A Thesis

entitled

Intersession Reliability of Quadriceps Corticospinal Excitability:

A Functional TMS Study

by

Kiana Marie Young

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Exercise Science

_____

Grant E. Norte, PhD, AT, ATC, CSCS Committee Chair

_____

David M. Bazett-Jones, PhD, AT, ATC, CSCS, Committee Member

_____

Amanda Murray, PhD, PT, DPT, Committee Member

_____

Dr. Amy Thompson, Interim Dean
College of Graduate Studies

The University of Toledo

May 2022

An Abstract of

Intersession Reliability of Quadriceps Corticospinal Excitability:
A Functional TMS Study

by

Kiana Marie Young

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the
Master of Science Degree in
Exercise Science

The University of Toledo
May 2022

Context: Altered corticospinal pathways contribute to quadriceps dysfunction following anterior cruciate ligament reconstruction (ACLR). To date, very few studies have explored the reliability of corticospinal excitability, as measured by transcranial magnetic stimulation (TMS), during functional tasks. Understanding the reliability of closed kinetic chain TMS measures can expand our knowledge of brain-to-muscle communication during activities of daily living by serving as a dynamic assessment tool to identify neuromuscular deficiencies that may relate to lower extremity injury in healthy, active individuals. **Objective:** To investigate the intersession reliability of TMS-derived outcomes of quadriceps corticomotor function during a single leg squat in healthy, active individuals. **Design:** A descriptive laboratory study with a test-retest design. **Subjects:** 18 healthy active females (21.83 $\pm$ 2.57 years, 166.40 ± 6.43 cm, 66.26 $\pm$ 14.38 kg). **Independent variable(s):** Time (day 1 and 14) and limb (dominant and non-dominant) were assessed. **Main Outcome Measure(s):** Active motor threshold (AMT) and normalized motor evoked potentials (MEPs) were assessed in the vastus medialis (VM).

Results: The dominant AMT produced the highest reliability ($ICC_{Consistency}$ = 0.734, $ICC_{Absolute}$ = 0.737, MDC = 6.77) with moderate agreement between sessions and acceptable degree of agreement through Bland-Altman plot analysis. However, the non-dominant AMT produced the lowest reliability ($ICC_{Consistency}$ = 0.179, $ICC_{Absolute}$ =0.188, MDC=18.39). Dominant ($ICC_{Consistency}$ = 0.179, $ICC_{Absolute}$ =0.188, MDC=18.39) and non-dominant ($ICC_{Consistency}$ = 0.249, $ICC_{Absolute}$ =0.238, MDC=15.53) MEPs had poor reliability. **Conclusion:** Although previous literature established that TMS is reliable when performing open kinetic chain tasks in the lower extremity, only the dominant limb AMT was moderately reliable in our study. We found that non-dominant AMT and bilateral MEP measurements have poor intersession reliability over a two-week period. Future research is likely warranted to improve the standardization of this technique prior to incorporating in outcomes research. **Word Count:** 290

Acknowledgements

I would like to first thank my advisor Grant Norte for his guidance, innovation, and support throughout the last two years. I would like to sincerely thank Dr. David Bazett-Jones and Dr. Adam Lepley for selflessly giving their time to serve on my committee. Last but most important, I would like to express my infinite gratitude to Justin Rush. This project would have been impossible without your endless encouragement, assistance, and mentorship. I cannot forget to acknowledge my Trini and US family and friends, especially Reann, Shalini, and Shelley, for always being there even on the tough days.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACL	Anterior Cruciate Ligament
ACLR	Anterior Cruciate Ligament Reconstruction
AMT	Active Motor Threshold
EMG	Electromyography
fTMS	Functional Transcranial Magnetic Stimulation
ICC	Intraclass Correlation Coefficients
LOA	Limit(s) of Agreement
MDC	Minimal Detectable Change
MEP	Motor Evoked Potential
MTAT	Motor Threshold Assessment Tool
MVIC	Maximal Voluntary Isometric Contraction
RMS	Root-Mean Square
RPE	Rate of Perceived Exertion
SEM	Standard Error of Measurement
SENIAM	Surface Electromyography for the Non-Invasive Assessment of Muscles
TMS	Transcranial Magnetic Stimulation
VM	Vastus Medialis

Chapter One

Intersession Reliability of Quadriceps Corticospinal Excitability: A Functional TMS Study

Introduction

Anterior cruciate ligament (ACL) injuries are common especially in females, who are at a 4 to 6 times greater rate than males to sustain an injury during high risk sports.[1] The quadriceps are important for physical performance during functional tasks, especially following an ACL injury.[2] This muscle group is essential for load distribution, shock attenuation, and force generation at the knee joint.[3] Quadriceps neuromuscular deficits occurs after an ACL injury.[4-7] One pathway that is frequently affected by ACL injury and reconstruction (ACLR) is the corticospinal tract. This pathway conducts motor impulses from the primary motor cortex down to the desired muscle, which has previously been referred to as corticomotor function. While quadriceps impairments are common, literature proposes that altered neural pathways may contribute to quadriceps dysfunction,[8] thus offering potential for novel assessment and intervention. For example, lesser corticospinal excitability is associated with lesser quadriceps voluntary activation[9] and rate of torque development,[10] contributing to quadriceps strength deficits and poor biomechanics with functional movements in individuals with ACLR.

Transcranial magnetic stimulation (TMS) is used to investigate corticomotor function by non-invasively stimulating the brain with a rapidly changing magnetic field delivered to the scalp through a coil.[11, 12] Single-pulsed TMS is used to measure active motor threshold (AMT) and motor evoked potential (MEP) amplitude by measuring the

electromyography (EMG) response following an external stimulus delivered to the motor cortex.[13] MEP amplitude indicates the strength of corticospinal projections to peripheral muscle[14] while AMT reflects the ability to excite descending neurons to generate a motor response.[15] Together, TMS outcome measures reflect the excitability of corticospinal pathway.[16] Individuals with ACLR have previously demonstrated increased AMT as well as decreased MEP amplitudes.[15] This indicates that there is a neural/brain component to muscle dysfunction as a larger stimulus is needed to excite the corticospinal pathway to relay information from the brain to the quadriceps to produce a voluntary muscle contraction.[12, 15, 17] Thus, there is lesser corticospinal excitability after injury, which implies that there is a neural component to muscle dysfunction, resulting decreased functional performance and possibly increased risk of re-injury.

Previous studies have found that AMT and MEP are reliable in assessing the integrity of the corticospinal tract during lower extremity muscle isometric contraction in healthy individuals.[17-21] However, to date, very few studies[17-20, 22] explore the reliability of TMS-derived indicators of corticomotor function during the performance of functional tasks, exclusively in open kinetic chain knee extension, which does not accurately represent the functional role of corticomotor excitability. Understanding the reliability of closed kinetic chain TMS measures can expand our knowledge of brain and muscle communication during sport-specific activities by serving as a dynamic assessment tool to identify another neuromuscular risk factor for lower extremity injury in healthy, active individuals. Therefore, the purpose of this study was to investigate the intersession reliability of quadriceps corticospinal excitability outcome measures (AMT and MEP amplitude) in the dominant and nondominant limbs during a single leg squat in healthy,

active females over 14 days. We hypothesized that during the performance of a single leg squat, AMT would have strong intersession reliability in the quadriceps, but MEP would have moderate intersession due to its high variability. [17]

Methods

Study design. A descriptive laboratory study with a test-retest design was used to investigate the reliability of TMS-derived outcome measures during a single leg squat in healthy females. Participants reported to the laboratory at the same time of day on two separate days and were tested with an identical protocol during each testing session. The independent variables were time (day 1 and day 14) and limb (dominant and non-dominant). The dependent variables were AMT and normalized MEP amplitudes at 120% AMT intensity.[10, 13]

Participants. 18 healthy volunteers were enrolled in this study. All participants were recruited from the University population and the local community. Volunteers were included if they were females, aged 18 to 30 years old and reported a score of 5 or greater on the Tegner Activity Scale. All volunteers were screened based on eligibility criteria and for the use of TMS.[23] Exclusion criteria consisted of participants that were pregnancy, had metal implants, intake of stimulants/medication that alters neural excitability, had a history of neurological or muscular disorder, and had any history of lower extremity injury within the last 12 months, lower extremity surgery, brain surgery, stroke, migraine, severe head injury, documented concussion within the last 12 months, and personal or familial seizure and/or epilepsy. All participants provided written and verbal informed consent for this

study, which was approved by the University of Toledo Institutional Review Board for Biomedical Research.

Instrumentation. Knee extension torque data were collected using a stationary isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, Inc., Shirley, NY) and digitized at 125 Hz via 16-bit data acquisition system (MP160, Biopac, Inc., Goleta, CA). Surface electromyography (EMG) of the vastus medialis (VM) was recorded using pre-gelled Ag-AgCl electrodes (EL503) in accordance with published guidelines, sampled at 2000 Hz, amplified at a gain of 1000, and bandpass filtered from 10-500 Hz. Force and EMG data were visualized in AcqKnowledge software (v. 5.0, Biopac, Inc.). A 2T magnetic stimulator (Magstim BiStim^2, Magstim Company, Ltd., Whitland, UK) with a 110 mm double cone coil was used to assess measures of corticospinal excitability.[10] All analyses and data visual aids were accomplished using SPSS Statistics (Version 25, IBM, Armonk, NY, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) respectively.

Procedures. The vastus medialis was tested on the dominant and nondominant limb. The dominant leg was determined by asking the subject which leg they would use if they were to kick a ball.[24, 25] The order in which limb was tested first was randomly assigned on the first day of testing and the order was maintained for the following testing session. The vastus medialis was identified as 80% of the distance from the anterior superior iliac spine and the anterior border of the medial ligament, according to Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines.[26]

EMG preparation. The participants' skin was shaved, debrided, and cleansed with an alcohol wipe to minimize impedance. Two electrodes were each placed on the prepared vastus medialis directly below and above the identified 80% vastus medialis. Another two electrodes were placed on the proximal tibia as the ground as shown in Figure 1.

Figure 1: EMG placement

Maximal Voluntary Isometric Contraction and Quadriceps EMG Activation. Participants were seated in a stationary dynamometer with the hips and the tested knee flexed at 85 degrees and 70 degrees[27] respectively. The participants were secured with straps to restrict excessive movement at their shoulders, lap, and ankle of the respective test limb. Participants were given a familiarization period by kicking against the stationary arm by kicking with 25%, 50%, 75% and 100% of their perceived maximal effort. To determine their MVICs, participants were instructed to extend the knee by kicking out as hard and fast as possible for three to five seconds[10] for three trials. The torque tracing was displayed on a TV monitor for visual feedback and verbal encouragement was provided by the investigator to foster maximum effort.[17] The peak torque was recorded during the three MVIC trials. The average of the middle one-second of the processed EMG signal of each MVIC was identified and 10 and 15 percent of the average were calculated. On the torque

tracing window of the proximal vastus medialis muscle activity, 10 and 15 percent MVIC threshold lines were displayed to provide visual aid to standardize the muscle activity for TMS derived outcome measurements.[7]

"Hot Spot" Mapping. We unfasted the shoulder and lap straps of the dynamometer and placed a swim cap (Adult Lycra Cap, Oceano, CA) over their heads and earplugs in their ears to muffle any external noise.[25] We ensured that horizontal line drawn on the swim cap was aligned with the participants' tragi and that the vertical line was aligned from the nasal bone to the occipital protuberance.[22] The participants were instructed to kick between the 10-15% MVIC threshold lines, as indicated by the visual feedback on the TV screen, and a stimulation of 50% TMS intensity was applied via the double cone coil to evoke an MEP. The coordinates on the cap were used to locate the spot where the largest MEP was produced, which was "the hot spot". This coordinate was utilized to determine the AMT during the functional task.

