants acquired the “knack” of performing maximal isometric contractions. Kroll (1981) later termed measurement schedule effects, the “quick jumps in strength phenomenon” (Kroll, 1981). If motor learning effects can contaminate baseline strength, Kroll (1963) suggested that it is important to include one or more pre-test sessions to subtract-out increases in strength due to task familiarization. Motor learning related increases in strength due to the measurement schedule alone are also associated with alterations in sEMG activity (Gabriel, Basford, & An, 1997; Green, Christie, & Gabriel, 2017; Green et al., 2014; McGuire, Green, Calder, Patterson, & Gabriel, 2014a; McGuire, Green, & Gabriel, 2014a), which is indirectly related to motor unit activity pattern.

The present study required four test sessions to ascertain how many repeated measurements would be required to obtain stable baselines scores, for both strength and motor unit discharge rates. Recall, stability is a critical characteristic of a reliable
measure. The pattern of means across test sessions in the absence of any treatment is therefore important for assessing stability of the measure. Maximal isometric wrist flexion strength in the present work exhibited a total increase of 10.0%, across the four test sessions. There was a significant 6.3% increase between sessions one and two, with another non-significant 3.9% increase thereafter. In contrast, maximal isometric dorsiflexion flexion strength exhibited a significant increases across all four test sessions.

This thesis presents evidence that the number of pre-test sessions may need to be greater for large versus small muscles. McGuire et al., (2014a) showed that the maximal isometric elbow flexion force continued to increase until the fourth test session (McGuire, Green, Calder, Patterson, & Gabriel, 2014b). No training was allowed between test sessions, and only a total of 15 maximal isometric contractions were performed prior to strength assessment on the four sessions. What is even more remarkable is that there was a two-week rest period between the third and fourth test sessions. Two-weeks are normally considered a complete period of detraining, even if the few contractions had resulted in any adaptation (Mujika & Padilla, 2001). The present study observed the same effect in the tibialis anterior, but with an even longer interval (i.e., six-weeks) between the third and fourth test sessions.

McGuire and colleagues (2014a) examined the effect of massed and distributed contractions in the acquisition of maximal isometric strength through motor learning. One group of participants performed 15 maximal isometric contractions of elbow flexors in one session, while another group completed the same number across three test sessions. It was hypothesized that massed practice (fifteen contractions/day) allowed for better entrainment of an internal model of the resistive exercise task performance. It was
then demonstrated that additional consecutive days of testing then allows for refinement and consolidation of the internal model through distributed practice (McGuire, Green, & Gabriel, 2014b).

Thus, the optimal measurement schedule for strength assessment for an intervention study does not have a pre-determined number of trials per session, nor does it have a set number of pretest sessions. The reason is that the maximal isometric strength has a skill component, which extends beyond absolute strength, to performance of the task itself, as assessed by force variability. In support, Green and colleagues (2014) recently demonstrated that, because there is a learning-related component to maximal isometric strength, stabilization of task performance in terms of force and sEMG variability should also be assessed prior to beginning any intervention (Green et al., 2014). Because the intraclass correlation coefficient is sensitive to the differences in means and/or variances, removing an unstable baseline can have a profound effect on overall reliability. Kroll (1963), for example, recalculated the intraclass correlation coefficient with the first three consecutive days of maximal isometric wrist flexion strength testing subtracted-out from the second set of three consecutive days of testing. The intraclass correlation coefficient changed from $R = 0.91$ to $R = 0.99$.

The intraclass correlation coefficients for maximal isometric wrist flexion force ($R = 0.99$) and dorsiflexion force ($R = 0.94$) observed in the present study are comparable to what has been demonstrated before. Green et al. (2015) reported an intraclass correlation coefficient for maximal isometric wrist flexion of $R = 0.90$, while MacIntosh et al. (2012) observed an intraclass correlation coefficient $R = 0.98$ for maximal isometric dorsiflexion force. Thus, despite the slight decreases in stability, the reliability of
maximal isometric strength remains excellent, because it exhibits a high degree of consistency within subjects, as assessed by the intraclass correlation coefficient.

Recall that the intraclass correlation is based on a ratio of the different sources of variance under consideration. Because maximal isometric voluntary contractions truly assess the functional capacity of the muscle, individuals can be fully differentiated based on this test (Kroll, 1970), so that the between subjects sum of squares is at least 50% of the total variance (Calder & Gabriel, 2007; Green et al., 2015). The variance across days (stability) is, however, expected to be greater than the variance of trials within days. The day-to-day variability is thought to reflect biological variability in the absence of any treatment. There is no specific guideline, but day-to-day variability can be as great as 35% of the total variance, particularly when there has been a “quick jump in strength” as observed here (Calder & Gabriel, 2007; Green et al., 2015). Since the experimental conditions within a day should remain constant, the trial-to-trial variability of maximal isometric strength is thought to represent some combination of measurement error and random error in performance. Previous studies have demonstrated that it can be close to 10% of the total variance, but is generally much less (Calder & Gabriel, 2007; Green et al., 2015). It therefore makes sense that, when trial-to-trial variability comprises a major portion of the total variance, there is a decrease in the intraclass correlation coefficient.

**Motor Unit Discharge Rate**

Maximal isometric strength was presented first because it serves as a gold-standard measure in terms of reliability analysis. Increases in maximal isometric strength have been shown to be associated with changes in sEMG activity that mirror the patterns
of change in MVC (McGuire et al., 2014a, 2014b). The overall reliability of sEMG, while very good, is generally lower than maximal isometric strength because sEMG is affected by technical factors, unrelated to the expression of muscle force (Mathur, Eng, & MacIntyre, 2005; Merletti, Lo Conte, & Sathyan, 1995; Yang & Winter, 1983). Technical factors that affect sEMG measurement may also have an impact on motor unit discharge rates obtained by decomposition of the sEMG signal.

Since there was a significant increase in maximal isometric strength during wrist flexion and dorsiflexion, it is reasonable to expect a parallel increase in motor unit discharge rates of the flexor carpi radialis and tibialis anterior, respectively (Kamen & Knight, 2004; Patten & Kamen, 2000). The largest difference in motor unit discharge rates across test sessions occurred between sessions one and three; it was 5.2% ($p = 0.22$) for the flexor carpi radialis and 7.8% ($p = 0.06$) for tibialis anterior. When the Day main effect was evaluated with the correct error term (Days $\times$ Subjects), the increases were non-significant. It is important to keep in mind that changes in motor unit discharge rates were at 60% MVC, not 100% MVC. Maximal contractions may have resulted in greater changes in MUDRs.

Statistically significant versus practically important changes are better defined for maximal isometric strength than they are for the motor unit discharge rates. There is evidence that suggest the observed increases in motor unit discharge rates were not random fluctuations. First and foremost, there was a monotonic increase that plateaued by session three, mirroring increases in maximal isometric strength. Second, the changes observed in the present study are consistent with previous indwelling studies documenting the response of motor unit discharge rates, where participants were
administered two-pretest sessions consisting of maximal isometric contractions, prior to a training intervention (Kamen & Knight, 2004; Patten & Kamen, 2000). Patten and Kamen (2000), for example, measured motor unit discharge rates in the adductor digiti minimi in response to resistance training in six young adults. There was a significant, increase in mean maximal motor unit discharge rates of 11% between the two pre-test sessions, which compares well with the 7.8% increase for the tibialis anterior at 60% MVC.

The stability of motor unit discharge rates observed in the present study are on the same order of magnitude as Patten and Kamen (2000). It is important to emphasize that the comparison is based on repeated measurement in the absence of training; this is an important distinction, to truly assess inherent variability of the measure. The expected lack of stability in motor unit discharge rate was lower than that for maximal isometric strength, yet the overall reliability was considerably lower. The main reason for this was that the true score variance for motor unit discharge rates was dramatically lower (< 50% of the total variance) than for maximal isometric strength. The true score variance is based on the between subjects sum of squares, which reflects how different one subject is from another. Recall, one characteristic of a good measure is that it discriminates between subjects when differences “truly” exist. When the true score variance is low, it means that the scores of one subject overlap with those of another.

The question now arises: is it because there is a great deal of variability of scores within subjects? The coefficient of variation (CV) of the motor unit discharge rates scores within subjects ranged between 5 and 17, which is quite low for a sEMG measure (Green et al., 2015; McIntosh & Gabriel, 2012). Moreover, maximal isometric strength
generally had higher individual values for the coefficient of variation than motor unit discharge rate, but was still considered a more reliable measure. Inspection of Figures 11 to 14 reveals a subtler aspect of reliability analysis described in the literature review. The individual mean motor unit discharge rate was close in magnitude to the group mean (Figure 15 and 16). The opposite is true for maximal isometric strength (Figure 13 and 14). Even though the individual values for the coefficient of variation are greater for maximal isometric strength, the individual means have a broader distribution around the group mean. Thus, it is still easy to distinguish between individuals, and the overlap in scores is much less than that for motor unit discharge rates.