Figure 2: Patient positioning for Hot Spot mapping

Functional TMS (fTMS). The participants were removed from the dynamometer and repositioned to stand facing a TV monitor 2 m away. The previously established 10% and 15% MVIC threshold lines of the processed proximal vastus medialis EMG signal

were displayed on the monitor. Participants were instructed to place their hands on their hips, transfer their weight to the test limb with non-test limb extended forward, and perform a single leg squat until their muscle activity was within the threshold lines. The participants descended to the appropriate muscle activation for 2 seconds prior to the stimulus being delivered. After each stimulus was delivered, participants reset to a double-limb stance. The investigator stood on a 0.37 m step behind the participant, holding the coil, and administered a single TMS pulse when the participant paused and maintained the required muscle activity. The TMS Motor Threshold Assessment Tool (MTAT), obtained online from http://www.clinicalresearcher.org/software.html, was used to determine the AMT. For each single leg squat, the TMS intensity was changed to the percentage determined by the TMS MTAT[28] until the lowest intensity able to produce a measurable MEP was obtained, representing the AMT for the test limb.[8] Immediately after obtaining the AMT, the stimulus intensity was increased to 120% of the AMT. Participants continued to perform single-leg squats until eight measurable MEPs were recorded at 120% of the AMT. A measurable MEP was defined as the peak-to-peak amplitude of the EMG waveform recorded after TMS stimulus, which corresponded with the latency of the previous trial. A 2D camera (iPod, Apple Inc., Cupertino, CA) was placed on a 0.8m tripod, 2.01m away from the participant's test limb to record the participants' peak knee joint angle during each successful single leg squat to document the amount of knee flexion required to achieve 10-15% muscle activity. After each limb was tested, the participant's rate of perceived exertion (RPE) was noted.

Figure 3: Patient positioning for functional TMS testing

Data Processing. The recorded MEP was normalized to the root-mean square (RMS) EMG, which was measured as the mean amplitude of 100 ms immediately prior to the stimulus.

Statistical Analysis. Intraclass Correlation Coefficients (ICC) for internal consistency ($ICC_{Consistency}$) and absolute agreement ($ICC_{Absolute}$)[29] were used to assess intersession reliability for each TMS-derived outcome measure in the dominant and non-dominant vastus medialis. ICCs were computed following removal of outliers following analysis of the correlation. Outliers were identified as being greater than two standard deviations away from the group mean. Reliability coefficients were interpreted as: poor (<0.5), moderate (0.5-0.75), good (0.75-0.9), and excellent (≥0.90).[29] Standard error of measurement (SEM) was calculated to examine any variation in results. The minimal detectable change (MDC) score was calculated to identify the 95% confidence level of a change occurring beyond that which could be associated with measurement error. Bland-Altman plots were used to investigate the absolute agreement between sessions.[30] The peak knee flexion angle of the 18 participants during each single squat at 120% AMT and the rating of perceived exertion after testing each leg were calculated for descriptive data (mean and standard deviation) and compared between sessions using dependent t-tests.

Results

Participants. Eighteen female participants were recruited between the ages of 18 and 30. Participants' demographic data is described in Table 1. All participants completed all testing sessions, creating a dropout rate of 0%. On day 1, participants obtained a knee flexion joint angle of 28.61 ± 10.42° on the dominant limb and 27.42 ± 9.67° on the non-dominant limb. We saw similar knee flexion angles on day 14 (dominant = 27.68 ± 8.33, non-dominant = 29.47 ± 8.28). There were no significant differences between day 1 (dominant = 8.36 ± 1.43; non-dominant = 8.08 ± 1.41) and day 14 (dominant = 7.33 ± 1.29, non-dominant = 7.33 ± 1.16) in participants' self-reported RPE.

Table 1: Participant Demographics

Participants (n=18)	Mean ± SD
Age (years)	21.83 ± 2.57
Height (cm)	166.40 ± 6.43
Mass (kg)	66.26 ± 14.38
Dominant limb	18 Right/0 Left
Tegner	5.83 ± 1

Abbreviations: cm, centimeters; kg, kilograms; Tegner, Tegner activity scale

Active Motor Threshold. All subject data were used to calculate the AMT ICCs and Bland-Altman plots at the day 1 and day 14 time points. All measures of reliability can be found in Table 2. The dominant VM AMT demonstrated moderate reliability ($ICC_{Consistency}$ = 0.734, $ICC_{Absolute}$ = 0.737, $p < 0.001$), and all data points fell within the limits of agreement (LOA) in the Bland-Altman plot (Figure 4). The non-dominant VM AMT ($ICC_{Consistency}$ = 0.382) was lower than the dominant limb ($ICC_{Consistency}$ = 0.734) and had one Bland-Altman data point fall outside of the LOA (Figure 5).

Motor Evoked Potential. Data from 17 subjects were used for analysis of the MEPs collected at 120% of AMT at the day 1 and day 14. Both ($ICC_{Consistency}$ = 0.179, $ICC_{Absolute}$ = 0.188, p=0.349), and non-dominant ($ICC_{Consistency}$ = 0.249, $ICC_{Absolute}$ = 0.238, p=0.287), VM muscles demonstrated poor reliability. The dominant VM Bland-Altman plot displayed one data point outside the limits of agreement (Figure 6) whereas the non-dominant VM displayed two one data point outside the limits of agreement (Figure 7).

Table 2: Intersession Reliability of TMS-derived Outcomes Over 2 Weeks

Measure	n	ICC (Consistency)	95% CI		ICC (Absolute)	95% CI		p-value	SEM	MDC
			Lower	Upper		Lower	Upper			
Dom AMT	18	0.734	0.42	0.89	0.737	0.42	0.89	< 0.001	2.44	6.77
Dom MEP	17	0.179	-1.27	0.70	0.188	-1.44	0.71	0.349	6.63	18.39
Non AMT	18	0.382	-0.09	0.713	0.357	-0.07	0.69	0.053	4.58	12.70
Non MEP	17	0.249	-1.07	0.73	0.238	-0.93	0.72	0.287	5.57	15.43

Good
Moderate
Poor

Abbreviations: AMT 120%, Active Motor Threshold at 120% intensity; Dom, dominant limb; ICC, Interclass Correlation Coefficient; MEP, Motor Evoked Potential; n, number of participants included in analysis; Non, non-dominant limb; VM, vastus medialis

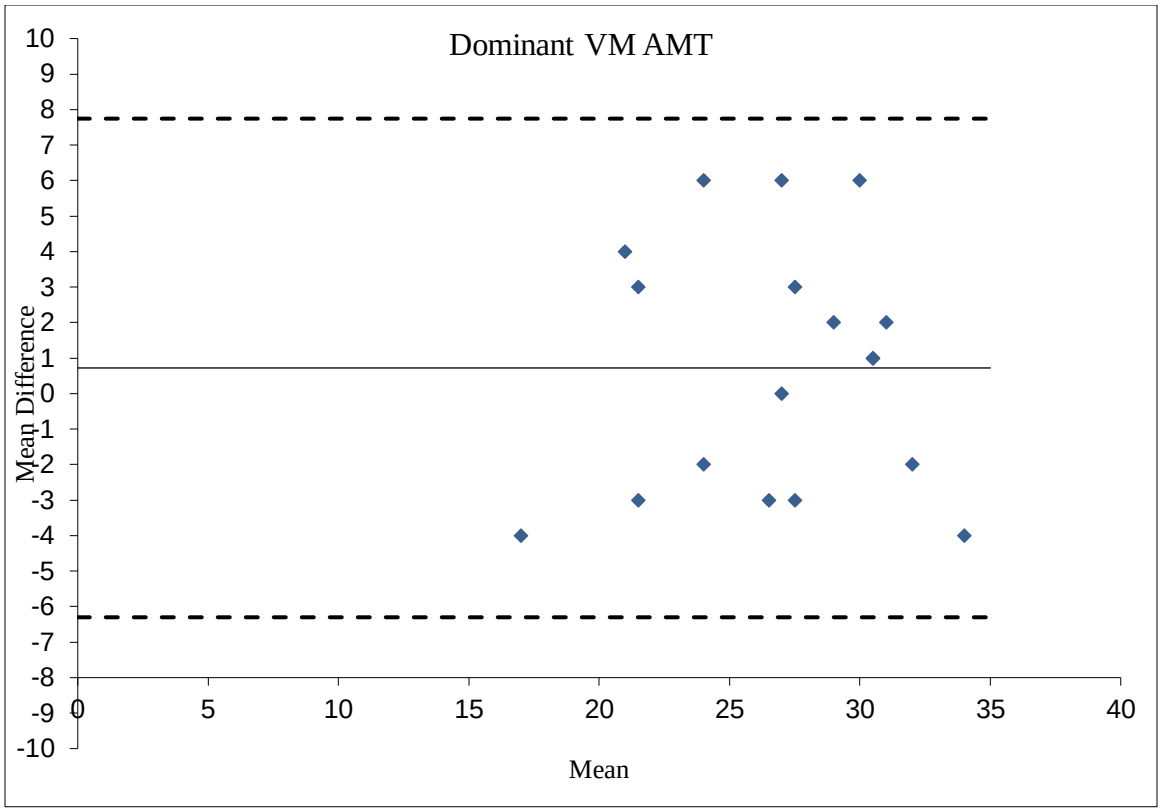


Figure 4: Dominant Vastus Medialis Active Motor Threshold Bland Altman plot. The solid line represents the mean difference of all AMT values between the 14 days. The dotted lines represent the LOA, which represent two standard deviations from the mean AMT.

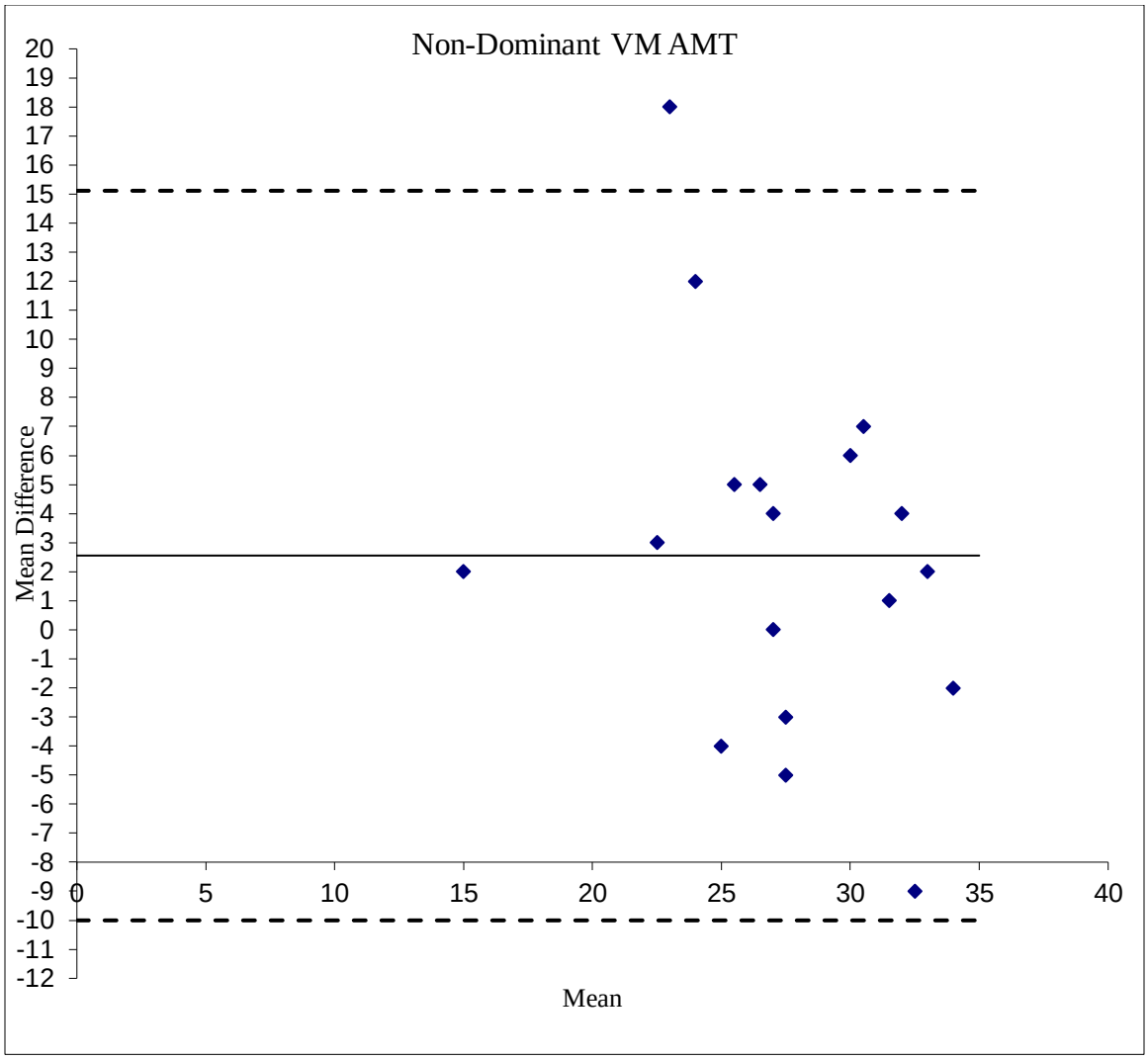


Figure 5: Non-Dominant Vastus Medialis Active Motor Threshold Bland Altman plot. The solid line represents the mean difference of all AMT values between the 14 days. The dotted lines represent the LOA, which represent two standard deviations from the mean AMT.

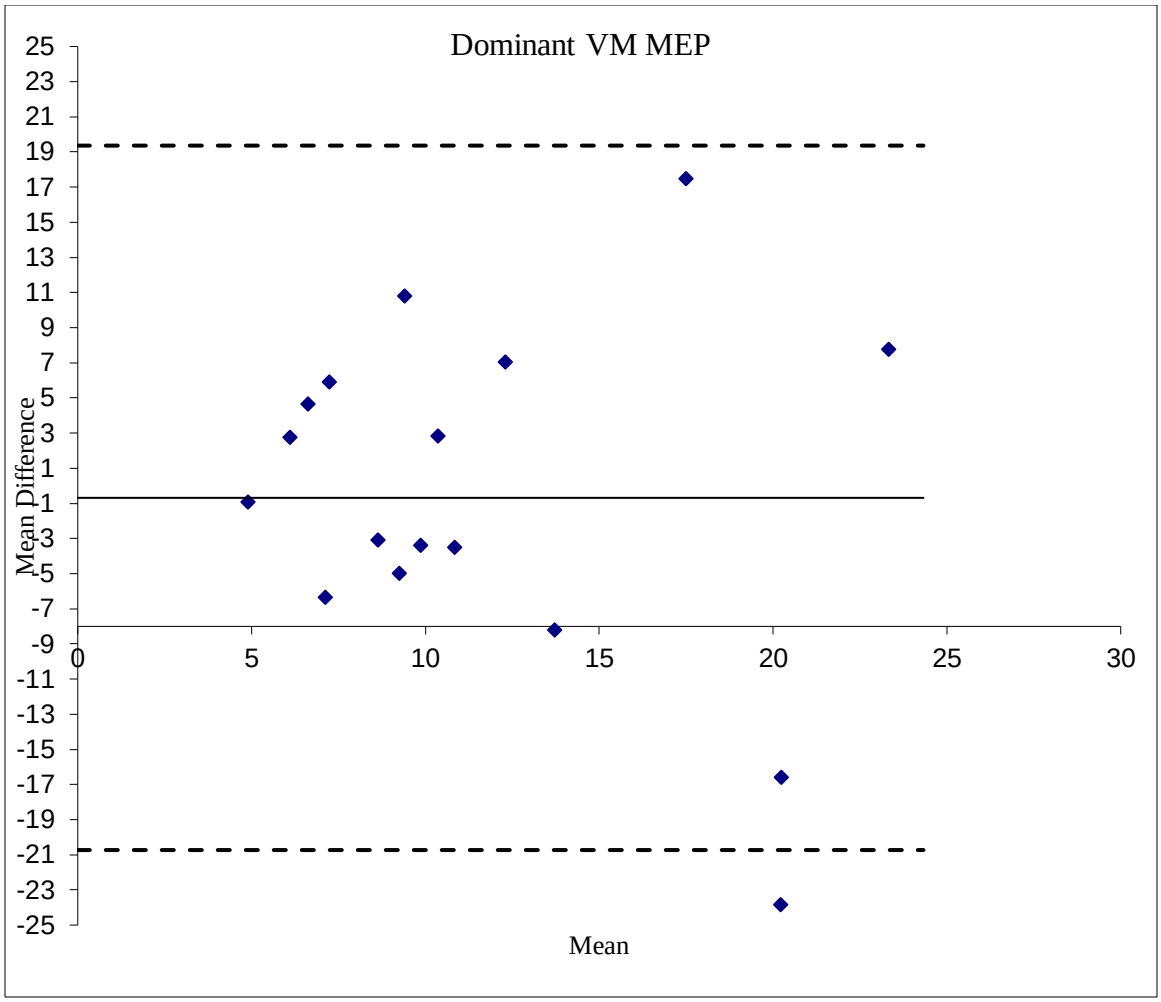


Figure 6: Dominant Vastus Medialis Motor Evoked Potential Bland Altman plot. The solid line represents the mean difference of all MEP values between the 14 days. The dotted lines represent the LOA, which represent two standard deviations from the mean MEP.

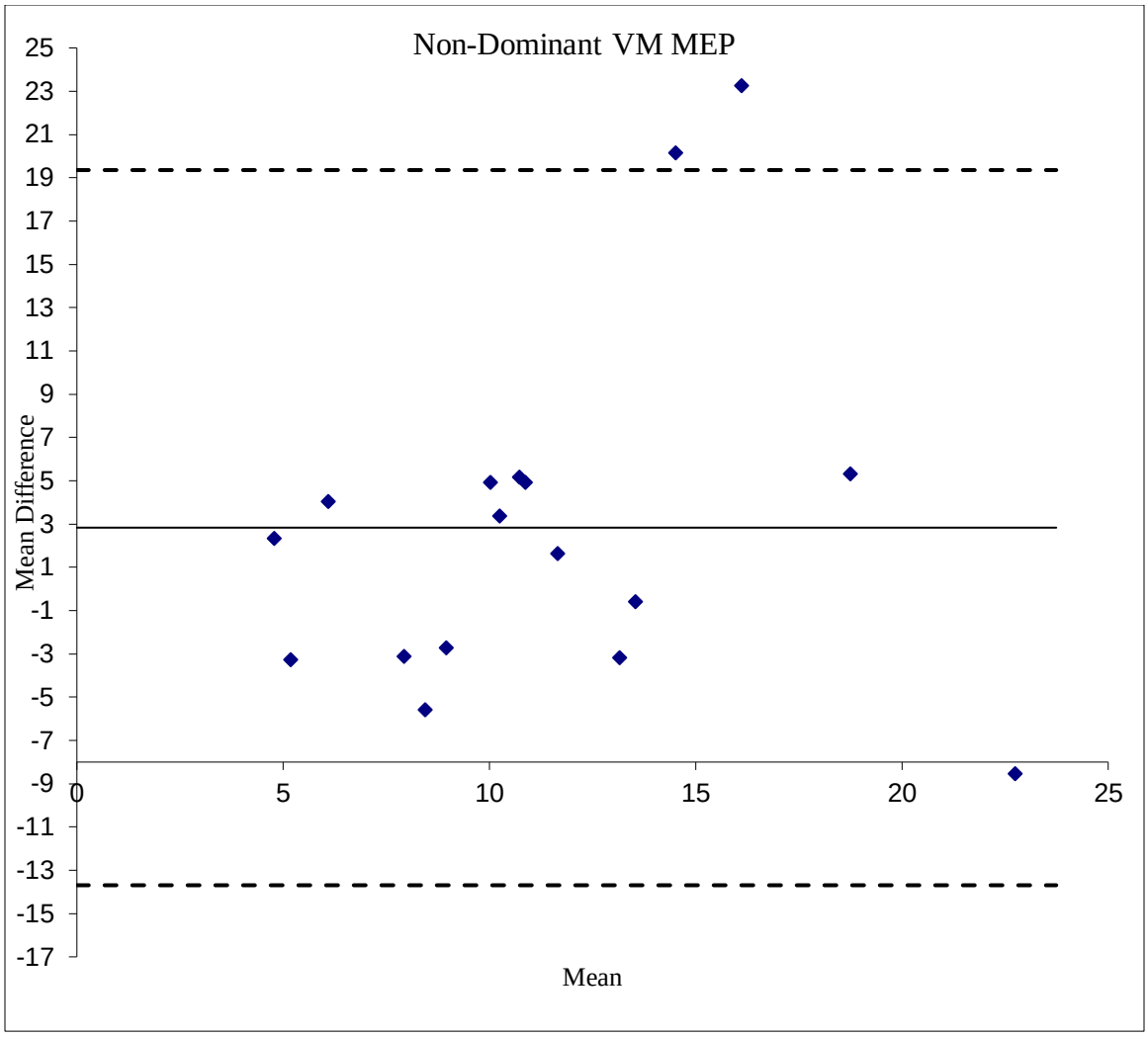


Figure 7: Non-Dominant Vastus Medialis Motor Evoked Potential Bland Altman plot. The solid line represents the mean difference of all MEP values between the 14 days. The dotted lines represent the LOA, which represent two standard deviations from the mean MEP.

Discussion

The aim of this study was to determine the intersession of reliability of quadriceps corticomotor function using TMS derived outcome measures (AMT and MEP amplitude) during a single leg squat. Our primary finding was that healthy active females' quadriceps AMTs of the dominant and non-dominant limb demonstrated moderate and poor intersession reliability, respectively. Additionally, the normalized MEPs at 120% AMT of the dominant and non-dominant limbs demonstrated poor intersession reliability. These results somewhat conflicted with our *a priori* hypotheses.

Previous research has demonstrated that corticospinal excitability measures in the quadriceps are reliable via acceptable ICC values and a good fit within Bland-Altman plots, which collectively gauge the degree and strength of associations.[17] Our results contradicted what Proessl et al. found that TMS-derived outcome measures are more variable with a single leg task than a double limb task, which had greater test-retest reliability.[22] There was varied reliability between limb corticospinal excitability during fTMS data collection. For our AMT, only the dominant limb was reliable seen with its moderate ICC values and all data points within a small range on a Bland-Altman plot. However, contrary to the reliability findings from a squatting task[22], our MEP data from the single leg squat had poor ICC values and data outside of the limits of agreement of the Bland-Altman plots. The stronger reliability in the dominant limb during the single leg squat could be attributed to better neuromuscular control and balance with use of the dominant leg. Although Takeno et al. researched upper extremity muscles[31], their study supported that AMT can be influenced by limb dominance.

Active Motor Threshold. The ICC fTMS calculation revealed that the dominant VM had the higher degree and strength of association between AMT measures over a two-week period. Additionally, the dominant VM AMT was statistically significant with an acceptable MDC of 6.765. Scheurer et al. estimated a minimal detectable change range of 2.9–7.5% for quadriceps AMTs measured in an open-chain to represent a physiological difference.[10] Thus, our results suggest that the dominant AMT is sufficiently sensitive to be a reliable metric to assess the corticospinal excitability. However, the non-dominant VM produced the poorest reliability and a higher percentage of data points outside of the limits of agreement in the Band Altman plot, indicating that a large range between limits of agreement. This magnitude of difference in reliability between limb dominance was not previously reported in a seated knee extension.[17] Luc et al. found moderate to strong reliability in both limbs during this open kinetic chain task.[17] Furthermore, leg dominance affects motor behavior during an unstable task such as balancing.[32] The large variation in non-dominant AMT can possibly be attributed to the unfamiliarity with using the non-dominant limb to perform functional closed kinetic chain tasks. A single leg squat is a balance and strength task, which requires more motor control and increased corticospinal excitability of the quadriceps to recruit more motor neurons to complete the functional movement.[33]

Motor Evoked Potentials. All normalized MEP at 120% AMT data had poor ICC statistics. The dominant MEPs were as reliable as the non-dominant MEPs. Both dominant and non-dominant quadriceps revealed MDC values greater than 5, indicating that a larger sample size and greater effect sizes may be required to detect changes in MEPs. All Bland-

Altman plot analyses for all MEPs revealed wide LOA ranges with more than 5% of data points falling outside of the LOA, compared to the previously recorded AMTs. Also, the non-dominant VM had several data points close to the mean displayed on the Bland-Altman plot analysis. Unlike our normalized MEP amplitude, MEPs were formerly found to be a reliable measure during seated knee extension[17] and a double limb squat[22]. Our results agreed with a previous study which demonstrated that corticospinal excitability is sensitive to functional movement and can influence neuromuscular activity.[22] It has been hypothesized that TMS- derived outcomes measures is influenced by body position and type of task.[33, 34] Furthermore, another factor with could contribute the MEP variability was muscle length as corticospinal excitability differs at various muscle lengths.[35] Therefore, MEP amplitude is not a reliable metric to assess the corticospinal excitability.