Figures 15 and 16 illustrate the case where the individuals within the sample are homogeneous. When individuals within a sample are homogenous, even though the variability of scores within subjects is low (high consistency), the scores between individuals will overlap. The between subjects sum of squares will be low, because the scores of subjects are close together. The true score variance and resulting intraclass correlation coefficient were therefore artificially deflated, even though subjects had very consistent scores. Taken all together, the stability and consistency results for motor unit discharge rates obtained by sEMG decomposition can be considered very reliable.

The reliability findings for motor unit discharge rate are consistent with the observations for muscle fiber conduction velocity, where the normal physiological values assume a very narrow range (McIntosh & Gabriel, 2012). McIntosh and Gabriel (2012) reported an intraclass correlation coefficient of \( R = 0.98 \) for maximal isometric dorsiflexion strength, but muscle fiber conduction velocity in the tibialis anterior was artificially deflated at \( R = 0.83 \). Submaximal motor unit discharge rates also have a
relatively narrow range of values at a given level of force (Connelly et al., 1999; Kirk, 1995). Based on the force-frequency curve for in vivo muscle electrical stimulation (Zuurbier, Lee-de Groot, Van der Laarse, & Huijing, 1998), it is reasonable to observe similar motor unit discharge rates, when participants are required to generate the same percentage of their own maximum contraction.

Even though homogeneous scores reduced the true score variance, the day-to-day (33-39%) error variances for motor unit discharge rates were considerably higher than for maximal isometric contractions (<19%), but consistent with what might typically be expected for sEMG measures (Green et al., 2015; McIntosh & Gabriel, 2012). The stability of the means across test sessions impacts the magnitude of the intraclass correlation coefficient through the “relative” contribution of the variance due to days, to the total variance. The day-to-day error not only reflects biological variability and measurement schedule effects (i.e., task learning), but also variations in environmental conditions (i.e., temperature and humidity) and electrode placement and replacement (Bell, 1993; Yang & Winter, 1983). Reliability analysis of voluntary versus evoked contractions have demonstrated that the largest contribution to day-to-day error in sEMG measurement is the way in which voluntary contractions are performed (Inglis et al., 2017), which ultimately affects motor unit activity patterns (Kamen & Knight, 2004; Patten & Kamen, 2000). Indirect support is given by the observation that, with careful methodological controls, the reliability of sEMG obtained during evoked contractions can be nearly as high as maximal isometric strength (Calder et al., 2005; Christie et al., 2010; Inglis et al., 2017)
The trial-to-trial error variance (18-25%) for motor unit discharge rate was considerably greater as percentage of the total variance, than it was for maximal isometric strength (<3%). However, the error variance due to trials was on the same order of magnitude for other sEMG measurements (Green et al., 2015; McIntosh & Gabriel, 2012). Since experimental conditions should remain constant throughout the duration of each test session, trial-to-trial error variance is conceptually thought to represent the random error of measurement (Kirk, 1995). The error variance due to trials for maximal isometric strength can provide insight to the same for motor unit discharge rates.

While the test position of each participant was strictly controlled and monitored, slight postural adjustments that resulted in trial-to-trial functions in maximal isometric strength would be associated with commensurate changes in underlying muscle activity (Rummel, 1974; Yang & Winter, 1983). The same is true for when participants focus their attention during the task, from one trial to the next (Marchant, Greig, & Scott, 2009; Vance, Wulf, Tollner, McNevin, & Mercer, 2004). Thus, the voluntary components of motor performance are believed to be the main source of random error. Indirect support for this hypothesis is given by the small (~5%) trial-to-trial error variance observed for evoked contractions (Calder et al., 2005; Christie, Inglis, Boucher, & Gabriel, 2005). Complicating matters further, strength assessment is affected by instructions, verbal encouragement, and visual feedback (Jung & Hallbeck, 2004), and unfortunately, not all participants respond similarly to these same methodological controls (Binboğa, Tok, Catikkas, Guven, & Dane, 2013).
Conclusions