Limitations. During the fTMS assessment, we did not record the muscle activity of the antagonists or synergists muscles at the hip, knee, and ankle.[22] That unaccounted muscle activity during the single leg squat could influence the reliability of the MEP values. Additionally, although we instructed participants to maintain a consistent schedule during two weeks of testing, it was difficult to control their activities of daily living between testing sessions. Any new change such as less sleep, decreased activity, increased caloric intake, increased stress, and increased caffeine intake could account for the variability in the TMS derived outcome measures. Furthermore, with repetitive testing, participants may have physiological muscle fatigue despite reporting a subjectively low RPE level of exertion score.

Clinical Significance. Our findings show that dominant AMT measures can be useful in outcomes-based research. Measuring corticospinal excitability during a functional task provides insight into corticospinal neural activity involved in the brain and muscle communication during everyday physical activity. Closed kinetic chain TMS measurement of the dominant limb can serve as a more dynamic assessment tool to identify another neuromuscular risk factor for lower extremity injury in healthy, active individuals. When investigating the clinical implications of fTMS, future research should control more variables to increase the reliability of a functional task.

Conclusion

This study aimed to determine if quadriceps corticospinal excitability outcome measures in the dominant and nondominant limbs are reliability during a single leg squat in healthy, active females over a 14-day period. Unlike previous literature which established that quadriceps TMS-derived outcomes are reliable during a seated knee extension[17, 22] and a double limb squat[33], only dominant vastus medialis AMT was moderately reliable, which may provide more insight into corticomotor function during activities of daily living. However, we also found that non-dominant AMT and bilateral MEP measurements were not reliable metrics to assess the corticospinal excitability. Accordingly, future research is likely warranted to improve the standardization of this technique prior to incorporating in outcomes research. .

References

1. Hewett TE, Myer GD, Ford KR, et al. Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes: a prospective study. *Am J Sports Med*. Apr 2005;33(4):492-501. doi:10.1177/0363546504269591
2. Hunnicutt JL, McLeod MM, Slone HS, Gregory CM. Quadriceps Neuromuscular and Physical Function After Anterior Cruciate Ligament Reconstruction. *Journal of Athletic Training*. 2020;55(3):238-245. doi:10.4085/1062-6050-516-18
3. Griffin LY, Albohm MJ, Arendt EA, et al. Understanding and preventing noncontact anterior cruciate ligament injuries: a review of the Hunt Valley II meeting, January 2005. *Am J Sports Med*. Sep 2006;34(9):1512-32. doi:10.1177/0363546506286866
4. Pietrosimone BG, McLeod MM, Lepley AS. A theoretical framework for understanding neuromuscular response to lower extremity joint injury. *Sports Health*. Jan 2012;4(1):31-5. doi:10.1177/1941738111428251
5. Ingersoll CD, Grindstaff TL, Pietrosimone BG, Hart JM. Neuromuscular consequences of anterior cruciate ligament injury. *Clin Sports Med*. Jul 2008;27(3):383-404, vii. doi:10.1016/j.csm.2008.03.004
6. Pamukoff DN, Pietrosimone BG, Ryan ED, Lee DR, Blackburn JT. Quadriceps Function and Hamstrings Co-Activation After Anterior Cruciate Ligament Reconstruction. *J Athl Train*. May 2017;52(5):422-428. doi:10.4085/1062-6050-52.3.05

7. Pietrosimone BG, Lepley AS, Ericksen HM, Gribble PA, Levine J. Quadriceps strength and corticospinal excitability as predictors of disability after anterior cruciate ligament reconstruction. *J Sport Rehabil*. Feb 2013;22(1):1-6. doi:10.1123/jsr.22.1.1

8. Pietrosimone BG, Lepley AS, Ericksen HM, Clements A, Sohn DH, Gribble PA. Neural Excitability Alterations After Anterior Cruciate Ligament Reconstruction. *J Athl Train*. Jun 2015;50(6):665-74. doi:10.4085/1062-6050-50.1.11

9. Ward SH, Blackburn JT, Padua DA, et al. Quadriceps Neuromuscular Function and Jump-Landing Sagittal-Plane Knee Biomechanics After Anterior Cruciate Ligament Reconstruction. *J Athl Train*. Feb 2018;53(2):135-143. doi:10.4085/1062-6050-306-16

10. Scheurer SA, Sherman DA, Glaviano NR, Ingersoll CD, Norte GE. Corticomotor function is associated with quadriceps rate of torque development in individuals with ACL surgery. *Exp Brain Res*. Feb 2020;238(2):283-294. doi:10.1007/s00221-019-05713-w

11. Rossini PM, Rossini L, Ferreri F. Brain-behavior relations: transcranial magnetic stimulation: a review. *IEEE Eng Med Biol Mag*. Jan-Feb 2010;29(1):84-95. doi:10.1109/MEMB.2009.935474

12. Van Wittenberghe IC, Peterson DC. Corticospinal Tract Lesion. *StatPearls*. 2020.

13. Brownstein CG, Ansdell P, Skarabot J, Howatson G, Goodall S, Thomas K. An optimal protocol for measurement of corticospinal excitability, short intracortical inhibition and intracortical facilitation in the rectus femoris. *J Neurol Sci*. Nov 15 2018;394:45-56. doi:10.1016/j.jns.2018.09.001

14. Groppa S, Oliviero A, Eisen A, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol*. May 2012;123(5):858-82. doi:10.1016/j.clinph.2012.01.010

15. Needle AR, Lepley AS, Grooms DR. Central Nervous System Adaptation After Ligamentous Injury: a Summary of Theories, Evidence, and Clinical Interpretation. *Sports Med*. Jul 2017;47(7):1271-1288. doi:10.1007/s40279-016-0666-y

16. Rossini PM, Rossi S, Babiloni C, Polich J. Clinical neurophysiology of aging brain: from normal aging to neurodegeneration. *Prog Neurobiol*. Dec 2007;83(6):375-400. doi:10.1016/j.pneurobio.2007.07.010

17. Luc BA, Lepley AS, Tevald MA, Gribble PA, White DB, Pietrosimone BG. Reliability of corticomotor excitability in leg and thigh musculature at 14 and 28 days. *J Sport Rehabil*. Nov 2014;23(4):330-8. doi:10.1123/jsr.2013-0069

18. Cacchio A, Cimini N, Alosi P, Santilli V, Marrelli A. Reliability of transcranial magnetic stimulation-related measurements of tibialis anterior muscle in healthy subjects. *Clin Neurophysiol*. Feb 2009;120(2):414-9. doi:10.1016/j.clinph.2008.11.019

19. O'Leary TJ, Morris MG, Collett J, Howells K. Reliability of single and paired-pulse transcranial magnetic stimulation in the vastus lateralis muscle. *Muscle Nerve*. Oct 2015;52(4):605-15. doi:10.1002/mus.24584

20. Temesi J, Ly SN, Millet GY. Reliability of single- and paired-pulse transcranial magnetic stimulation for the assessment of knee extensor muscle function. *J Neurol Sci*. Apr 15 2017;375:442-449. doi:10.1016/j.jns.2017.02.037

21. van Hedel HJ, Murer C, Dietz V, Curt A. The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for torque and motor task. *J Neurol*. Aug 2007;254(8):1089-98. doi:10.1007/s00415-006-0493-4

22. Proessl F, Beckner ME, Sinnott AM, et al. Reliability of corticospinal excitability estimates for the vastus lateralis: Practical considerations for lower limb TMS task selection. *Brain Res*. Mar 2 2021;1761:147395. doi:10.1016/j.brainres.2021.147395

23. Rossi S, Antal A, Bestmann S, et al. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clin Neurophysiol*. Jan 2021;132(1):269-306. doi:10.1016/j.clinph.2020.10.003

24. Pietrosimone BG, Hammill RR, Saliba EN, Hertel J, Ingersoll CD. Joint angle and contraction mode influence quadriceps motor neuron pool excitability. *Am J Phys Med Rehabil*. Feb 2008;87(2):100-8. doi:10.1097/PHM.0b013e31815882e0

25. Pietrosimone BG, Gribble PA. Chronic ankle instability and corticomotor excitability of the fibularis longus muscle. *J Athl Train*. Nov-Dec 2012;47(6):621-6. doi:10.4085/1062-6050-47.6.11

26. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol*. Oct 2000;10(5):361-74. doi:10.1016/s1050-6411(00)00027-4

27. Pincivero DM, Salfetnikov Y, Campy RM, Coelho AJ. Angle- and gender-specific quadriceps femoris muscle recruitment and knee extensor torque. *J Biomech*. Nov 2004;37(11):1689-97. doi:10.1016/j.jbiomech.2004.02.005

28. Ah Sen CB, Fassett HJ, El-Sayes J, Turco CV, Hameer MM, Nelson AJ. Active and resting motor threshold are efficiently obtained with adaptive threshold hunting. *PLoS One*. 2017;12(10):e0186007. doi:10.1371/journal.pone.0186007

29. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med*. Jun 2016;15(2):155-63. doi:10.1016/j.jcm.2016.02.012

30. Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet*. Oct 21 1995;346(8982):1085-7. doi:10.1016/s0140-6736(95)91748-9

31. Takeno K, Norte GE, Glaviano NR, Khuder S, Ingersoll CD. Within- and Between-Session Reliability of Corticospinal Excitability in the Upper Extremity. *Measurement in Physical Education and Exercise Science*. 2022:1-12. doi:10.1080/1091367X.2022.2070434

32. Promsri A, Haid T, Werner I, Federolf P. Leg Dominance Effects on Postural Control When Performing Challenging Balance Exercises. *Brain Sci*. Feb 25 2020;10(3)doi:10.3390/brainsci10030128

33. Terada M, Kosik KB, McCann RS, Drinkard C, Gribble PA. Corticospinal activity during a single-leg stance in people with chronic ankle instability. *J Sport Health Sci*. Aug 28 2020;doi:10.1016/j.jshs.2020.08.008

34. Soto O, Valls-Sole J, Shanahan P, Rothwell J. Reduction of intracortical inhibition in soleus muscle during postural activity. *J Neurophysiol*. Oct 2006;96(4):1711-7. doi:10.1152/jn.00133.2006

35. Skarabot J, Tallent J, Goodall S, Durbaba R, Howatson G. Corticospinal excitability during shortening and lengthening actions with incremental torque output. *Exp Physiol*. Dec 2018;103(12):1586-1592. doi:10.1113/EP087347

Appendix A

The Problem

Problem Statement

The knee is the most commonly injured joint and more than 66% of activity-related injuries occur in the lower extremity.[1] An anterior cruciate ligament (ACL) rupture is one of the most common traumatic knee injuries occurring in the United States.[2] Following an ACL injury, quadriceps dysfunction is common and contributes to self-reported physical disability.[3-6] ACL injury and reconstruction not only produce abnormal gait biomechanics with higher loading rates, contributing to cartilage degradation and osteoarthritis development[7] but also affect corticomotor function of lower extremity muscles.[8, 9] This neural impairment in individuals with ACL reconstruction manifest as inhibition or abnormal facilitation of uninjured musculature surrounding the injured joint, as a protective mechanism.[3, 10] Research demonstrates that after injury, the quadriceps do not return to preinjury levels of neuromuscular function, causing long-term changes in muscle function and lower extremity movement, increasing the risk of reinjury.[3, 11, 12]

Transcranial magnetic stimulation (TMS) is used to assess corticospinal excitability by non-invasively stimulating the brain with a rapidly changing magnetic field delivered to the scalp through a coil.[13-15] A single TMS pulse applied over the contralateral primary motor cortex excites the cortical neurons to relay information through the descending corticospinal tract and alpha motor neuron to the peripheral muscle, eliciting a motor evoked potential (MEP).[16-19] TMS can also be used to identify the lowest intensity needed to produce a measurable MEP from the target muscle during a tonic muscle contraction, which is known as the active motor threshold (AMT).[10, 20] Individuals with an ACL

reconstruction have higher AMTs and lower MEP amplitudes,[10, 13] indicating that there is decreased brain to muscle communication, resulting in quadriceps weakness,[21] dysfunction, and lower quadriceps rate of torque development in the injured limb.[22] These factors can potentially increase the risk of reinjury.

Existing literature has shown that TMS is a reliable measurement of corticospinal excitability.[15, 23-26] However, when corticospinal excitability of the quadriceps is measured, participants are seated in an isokinetic dynamometer and are in a resting state and/or performing a sub-maximal voluntary isometric contraction. Utilizing TMS in a functional position is important as it may be a useful tool to identify risk factors for lower extremity injury, specifically to the ACL, in healthy active individuals. Additionally, functional TMS (fTMS) has the potential to expand our understanding of neural activity involved in the brain and muscle communication as well as to serve as an outcome measure for return to play decisions. To date, very few studies have explored whether AMT and MEP are reliable measures of the corticospinal excitability during the performance of functional tasks (e.g., single-leg squat). Therefore, the purpose of this study was to investigate the intersession reliability of TMS-derived outcomes of in assessing quadriceps corticomotor function during a single leg squat in healthy, active individuals.

Research Question

Are TMS-derived outcomes reliable when assessing quadriceps corticomotor function during a single leg squat in healthy, active individuals?

Experimental Hypothesis

We hypothesize that AMT will have strong intersession reliability in the quadriceps, but MEP will have moderate intersession reliability during the performance of a functional task.

Assumptions

- Participants will exert maximum effort during the maximum voluntary isometric contraction (MVIC).
- Electromyography (EMG) data accurately reflects the physiology of the entire vastus medialis.
- Participants will perform the single leg squat to 10 to 15% of their maximal muscle contraction.
- The hotspot represents the most excitable spot of the primary motor cortex.
- Participants did not consume any stimulant or depressants such as caffeine, alcohol, and/or tobacco within 24 hours of data collection.

Delimitations

- The participant population will include individuals between the ages of 18 and 30 years deemed physically active by a Tegner Activity Scale rating of 5 or greater.
- Participant does not have a prior lower extremity injury within the last 12 months.
- Participant does not have a previous medical history of lower extremity surgeries.
- Participant has not had a concussion within the last 12 months.

- Participant does not have a history of neurosurgery, stroke, migraines, and/or severe head injury.
- Participant does not have a known history of cardiopulmonary disorder and/or neurological or psychiatric disorders.
- Participant does not have a current form of neuropathy.
- Participant does not have an active or inactive implanted biomedical device.
- Participant is not pregnant.
- Participant is not currently taking any medications which may influence that alters neural excitability.

Operational Definitions

1. Active Motor Threshold (AMT) – the lowest stimulus intensity needed to elicit a measurable motor evoked potential response from the target muscle during a tonic contraction.
2. Corticospinal Excitability – the ability of cortical neurons to relay information from the primary motor cortex to the peripheral muscle.
3. Functional Transcranial Magnetic Stimulation (fTMS) – the assessment of TMS-derived outcome measures during a dynamic task, such as a squat.
4. Hotspot – the location on the primary motor cortex where the lowest intensity evoking the highest amplitude of motor evoked potential.
5. Intersession Reliability – the degree to which the result of a measurement is accurate when compared between two different time points.

6. Motor Evoked Potential (MEP) – the amplitude of the electromyography (EMG) response needed to elicit a muscle contraction.
7. Transcranial Magnetic Stimulation (TMS) – a painless, non-invasive, non-imaging technique, based on electromagnetic induction of an electric field in the brain. This technique stimulates the brain through a rapidly changing magnetic field delivered to the scalp through a coil.

Significance of Study

Quadriceps dysfunction in individuals with ACL reconstruction (ACLR) contributes to poor self-reported knee function, less physical activity, and abnormal gait patterns. It is also a risk factor for osteoarthritis development. This study is significant because traditional approaches to resolve quadriceps dysfunction do not focus on the neural causes. Existing literature has demonstrated that corticomotor function changes following injury, but an assessment of quadriceps corticomotor excitability during a functional task, such as a single leg squat, does not exist. By understanding corticospinal excitability of the quadriceps of healthy active individuals during a single leg squat, we will be able to identify potential risk factors for lower extremity injury.

Appendix B

Literature Review

What is Corticomotor Function?

Corticomotor function is the excitability of the corticospinal motor system,[21] which is encompassed of intracortical and corticospinal pathways.[27] The corticospinal tract begins in the primary motor cortex (M1) and the descending axons, travel to the ventral horn to synapse onto the lower motor neurons, which exit the spinal cord to contract muscle.[17] This corticospinal pathway is essential in the production of voluntary muscle function.[2] A decrease in corticospinal excitability would increase the need for greater synaptic input into the motor cortex.[16] The intracortical pathway is the excitability of short inhibitory and facilitatory interneuronal circuits within the motor cortex.[28] Knowledge of intracortical facilitation and inhibition helps to identify the activity of interneurons in the cortex and detects neuroplastic changes in cortical excitability through intracortical neurotransmitter receptors, specifically gamma aminobutyric acid and N-methyl-D-aspartate.[13, 29]

What Assessments Are Used to Measure of Corticomotor Function?

Electroencephalography (EEG), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI) are primary non-invasive functional neuroimaging modalities used to assess brain activity.[30] They allow for the visualization and analysis of the brain function and structure, specifically brain metabolism and neural firing.[30, 31] Transcranial magnetic stimulation (TMS) is a painless, non-invasive, non-imaging technique, based on Faraday's law of electromagnetic induction.[20, 32] A simple TMS device

is comprised of a few circular turns of copper wire connected to the terminals of a large electrical capacitance via a switch.[32] When TMS is delivered over the primary motor cortex with adequate intensity, an electrical current is transmitted through a round coil of wire placed over the scalp.[20, 33] This current generates a rapidly changing magnetic field over the scalp induced by a figure-of-eight coil also known as double-cone coil, which penetrates to the underlying cortical region without attenuation through the skull.[14, 20, 34] Coil shape and size, magnetic field strength, and frequency and duration of magnetic pulses delivered influences TMS.[14] Thus, TMS helps in the understanding of the primary motor cortex and excitability of descending motor pathways in response to musculoskeletal injury.[13]

TMS can be applied as one stimulus at a time (single-pulse TMS), in pairs of stimuli separated by a variable interval to the same or different brain areas (paired-pulse TMS), or in trains of repetitive stimuli at various frequencies (rTMS).[20, 34] Single-pulse TMS allows routine evaluations of the excitability and conductivity of corticospinal motor pathways.[34] Single-pulse TMS allows for corticospinal excitability measurements through assessment of motor threshold (MT), motor evoked potential (MEP) amplitude, and cortical silent period duration (cSP). MEP is the amplitude of the electromyography (EMG) response needed to elicit a muscle contraction.[35] A low MEP can be interpreted as less stimulus directed to the descending motor pathway, leading insufficient neural drive to generate a contraction. The cortical motor threshold is the lowest stimulus intensity needed to elicit a measurable MEP response from the target muscle.[20] MT can be measured with the muscle at rest (RMT) or during tonic contraction (AMT).[36] MT is increased when the excitation thresholds increase in pyramidal cells in the motor cortex.[20] Evidence demonstrates that

MT is higher in patients with ACLR and chronic ankle instability (CAI), indicating that a larger stimulus is needed to excite descending cortical neurons.[13] Thus, a high MT implies that there is increased inhibition of supraspinal regions associated with motor function as well as a decreased motor cortex excitability and decreased ability to produce a motor response, resulting in muscle weakness.

Stimulation of the primary motor cortex induces an electric current in the brain tissue which produces a depolarization of neurons that is transmitted via the corticospinal tract to the contralateral peripheral muscle where a motor response can be recorded.[15] MEP reflects the conduction of the descending corticospinal tract and determines the hotspot of the motor cortex. The motor hotspot is the optimal stimulation point where the lowest intensity evokes the highest amplitude of MEP.[37] When the TMS stimulator is set at 50% output, the hotspot can be used to determine the MT by decreasing stimulus intensity until a barely discernable MEP is achieved.[24] The recorded MEP was normalized to the root-mean square EMG, which was measured as the mean amplitude of 100 ms immediately prior to the stimulus.

In the paired-pulse paradigm, TMS stimulation can be delivered to a single cortical target using the same coil or to two different regions of the motor cortex using two different coils.[34] Paired-pulse TMS allows for study of excitability of short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF).[26] SICI occurs when an MEP generated by a subthreshold conditioning stimulus followed by a suprathreshold test stimulus at short interstimulus interval (<5ms) whereas LICI happens during a longer interval (>50ms).[38, 39] _ENREF_21Higher levels of intracortical inhibition is associated with lower cortical excitability[39], resulting in muscle

inhibition. ICF uses paired pulses similarly to intracortical inhibition but has an interstimulus interval between the conditioning and testing pulses (>5ms).[38] Less ICF indicates less facilitation of the muscle, also leading to muscle inhibition.

What Is the Evidence of Corticomotor Impairment?

Dysfunction in the corticomotor pathways affects the excitability of the motor cortex, the functional integrity of intracortical neuronal structures, the conduction along corticospinal tract, as well as the function of nerve roots and peripheral motor pathway to the muscles.[20] Impaired corticomotor function alters muscle contractile ability, contributing to motor deficits and persistent dysfunction.[13] Disability is evident after joint injury and/or surgery, including anterior cruciate ligament (ACL) ruptures and reconstructions, meniscectomy, osteoarthritis (OA), total knee arthroplasties, acute ankle sprain, and chronic ankle instability.[3] Neuromuscular deficits such as muscle weakness, atrophy, and altered function are common following joint injury and change functional performance.[3] Literature has demonstrated that knee function does not return to pre-injury levels and experience limitations post ACL reconstruction (ACLR).[11] Furthermore, poor quadriceps function influences gait biomechanics.[7] Since the quadriceps resists perturbation[27], produces dynamic stability[27], and attenuates joint forces[27], decreased quadriceps rate of torque development (RTD) results in high-rate loading during walking and running.[7] Increased loading rates exacerbate cartilage degradation, contributing to the development of knee OA after ACLR.[7]

Additionally, maximal voluntary isometric contraction (MVIC), electromechanical delay (EMD), central activation ratio (CAR), and coefficient variance (CV) are common

measures of neuromechanical function.[27] MVIC is a measure a measure of muscle strength. When measuring AMTs, participants are generally asked to sub-maximally contract the target muscle to about 5% of the MVIC.[10, 27] Deficits in muscle strength as a result of impaired corticomotor function limits MVIC ability, which indicates that the decline in efficiency of the muscle to generate force.[40] RTD is the ability of a muscle to produce torque rapidly.[22] It estimates explosive voluntary force production[41] by measuring the slope of the torque–time curve (Δ torque/ Δ time) obtained during isometric conditions.[42] RTD are classified as RTD is often classified as "early" and "late" for time intervals ≤100 ms and ≥200 ms, respectively.[42, 43] Early RTD is associated with neural activation transmitted by motor neurons to muscles. Thus, deficits in Early RTD are related to the ability of the central nervous system to maximally activate the muscle in the early interval after initiation of the contraction.[44] However, late RTD is related to maximal strength.[45] This can affect the ability of the limb to generate sufficient moment especially during dynamic tasks.[22] Research has shown that lesser early-phase RTD is related with sport-specific tasks and less excitable corticospinal projections to quadriceps motor neurons after ACLR.[27] Following ACLR with patellar tendon autograft, patients have lower RTD and rate of knee extension moment in the reconstructed limb, as a result of changed knee loading and potentially increases injury risk and future development of post-traumatic osteoarthritis.[22] A decrease in function of the excitation-contraction coupling process is linked to prolonged EMD following ACLR. EMD is the time lapse between the onset of muscle electrical activation and the onset of force production.[46] Individuals with ACLR have an increased EMD, which implies that there is muscle impairment in the quadriceps as well as a

decreased force attenuation at the knee joint. Less electromechanical delay is ideal for optimal neuromuscular function.