Maximal isometric contractions of the wrist flexor and dorsiflexors resulted in a “quick jump in strength” due only to the measurement schedule. Increases in maximal isometric strength were associated with modest changes in motor unit discharge rates at 60% MVC. The majority of the changes occurred early in the measurement schedule (sessions 1 and 2). The slight decrease in stability in maximal isometric strength was compensated for by excellent consistency, as evidenced by high intraclass correlation coefficients ($R^2 \geq 0.94$). Changes in motor unit discharge rates mirrored maximal isometric strength, but the increases in means were non-significant due to submaximal testing. The consistency of motor unit discharge rates as revealed by the coefficients of variation for individual subjects was not reflected in the overall intraclass correlation coefficient for both muscles ($R = 0.79$). The true score variance, the most important component of the intraclass correlation, was low due to homogeneous scores, artificially deflating the coefficient. Homogeneous scores were due to the narrow range of motor unit discharges obtained when participants all performed isometric contractions at the same level of force (60% MVC). Motor unit discharge rates can be considered to be highly reliable in a healthy, college-age population over a longer period of time, as would occur with an intervention study. If broader application of non-invasive MUP detection is a goal, more research is necessary. The reliability observed in this thesis may not apply to other populations with greater subcutaneous tissue, altered MUDRs as would occur with aging, or with neuromuscular disorders where shape of MUP changes trial-to-trial.
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Appendix A: Research Ethics Board Clearance Certificate
Certificate of Ethics Clearance for Human Participant Research

DATE: 5/1/2018

PRINCIPAL INVESTIGATOR: GABRIEL, David - Kinesiology

FILE: 16-313 - GABRIEL

TYPE: Masters Thesis/Project

STUDENT: David Gabriel

SUPERVISOR: David Gabriel

TITLE: Validation of a surface EMG decomposition algorithm for motor unit identification

ETHICS CLEARANCE GRANTED

Initial Clearance Date: 5/24/2017
Type of Clearance: RENEWAL

Expiry Date: 5/1/2019

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

Renewed certificate valid from 5/1/2018 to 5/1/2019.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 5/1/2019. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Stephen Cheung, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.
Appendix B: Informed Consent Form
Title of Project: Reliability of surface EMG detected motor unit firing during maximal voluntary contractions

Principle Investigator: David A. Gabriel, Ph.D., FACSM
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This study has been reviewed and approved by the Brock Research Ethics Board (#XX-XXX). The Brock Research Ethics Board requires written informed consent from participants prior to participation in a research study so that they can know the nature and risks of participation and can decide to participate or not to participate in a free and informed manner. You are asked to read the following material to ensure that you are informed of the nature of this research study and how you will participate in it if you consent to do so. Signing this form will indicate that you have been so informed and that you give you consent.

Introduction

You are being asked to participate in research being conducted by David A. Gabriel, Ph.D. The on-going research program is focused on the relationship between skeletal muscle force and the electrical activity that it generates. The electrical signal of skeletal muscle is measured from the skin surface, similar to electrocardiography (EKG), which measures the electrical activity of cardiac (heart) muscle. The skeletal muscle electrical signal is termed, electromyography (EMG). The signal can then be analyzed in a number of different ways, and each method has an impact on the EMG-to-force relationship. For this reason, the data from this study will be retained for the purpose of identifying new signal processing techniques and methodologies, as well as for future external review of any publications resulting from this study. Data will be kept for the duration of my academic career, or until such time they are no longer useful to me.

The main purpose is to examine the mechanisms underlying the cross education rehabilitation technique. Cross education is the gain in strength in an untrained limb following a unilateral training program.

This study will investigate the cross education phenomenon by implementing a 6 week unilateral training program. The focus of the study will be the wrist flexors and ankle
Participants will be assigned to complete a training protocol on either their dominant wrist or ankle. Training can be completed outside of the laboratory using standard gym equipment. It will consist of 4 days/week using a weight at 80% of maximal strength for 3 sets of 10-12 reps.

Testing will be conducted on both arms (wrist flexion/extension) and both legs (ankle flexion/extension) regardless of the limb being trained. There will be 4 testing sessions: one familiarization session, a baseline session within 24-48 hours, a post-training session following 6 weeks of training, and a retention session following 6 weeks of detraining. Each testing session will last approximately 3 hours.