Previously, motor dysfunction of an uninjured musculature surrounding an injured joint has been theorized to be a result of arthrogenic muscle inhibition (AMI).[3, 13] MI is the mechanism where a muscle fails to act due to neural inhibition, changes in the discharge of articular sensory receptors, altered spinal reflex excitability, and abnormal cortical activity.[47] These changes in sensory discharge and spinal inhibition are theorized to elicit changes in the supraspinal regions. AMI occurs secondary to mechanoreceptor damage, swelling, and pain that serves to inhibit descending motor neurons.[13] AMI shuts down the efferent motor pathway and obstructs muscle rehabilitation by preventing muscle activation, gains in strength, and restoration of normal proprioceptive function.[48] This results in chronic deficiencies, the potential for reinjury, and the risk of chronic degenerative joint conditions.[48]

Muscle inhibition is manifested as a consequence of corticomotor impairment following injury. Existing literature suggests that reflexive excitability is likely affected in the early stages of injury such as 0 to 6 months following ACL surgical reconstruction, while corticomotor excitability is altered in chronic states of injury as far 4 years post-surgery.[13] However, there is limited evidence about the functional implications of corticomotor impairment, especially in uninjured individuals. Table B.1 provides a comparison of evidence of corticomotor impairment. Understanding how neuromuscular alterations affect function is essential for proper clinical management of lower extremity joint injuries.

What Is the Evidence of Transcranial Magnetic Stimulation Reliability?

There are few reliability studies, which examine TMS outcome measures in the lower extremity. Table B.2 compares the current literature, which demonstrates that AMT has strong reliability and MEP has good reliability. To date, no investigations explored whether TMS outcome measures such as AMT and MEP are reliable assessments of the corticomotor pathway during functional tasks, similar to what is performed during sport-specific activity e.g., single-leg land.

Table B.1. The table below displays the existing evidence of the impairment of TMS outcome measures in the lower extremity musculoskeletal pathology.

Lower extremity Musculoskeletal Pathology	Population	Muscle tested	Task Performed	Impairment			Clinical Manifestation
				Spinal-Reflex Excitability	Corticomotor Excitability	Neuromechanical Measure	
ACLR[10]	Individuals with unilateral ACLR	vastus medialis	- Seated for AMT testing - Supine for reflex testing	higher bilateral H:M ratio was in the ACL-R than the control	Higher AMT in the injured than in the uninjured limb in the ACL-R group and in the matched limb of the control group	Lower bilateral quadriceps CAR in the ACL-R compared with the control group	Corticomotor deficits were present after surgery and require higher excitability to maintain voluntary activation.
ACLR[49]	Individuals with unilateral ACLR & healthy control	Quadriceps	- Seated isometric knee extension contraction for AMT testing - Supine with knees flexed to 15° for quadriceps	Normal H:M ratio	Greater quadriceps AMT in the ACLR limb than the contralateral limb	- Greater asymmetry in knee-extension MVIC torque & quadriceps CAR in ACLR group - Normal knee-extension MVIC torque	Asymmetry in quadriceps strength, activation, & cortical excitability persisted in individuals with ACLR beyond return to recreational activity, resulting in reduced self-reported function & increased rate of subsequent joint injury in healthy,

			H-reflex testing				active individuals after ACLR
ACLR[50]	Individuals with unilateral ACLR	Quadriceps	Stationary maximal effort, submaximal effort, or a resting state	H:M ratio increased with peak vertical ground reaction force	No between-limbs differences for quadriceps AMT.	In ACLR limb, greater MVIC was associated with greater peak knee-flexion angle and less peak vertical ground reaction force. Greater CAR was associated with greater peak internal knee-extension moment.	Greater quadriceps MVIC and CAR may provide better energy attenuation during a jump-landing task. Individuals with greater peak vertical ground reaction force in the ACLR limb possibly require greater spinal-reflex excitability to attenuate greater loading during dynamic movements.
CAI[8]	Individuals with unilateral CAI & healthy control	Fibularis Longus	Seated with a slightly flexed knee joint and the ankle secured in 10° of	N/A	- Higher RMT in CAI group bilaterally. - Moderate negative correlation between RMT and Functional Ankle Disability	N/A	Corticospinal excitability deficits may be influential in altering function.

			plantar flexion.		Index (FADI) and FADI Sport		
CAI[51]	chronic ankle instability group, lateral ankle sprain coper group, and control	Tibialis anterior	single leg standing	N/A	Longer cSP at 100% intensities of AMT and lower normalized MEP at 120% intensities of AMT in CAI group compared to lateral ankle sprain copers and controls. No significant difference in cSP at 100% & 120% intensities of AMT & MEP at 100% & 120% intensities of AMT among all groups.	N/A	Increased difficulty in controlling the tibialis anterior muscle during a single-leg stance, influencing postural control performance in those with CAI.

What Is the Evidence of Transcranial Magnetic Stimulation Reliability?

Table B.2. The table below displays the existing evidence of TMS reliability studies of lower extremity muscles.

Study Title	Reliability of transcranial magnetic stimulation-related measurements of tibialis anterior muscle in healthy subjects		Reliability of Corticomotor Excitability in Leg and Thigh Musculature at 14 and 28 Days		Reliability of Single and Paired-Pulse Transcranial Magnetic Stimulation in The Vastus Lateralis Muscle		Reliability of corticospinal excitability estimates for the vastus lateralis: Practical considerations for lower limb TMS task selection		Reliability of single- and paired-pulse transcranial magnetic stimulation for the assessment of knee extensor muscle function	The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for torque and motor task
Study Authors	Cacchio et al, 2009 [24]		Luc et al, 2014[15]		O'Leary et al., 2015[26]		Proessl et al, 2021[52]		Temesi et al, 2017 [25]	van Hedel et al, 2007 [23]
Population	50 healthy subjects (age 44.8 years ± 16.5 years)		20 healthy volunteers		16 men (age 26 ± 5 years)		19 men (age: 25 ± 5 years)		20 healthy adults (age 23 ± 5 years)	20 healthy subjects
Muscle Tested	anterior tibialis		- vastus medialis oblique - fibularis longus		vastus lateralis		vastus lateralis		- vastus lateralis - rectus femoris - vastus medialis	anterior tibialis
Task Performed	Seated		Seated knee extension		Seated maximal voluntary isometric contractions		- Isometric knee extension - Squat		Seated knee extension	- Isotonic–isometric contraction - Continuously increasing isometric TA contraction
TMS	MT	MEP	SPmax	AMT	MEP	RMT AMT	SICI LICI	MEP	MEP SICI	MEP

Outcomes Assessed						MEP cSP	ICF MVC		LICI		
Time Points Measured	3 sessions: - 2 sessions, at least 1.5 hrs apart - 1 session 4 wks later			3 sessions: - baseline - day 14 - day 28		2 sessions: -1st session 3 measurements at 4 hr intervals -2nd session at least 5 days apart from 1st		2 sessions, 24 hours apart	3 sessions: 1 familiarization session 2 sessions the same time of day with 2–14 days between sessions		2 sessions, at least 7 days apart
Type of Reliability	- Inter-investigator - Intra-investigator - Between-Session			Intersession		- Within-Day - Between-Day		Intersession	- Intrasession - Intersession		Intrarater
ICC Value	Intra: 0.98	0.93	0.95	Dominant VMO: Day 14: AMT: 0.96 MEP:0.93 Day 28: AMT: 0.93 MEP: 0.71	Dominant Fibularis: Day 14: AMT: 0.96 MEP: 0.24 Day 28: AMT: 0.92 MEP: 0.047	Within RMT:0.99 AMT:0.98 MEP:0.85 cSP:0.97 SICI80:0.84 LICI:0.96 ICF100:0.73 MVC:0.82	Between RMT:0.91 AMT:0.92 MEP:0.82 cSP:0.83 SICI80:0.68 LICI:0.47 ICF100:0.56 MVC:0.70	KE: 0.92 Squat: 0.68	Inter-session: vastus lateralis: MEP:0.956 SICI:0.538 LICI:0.871 rectus femoris: MEP:0.799 SICI:0.357 LICI:0.864	Intra-session: vastus lateralis: MEP:0.985 SICI:0.630 LICI:0.942 rectus femoris: MEP:0.956 SICI:0.827 LICI:0.826	10% MVC: ICC_{all}: 0.29 $ICC_{healthy}$: 0.28 20% of MVC: ICC_{all}: 0.48 $ICC_{healthy}$: 0.46 40% MVC: ICC_{all}: 0.35 $ICC_{healthy}$: 0.37 60% MVC: ICC_{all}: 0.22 $ICC_{healthy}$: 0.28
	Inter: 0.94	0.79	0.89								
	Re-test: 0.97	0.92	0.95								

									vastus medialis: MEP:0.84 SICI:0.565 LICI:0.879	vastus medialis: MEP:0.966 SICI:0.570 LICI:0.933	
Reliability Category	Good intra- and inter-investigator reliability for motor threshold, MEP latency, and SPmax in healthy subjects.			Strong AMT reliability, Weak reliability at 100 and 105% of AMT on both day 14 and 28.		MEP amplitude and cSP duration showed good within and between-day reliability. Intracortical facilitation showed moderate to good within-day reliability but poor to moderate reliability between days.		Knee extension showed better test–retest reliability and agreement for MEP_{MAX} than the squat but force and EMG were similarly reliable. Force and MEP_{MAX} were also greater during knee extension.	Single-pulse MEPs elicited by strong and weak single pulses had excellent relative reliability and variability for strong single-pulse TMS was less than for weak single-pulse TMS		Good reliability lower leg motor evoked potential amplitudes

Appendix C

Additional Methods

Specific Testing Protocol

1. Biopac Setup

   a. Connect STM 100C, EMG 100C, UIM to MP160 unit

   b. Connect STMISOC to the STM100C via output jack

   c. Connect MP160 to the computer using a LAN wire

   d. Turn on MP160 unit and the computer

   e. STM100C Settings

      i. Source = OUT

      ii. Level = 100%

      iii. Polarity = POS

      iv. Current = DC

   f. EMG100C Settings

      i. Gain = 1000

      ii. LP = 5kHz

      iii. 100HzHP = off

      iv. HP = 1.0 Hz

g. STMISOC Settings

   i. Voltage Monitor = 0.5 V

   ii. Voltage Switch = Voltage (1:10) 200 V Max

h. Plug active and dispersive electrodes into the STMISOC

2. Acqknowledge

a. Open Acqknowledge for Windows on computer 1

   i. Open Torque_EMG template

   ii. Select the attached MP160 unit

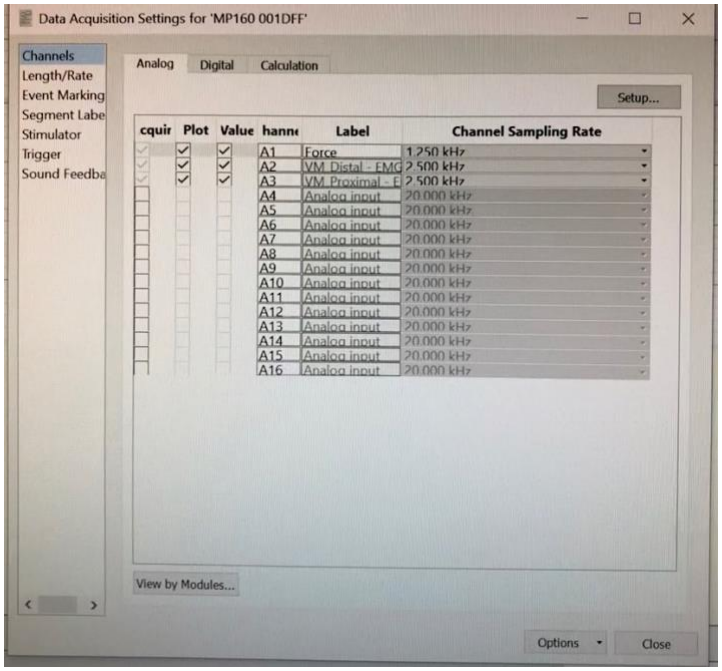
b. MP160 | Set-up data acquisition | Channels | Analog

   i. Channel 2

      1. Sample Rate = 2000 Hz

      2. Label = proximal vastus medialis

      3. Check all boxes associated with this channel



c. MP150 | Show Manual Control

   i. Analog Outputs: Out 1 = 0.0

   ii. Analog Outputs: Out 2 = 10.0

   iii. Open data journal and stimulator window

d. MP160 | Torque window

   i. Sample Rate = 1250 Hz

   ii. Constant = 145

   iii. Low pass

   iv. Window = Blackman -61 dB

   v. Freq.Co = 15 Hz

   vi. Number of Coefficients | Optimize for sample rate

e. Click start button to confirm proper setup

3. Subject Preparation - Electromyography

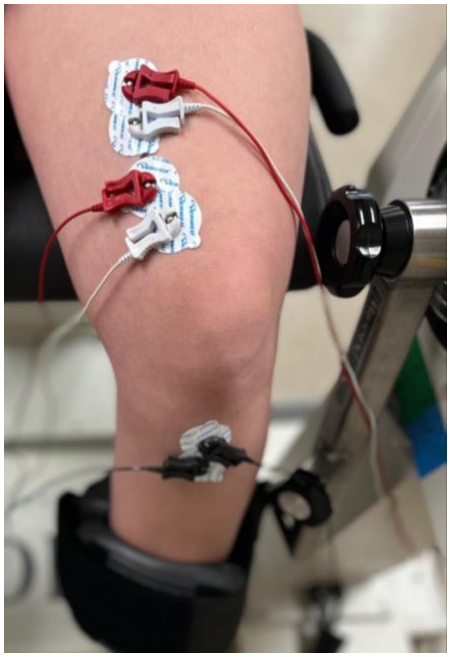
a. Position subject supine on the treatment table
b. Measure and identify the vastus medialis with a marker 80% of the distance from the anterior superior iliac spine and the joint space in front of the anterior border of the medial ligament
    i. Shave an 8cm x 2cm area surrounding the identified spot
    ii. Debride skin with an abrasive pad or gauze
    iii. Clean with isopropyl alcohol
c. Move the subject to a seated position in the Biodex
d. Identify the vastus medialis during manually resisted isometric knee extension contraction
    i. Extend the knee without rotating the thigh while applying pressure against the leg above the ankle in the direction of flexion
e. Place two surface EMG electrodes over the prepared distal vastus medialis directly below the 80% marker and another two electrodes over the prepared proximal vastus medialis directly above the 80% marker
    i. Parallel with muscle fiber orientation
    ii. Interelectrode distance of 2.0 cm
f. Identify a prominent bony area on the anteromedial tibia for the ground (reference) electrode
    i. Shave a 4cm x 2cm area
    ii. Debride skin with an abrasive pad or gauze
    iii. Clean with isopropyl alcohol
g. Place two disposable surface EMG electrodes each on the prepared tibial area

h. Attach the leads from channel 2 of the EMG100C unit to the proximal vastus medialis and the leads from the transcranial magnetic stimulation (TMS) EMG on computer 2 to the distal vastus medialis

   i. Proximal active electrode = Red lead

   ii. Distal active electrode = White lead

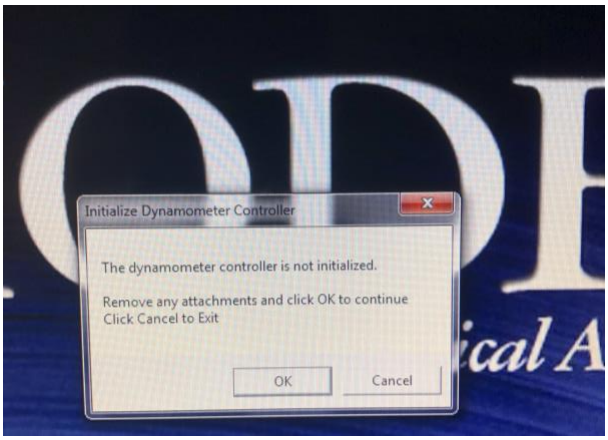
   iii. Ground (reference) electrode = Black lead



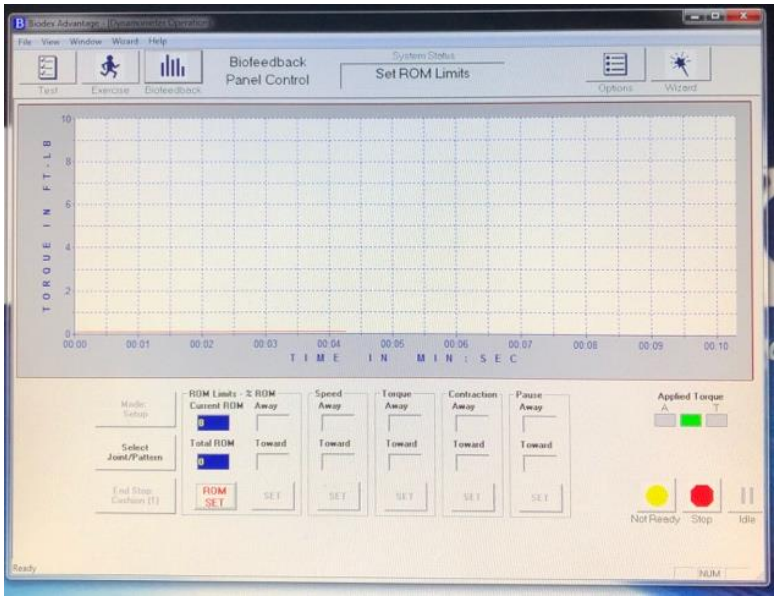
4. Knee Extension MVIC in Biodex

   a. Turn on the main and computer power switches

   b. Remove any attachments from the dynamometer input shaft and select “OK” to proceed with initialization

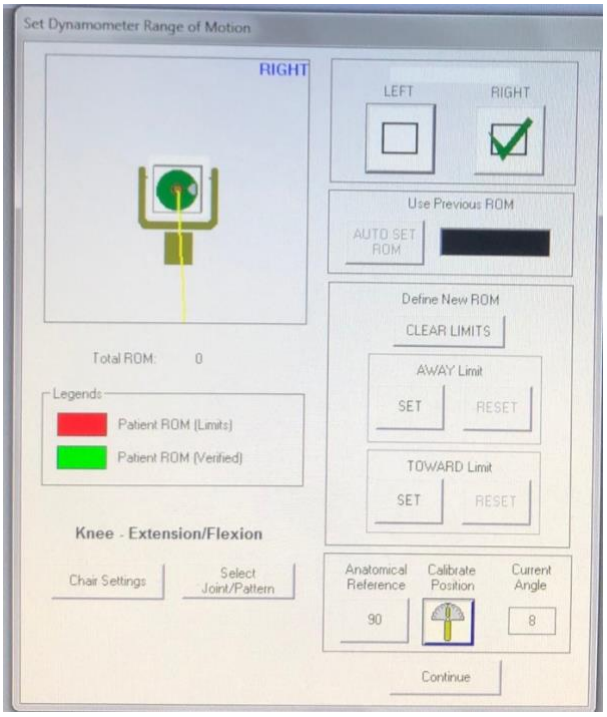


c. Select “Biofeedback”



d. Click “ROM SET”

e. Select testing side (left or right)



f. Place the knee attachment to the dynamometer

g. Use an inclinometer to measure 70 degree of knee flexion and press “Hold” to lock the position

h. Set ROM as 70 degrees for away and toward

i. Select Calibrate Position | Continue | Mode Isometric | Isometric

j. Test Biodex for biofeedback (push or pull on the leg attachment)

k. Seat the participant in the Biodex

    i. Raise/lower seat or move patient toward/away from dynamometer to align the patient’s lateral femoral condyle with the dynamometer shaft red dot

l. Stabilize patient with shoulder, waist, and shank straps

i. Ensure that the subject was comfortable and that their movement was restricted

ii. Provide instructions to the subject

1. “Sit up straight”
2. “Head back against the head rest”
3. “Cross arms across chest”

m. Open LabView Program on computer 1

i. Select File | Desktop | Faculty | Norte | Active Research Studies | 201820 - LEAP | Templates | LabView Program | SMR_CAR 2000Hz

n. Use LabView to display proximal vastus medialis torque onto television monitor in front of participant for visual feedback

o. Click the start button in the Acqknowledge window

p. Allow the subject to warm up by kicking against the stationary arm with 25%, 50%, 75% and 100% of maximal effort

q. Instruct subject to kick out as hard and fast as possible for 3-5 seconds

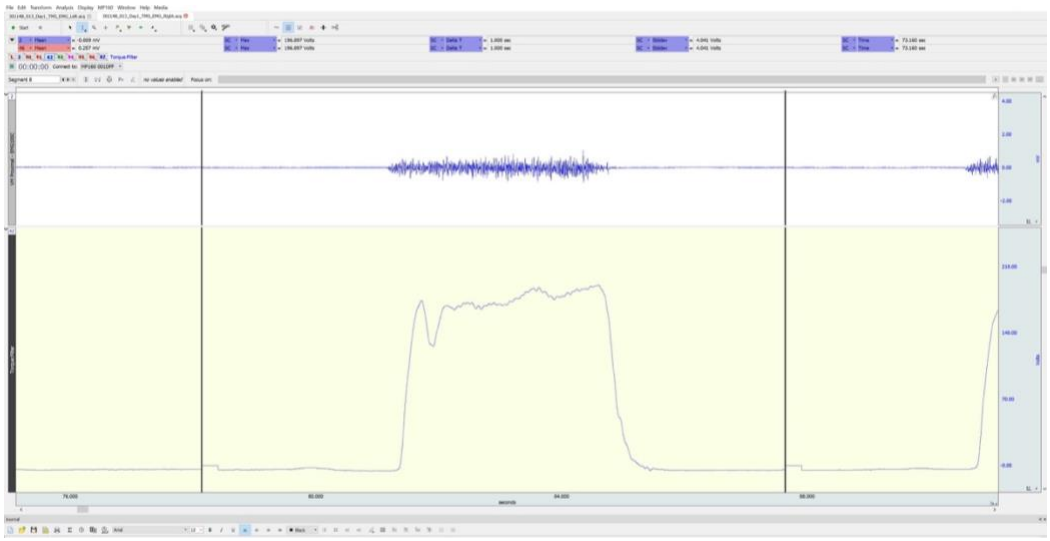
    i. Provide verbal encouragement during testing

r. Collect 3 MVIC trials

    i. Allow 30 seconds of rest between trials

s. Highlight each MVIC trial and record the mean, maximum, minimum, delta T, and time of the filtered proximal vastus medialis root mean square (RMS) channel in the data journal

    i. Repeat this procedure for the 3 recorded maximum trials



5. TMS – Hotspot Mapping

a. Keep EMG 100C set up and EMG electrodes in the same position from previous data collection

b. Use previous Acqknowledge Torque_EMG template

c. Create a 10% and 15% MVIC threshold line expression

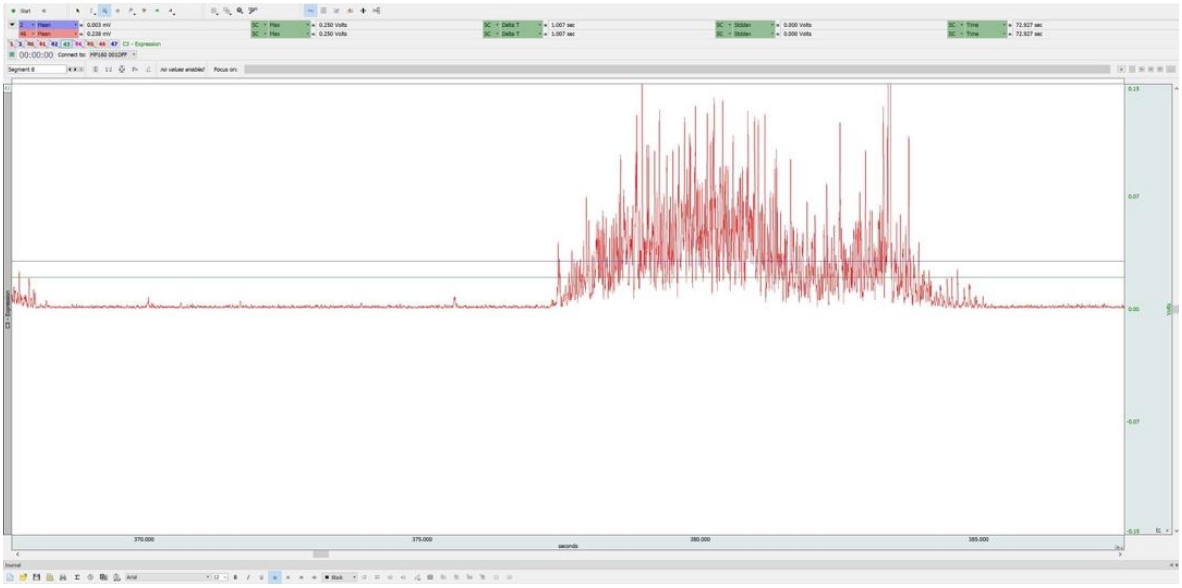
    i. Select MP160 | Set-up data acquisition | Analog | Expression | Set-Up

    ii. Paste % MVIC

iii. Multiply the average maximum MVIC by 0.10 to obtain 10% MVIC

iv. Repeat 7. f. i. to 7. f. iii. with 15% instead of 10%

d. Ensure that the respective 10 and 15% MVIC lines is displayed on the Acqknowledge window



e. Subject will be in the same position from previous data collection

i. Unstrap the hip and shoulder restraints only

f. Instruct participant to wear formable disposable ear plugs and a Lycra swim cap

i. Frontal line aligns with each tragus in the x-axis

ii. Sagittal aligns from the nasal bone to the external occipital protuberance in the y-axis

g. Turn on the front and back power switches of the Magstim BiStim2 magnetic stimulator

   i. Box 1 = 50% intensity

   ii. Box 2 = [no setting]