Upon completion of the 4 testing sessions, participants will receive $100 as compensation for their participation. It is important to remember that you are free to withdraw consent at any time without loss of access to any services or programs at Brock University to which you are entitled. Refusing or withdrawing from the study will not affect your current or future standing at Brock University.

Inclusion / Exclusion Criteria: the participant must…

• be age 18-30 years,
• be in overall good health,
• NOT have any neurological or orthopedic disorders,
• NOT be a current student of the investigators.

If you agree to participate in the study, you will be asked to complete a demographic questionnaire and the PAR-Q questionnaire. Your responses to the PAR-Q will determine if you are physically fit and can participate in a study that requires physical exertion. To participate in this study you will need to wear shorts and a t-shirt. During the first session, we need to take some preliminary measurements: age, height, weight, as well as length and circumference of the arms and legs.

Plan and Procedure

The following procedures will take place during each session:

1. First, the muscles of the right and left leg and arm will be prepared for exercise testing. Small areas on the belly of the tibialis anterior (muscle along shin bone), soleus (calf muscle), flexor carpi radialis (FCR, inner forearm muscle), and extensor carpi radialis (ECR, outer forearm muscle) will be shaved, lightly abraded and cleansed with alcohol. These areas correspond to the location of the electrodes that will be taped to the skin surface. The electrodes will measure the electrical activity of the muscles; similar to the more familiar electrocardiogram that measures the electrical activity of the cardiac (heart) muscles.

2. You will sit in a testing chair with your leg/arm secured in a jig designed to isolate dorsiflexion (upward flexion of foot) or wrist flexion (palm towards forearm) during an isometric (stationary, no movement at the joint) contraction. Adjustable straps on the chair will ensure stability and minimize extraneous movements. A load cell will
be attached to the footplate under the ball of the foot to record dorsiflexion force, or the hand bar to record wrist flexion force. The leg/arm not being tested will rest on a support. It is important not to hold your breath while strength testing.

3. Evoked potentials will be elicited at the peroneal nerve (leg nerve, accessed with a self-adhesive cathode on the skin at the head of the fibula bone) or median nerve (forearm nerve, accessed with a self-adhesive cathode on the skin at the elbow crease). An anode electrode will be placed on the other side of the joint opposite the cathode. An evoked potential (twitch) is a small amount of electrical stimulation being sent to the surface of the skin, which causes an involuntary twitch of the muscle. This stimulation is very brief (<1 ms) and feels like a short burst of ‘pins and needles’ at the location of stimulation, similar to when your foot or hand ‘falls asleep’. The stimulation level will be supramaximal to elicit a maximal M-wave contraction of the dorsiflexors/wrist flexors. This may take approximately 5 submaximal stimulations of increasing intensity for each of the muscles to determine a maximal stimulation level. Five (5) of these will be performed at the beginning of testing, and during and after each of the 3 maximal voluntary contractions. This will completed for both arms and both legs for a total of 44 per session.

4. For each limb you will perform 3 agonist (flexion) maximal isometric voluntary contractions, and 3 antagonist (extension) maximal isometric voluntary contractions. Each contraction will be 5-seconds in duration, separated by 1-minute of rest. There will then be 3 agonist ramp contractions to 60% of your maximal flexion force. Each contraction will be 18-seconds in duration, separated by 3-minutes of rest. A sample force trace (at approximately your maximal force) will be presented to you on a computer screen. You will be asked to track your force in a ramp (slow-rise) pattern to your maximal force, hold it steady for 6 seconds, followed by a ramp (slow-decline) back to resting position.

5. This will conclude the testing session. Before you leave the Electromyographic Kinesiology Laboratory, we will take a picture of your lower leg and forearm with the electrodes on, to be used for placement in the following sessions. These pictures will be deleted immediately following your completion of the study.

**Risks and Discomforts**

It is not possible to predict all possible risks or discomforts that volunteer participants may experience in any research study. Based upon previous experience, the present investigator anticipates no major risks or discomforts will occur in the present project.

1. **Skin irritation.** Skin irritation may result from mildly abrading the skin, cleaning the skin with alcohol, then applying surface electromyographic (sEMG) recording electrodes with electrolyte gel. Washing the electrolyte gel from skin surface and applying skin lotion immediately after the test session can minimize the irritation.
2. **Muscle soreness.** It is possible that you might experience slight muscle soreness within 48 hours of the test. If soreness does occur, it will be very mild and dissipate within 72 hours.

3. There are two possible risks associated with electrical stimulation in a healthy-able bodied population:

   a) The first concern is electrical safety which is maintained by grounding both the participant and laboratory equipment. Electrical safety is further enhanced by the use of an isolation unit that separates the participant from the stimulator.

   b) The second risk is that the participant perceives the electrical stimulus to the nerve as noxious, resulting in vasovagal syncope (i.e., fainting). If the electrical stimulation pads are placed correctly over the nerve, the actual physical discomfort is minimal. However, there is no way to predict how someone will respond subconsciously to the electrical stimulation. The student-investigator will constantly monitor the participant for how well the procedures are being tolerated and will discontinue the protocol if the participant expresses a desire to stop or if the initial signs of fainting are present. A participant has never fainted in the laboratory while following these guidelines. If fainting does indeed occur, the student investigator has been certified in CPR and first aid. Because this reaction is not under the control of the participant, they will be discontinued from further study.

4. **Systemic stress due to maximal exertion.** Maximal effort contractions are associated with an increase in blood pressure. You must make sure that you do NOT hold your breath during maximal exertions. If you have received medical clearance and/or are already physically active, the risks are minimal.

   *Understand that should any of these side effects occur, you are free to withdrawal from the study because of them. It is important to remember that you are free to withdrawal consent at any time without loss of access to any services or programs at Brock University to which you are entitled. The researchers’ first priority as an investigator is to maintain the emotional, psychological, and physical health of those participating in the study.*

**Voluntary Participation**

Participation in this study is voluntary. Refusal to participate will NOT result in loss of access to any services or programs at Brock University to which you are entitled. You will inform the investigator of your intention to withdrawal prior to removing yourself from this study.

**Potential Benefits**

Participants will receive no direct benefits from participating in this study. However, participants should know that their willingness to serve as a participant for this
experiment will help a Brock University researcher and other scientists develop new theories of exercise that will benefit individuals in the future.

**Costs and Compensation**

Upon completion of the four testing sessions participants will be compensated with $100.

**Discontinuation of Participation**

Participation in this research study may be discontinued under the following circumstances. The investigator, David A. Gabriel, Ph.D., may discontinue your involvement in the study at any time if it is felt to be in your best interest, if you not comply with study requirements, or if the study is stopped. You will be informed of any changes in the nature of the study or in the procedures described if they occur. It is important to remember that you are free to terminate your participation at any time, for any reason. Refusing or withdrawing from the study will not affect your current or future standing at Brock University.

**If you choose to discontinue participation in the study at any time: Do you agree to allow any data collected up to that point in this study to be used and retained for analysis of signal processing methods?**

- [ ] Yes, my data may be used for analysis and retained indefinitely for future analysis.
- [ ] No, I do not wish for my data to be used for analysis or retained for future analysis.

**Confidentiality**

Although data from this study will be published, confidentiality of information concerning all participants will be maintained. All data will be coded without personal reference to you. Any personal information related to you will be kept in a locked office, to which only the investigator has access.

**Data Retention**

The data from this study will be retained for the purpose of identifying new signal processing techniques and methodologies, as well as for future external review of any publications resulting from this study. Data will be kept for the duration of my academic career, or until such time they are no longer useful to me.

**Do you agree to allow your data from this study to be retained for future analysis of signal processing methods?**

- [ ] Yes, my data may be retained indefinitely for future analysis.
No, I do not wish for my data to be used for future analysis. (Note: data will be retained in case of future external publication review, but will not be re-analyzed in the future).

**Persons to Contact with Questions**

The investigator will be available to answer any questions concerning this research, now or in the future. You may contact the investigator, David A. Gabriel, Ph.D., by telephone during office hours at (905) 688-5550 extension 4362, or by email at dgabriel@brocku.ca. Also, if questions arise about your rights as a research subject, you may contact the Research Ethics Office at (905) 688-5550 extension 3035. If you wish to speak with someone not involved in the study, please call the Chair of the Department of Kinesiology at (905) 688-5550 extension 4538.

**Consent to Participate**

Certify that you have read all the above, asked questions and received answers concerning areas you did not understand, and have received satisfactory answers to these questions. Furthermore, you have completed the PAR-Q questionnaire indicating that you are physically able to participate. You willingly give consent for participation in this study.

**Name of Participant (Please Print):** ________________________________

______________________________  ________________________________

Signature of Participant  Date (day/month/year)

In addition to the considerations described in this document, the investigator fully intends to conduct all procedures with the subject’s best interest uppermost in mind, to ensure the subject’s safety and comfort.

I have fully explained the procedures of this study to the above volunteer. I believe that the person signing this form understands what is involved in this study and voluntarily agrees to participate.

______________________________  OR  ________________________________

Date (day/month/year)

______________________________  OR  ________________________________

David A. Gabriel, Ph.D., FACSM  Lara Green, MSc
*Department of Kinesiology*  *Faculty of Applied Health Sciences*
Appendix C: PAR-Q and Demographic Questionnaire
**PAR-Q Form** (to be filled out by participant)

Adapted from the Canadian Society for Exercise Physiology

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**PAR-Q+**

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor or a qualified exercise professional before becoming more physically active.

**SECTION 1 - GENERAL HEALTH**

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Has your doctor ever said that you have a heart condition or high</td>
<td></td>
<td></td>
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<tr>
<td>blood pressure?</td>
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<tr>
<td>2. Do you feel pain in your chest at rest, during your daily activities</td>
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<tr>
<td>of living, or when you do physical activity?</td>
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</tr>
<tr>
<td>3. Do you lose balance because of dizziness or have you lost consciousness</td>
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<tr>
<td>in the last 12 months? Please answer NO if your dizziness was associated</td>
<td></td>
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<tr>
<td>with over-breathing (including during vigorous exercise)?</td>
<td></td>
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<tr>
<td>4. Have you ever been diagnosed with another chronic medical condition</td>
<td></td>
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<tr>
<td>(other than heart disease or high blood pressure)?</td>
<td></td>
<td></td>
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<tr>
<td>5. Are you currently taking prescribed medications for a chronic</td>
<td></td>
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<tr>
<td>medical condition?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Do you have a bone or joint problem that could be made worse by</td>
<td></td>
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<tr>
<td>becoming more physically active? Please answer NO if you had a joint</td>
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<td></td>
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<tr>
<td>problem in the past, but it does not limit your current ability to be</td>
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<tr>
<td>physically active. For example, knee, ankle, shoulder or other.</td>
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<td></td>
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<tr>
<td>7. Has your doctor ever said that you should only do medically supervised</td>
<td></td>
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<tr>
<td>physical activity?</td>
<td></td>
<td></td>
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</tbody>
</table>
### SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you have Arthritis, Osteoporosis, or Back Problems?</td>
<td>If yes, answer questions 1a-1c</td>
<td>If no, go to question 2</td>
</tr>
<tr>
<td>1a. Do you have difficulty controlling your condition with medications or other physician prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b. Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolisthesis/pars defect (a crack in the bony ring on the back of the spinal column)?</td>
<td></td>
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</tr>
<tr>
<td>1c. Have you had steroid injections or taken steroid tablets regularly for more than 3 months?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Do you have Cancer of any kind?</td>
<td>If yes, answer questions 2a-2b</td>
<td>If no, go to question 3</td>
</tr>
<tr>
<td>2a. Does your cancer diagnosis include any of the following types: lung/brechogenic, multiple myeloma (cancer of plasma cells), head, and neck?</td>
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<tr>
<td>2b. Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?</td>
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<td></td>
</tr>
<tr>
<td>3. Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm</td>
<td>If yes, answer questions 3a-3e</td>
<td>If no, go to question 4</td>
</tr>
<tr>
<td>3a. Do you have difficulty controlling your condition with medications or other physician prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)</td>
<td></td>
<td></td>
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<tr>
<td>3b. Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)</td>
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<tr>
<td>3c. Do you have chronic heart failure?</td>
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<tr>
<td>3d. Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)</td>
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<tr>
<td>3e. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?</td>
<td></td>
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<tr>
<td>4. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre Diabetes</td>
<td>If yes, answer questions 4a-4c</td>
<td>If no, go to question 5</td>
</tr>
<tr>
<td>4a. Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)</td>
<td></td>
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<tr>
<td>4b. Have you any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?</td>
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<tr>
<td>4c. Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?</td>
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<tr>
<td>5. Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer’s, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)</td>
<td>If yes, answer questions 5a-5b</td>
<td>If no, go to question 6</td>
</tr>
<tr>
<td>5a. Do you have difficulty controlling your condition with medications or other physician prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)</td>
<td></td>
<td></td>
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<tr>
<td>5b. Do you also have back problems affecting nerves or muscles?</td>
<td></td>
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</tbody>
</table>
Please read the questions below carefully and answer each one honestly; check YES or NO.

<table>
<thead>
<tr>
<th>Question</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6. Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)</td>
<td></td>
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<tr>
<td>6b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?</td>
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<tr>
<td>6c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?</td>
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<tr>
<td>6d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?</td>
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<tr>
<td><strong>7. Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia</strong></td>
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</tr>
<tr>
<td>7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7c. Has your physician indicated that you exhibit sudden bouts of high blood pressure known as Autonomic Dysreflexia?</td>
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<tr>
<td><strong>8. Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event</strong></td>
<td></td>
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</tr>
<tr>
<td>8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8b. Do you have any impairment in walking or mobility?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8c. Have you experienced a stroke or impairment in nerves or muscles in the past 5 months?</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>9. Do you have any other medical condition not listed above or do you live with two chronic conditions?</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?</td>
<td></td>
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</tr>
<tr>
<td>9b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9c. Do you currently live with two chronic conditions?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.
PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed healthcare professional before becoming more physically active or engaging in a fitness appraisal and/or visit a qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better.
- You are pregnant – talk to your healthcare practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active or.
- You have health changes – please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 – DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

  The undersigned have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, healthcare provider, or other designee) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME __________________________________________ DATE __________________________

SIGNATURE _________________________________________ WITNESS _________________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ____________________________________________

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES

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CSEP approved Sept 12 2011 version
Appendix D: Questionnaire and Anthropometric Measurements
Demographics and Physical Activity Levels (to be filled out by participant)

Name ___________________ Gender _____ Age ________ Weight ________
Height _______ Dominant Hand: _______ Foot: _______ (determined by LPI)
Letter of Invitation _____ Consent Form Signed _______ PAR-Q+ _______
Preferred method of communication: phone / email __________________________

Physical Activity Level:

How many times per week / hours per session do you weight train? _______ x /
_______ hrs
What percentage of time do you weight train: upper body _____% lower body
_____%
Approximately, how long have you been weight training (years/months):
____________
How many times per week / hours per session do you do other activity: _____ x /
_____ hrs
What other activity are you participating in:
________________________________________
________________________________________________________________________

Anthropometric Measurements: Arm

Length: Forearm (olecranon process to malleolus) _______ cm
Hand Lever (base to middle finger) _______ cm
Circumference: Proximal (widest) _______ cm Distal (narrowest) _______ cm
**Anthropometric Measurements: Leg**

**Length:**
- Lower Leg (fibular head to lateral malleolus) ________ cm
- Foot Lever (medial malleolus to first metatarsal) ________ cm

**Circumference:**
- Calf (widest) ________ cm
- Soleus (directly under gastroc) ________ cm
**Lateral Preference Inventory**

**Handedness:** Which hand would you most often use to…

1. **Draw**  
   - ___Left  
   - ___Either  
   - ___Right  

2. **Throw a ball to hit a target**  
   - ___Left  
   - ___Either  
   - ___Right  

3. **Use an eraser on paper**  
   - ___Left  
   - ___Either  
   - ___Right  

4. **Remove (and pass) the top card when dealing from a deck**  
   - ___Left  
   - ___Either  
   - ___Right  

5. **Swing a tennis/squash/badminton racquet**  
   - ___Left  
   - ___Either  
   - ___Right  

**Footedness:** Which foot would you most often use to…

1. **Kick a ball to a target**  
   - ___Left  
   - ___Either  
   - ___Right  

2. **Pick up a pebble with your toes**  
   - ___Left  
   - ___Either  
   - ___Right  

3. **Step on a bug**  
   - ___Left  
   - ___Either  
   - ___Right  

4. **Step up onto a chair (first foot up)**  
   - ___Left  
   - ___Either  
   - ___Right  

5. **Write your name in sand**  
   - ___Left  
   - ___Either  
   - ___Right  

*Items 1-4 for handedness and footedness are from the Lateral Preference Inventory (Coren, 1993). Items #5 for handedness and footedness are common items used in preference inventories that have been added.*