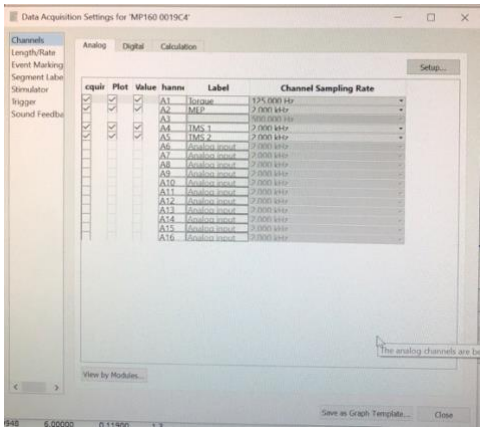
   iii. Delay = 0 ms

h. Connect EMG wires which correspond with the TMS

i. Open Acqknowledge for Windows on computer 2

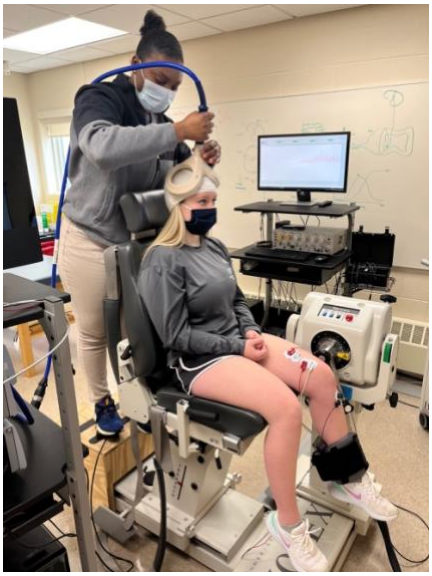
j. Open Torque_TMS template

   i. TMS channel 1 = 2000Hz



k. Turn off the lights and close any open doors to reduce external noise.

l. Position the TMS double cone coil above the participant's head

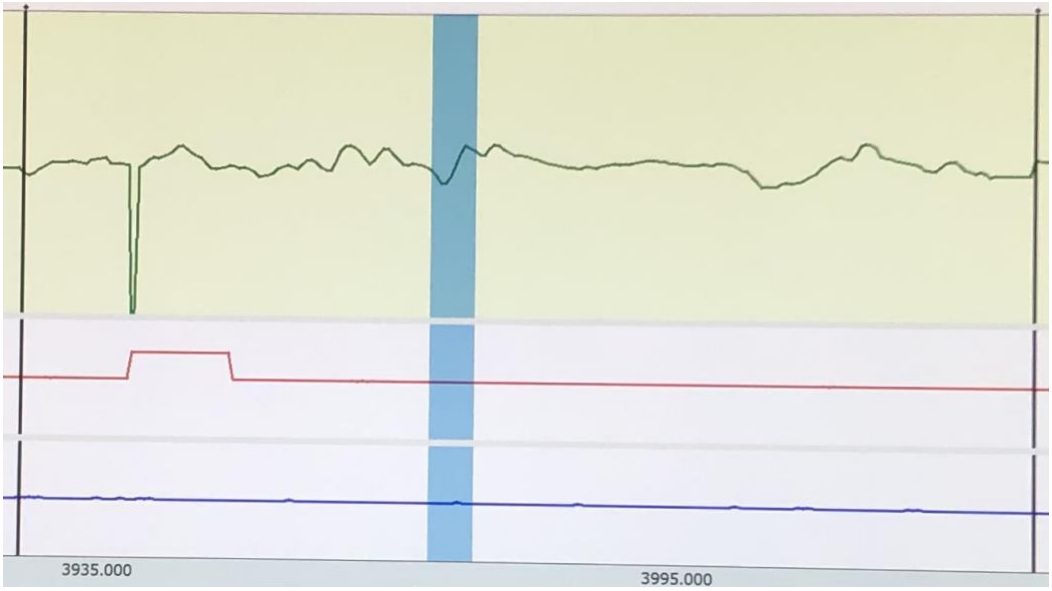


a. Click the start button in the data window on computer 1 and computer 2

b. Instruct the subject to kick to in between the 10 and 15% MVIC threshold line for 3-5s

c. Stimulate the participant with 50% TMS intensity once the EMG activity was at the required threshold

d. Assess motor evoked potential (MEP) wave on the Acqknowledge template



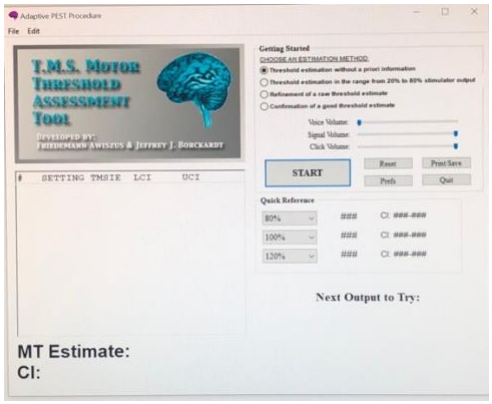
e. Repeat 6. q. to 6. t. along the coordinate system on the Lycra swim cap until the largest MEP amplitude was found

6. Functional TMS

a. Detach the EMG wires and remove the participant from the Biodex

b. Position the participant 2m in front of a visual feedback monitor screen and reattach the EMG wires

c. Open TMS Motor Threshold Assessment Tool (TMS MTAT) on computer 2



d. Instruct the participant to perform a single leg squat

i. “Place hands on hips”

ii. “Transfer weight to the test limb and bend knee until the EMG activity is within 10-15% threshold for 3-5s”

e. Stimulate the participant with the displayed default TMS stimulus intensity at the hotspot once the EMG activity was at the required threshold

f. Determine if an MEP was obtained

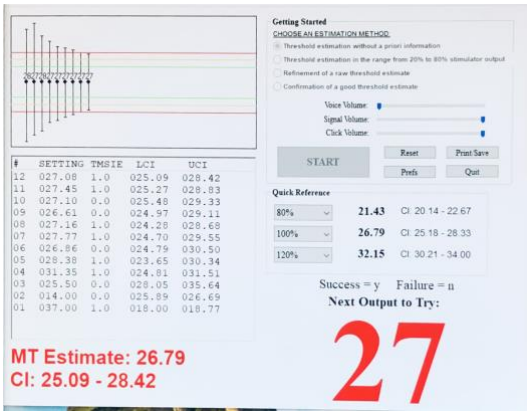
   i. If an MEP was measurable, select “yes” on TMS MTAT

   ii. If an MEP was not measurable, select “no” on TMS MTAT

g. Set TMS intensity to the percentage displayed on the TMS MTAT

h. Repeat 7. h. to 7. k. until the lowest AMT was achieved.

   i. Record this intensity as the active motor threshold (AMT)



i. Set the TMS intensity to 120% of the previously recorded AMT

j. Position an iPod 2m away from the participant on a tripod, 0.8m tall, to measure the knee joint angle.

   i. Unlock iPod, open “Camera”, switch to “Video”, and press the red record button

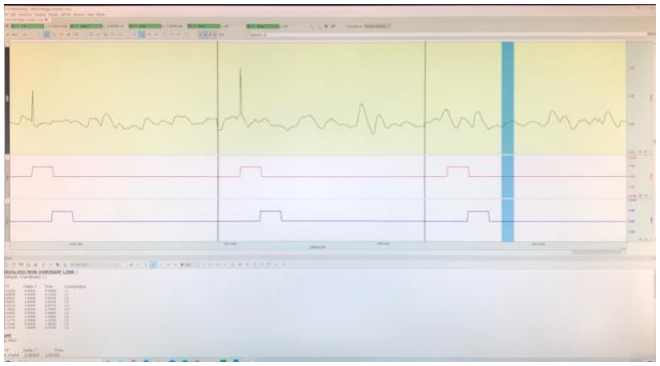
k. Instruct the participant to perform a single leg squat

l. Stimulate the participant with 120% AMT.

m. Record MEP

n. Repeat 7. n. to 7. p. until 8 successful trials were obtained

   i. A successful trial was a recordable MEP



Executive Summary

Executive Summary
Post-Professional Athletic Training Program
Motion Analysis & Integrative Neurophysiology Laboratory
University of Toledo

Title: Intersession Reliability of Quadriceps Corticospinal Excitability: A Functional TMS Study.

Principal Investigator:	Grant Norte, PhD, AT, ATC, CSCS (Associate Professor, School of Exercise and Rehabilitation Sciences)
Research Team:	Kiana Young, AT, ATC (Graduate Assistant) Justin Rush, MS, AT, ATC (Co-Investigator) David Bazett-Jones, PhD, AT, ATC, CSCS (Co-Investigator) Adam Lepley, PhD, ATC (Co-Investigator)

Purpose: To investigate the intersession reliability of transcranial magnetic stimulation (TMS) derived outcomes to assess quadriceps corticospinal excitability during a single leg squat in active healthy uninjured individuals.

Participants: Physically active healthy individuals from the University of Toledo and local Toledo community. Estimated sample size: 20

Inclusion Criteria:
- 18 to 30 years old
- Healthy males and females
- Physically active as indicated by a Tegner Activity Scale rating of 5 or greater

Exclusion Criteria:
- Lower extremity injury within the last 12 months
- Previous medical history of lower extremity surgeries
- History of brain surgery, stroke, migraine, and/or severe head injury
- Concussion within the last 12 months
- Personal or familial history of seizure and/or epilepsy
- History of cardiac disease
- Neurological or muscular disorder
- Medication that alters neural excitability
- Metal implants
- Pregnant

Study Design: Descriptive laboratory study with a test-retest design

Independent Variables:

- Time at 2 levels: 1st session & 14-day follow-up session
- Limb at 2 levels: dominant & non-dominant

Dependent Variables:

- Active motor threshold (AMT, %)
- Motor evoked potential (MEP)

Procedures:

1. Recruit healthy active individuals.
2. Complete informed consent.
3. Screen using Tegner Activity Scale and review eligibility criteria
4. Shave, debride, and clean the skin and place EMG electrodes on distal and proximal vastus medialis (VM).
5. Position participant in Biodex with 70 degrees of knee flexion and place restraints at shoulders, lap, and ankle of test limb.
6. Instruct participant to "kick out as hard and fast as you can."
7. Record the maximum voluntary isometric contraction (MVIC) in AcqKnowledge software and calculate the mean of 3 trials.
8. Place cap on participant's head and instruct the participant to insert ear plugs in ears.
9. Instruct the participant to kick out to 10 to 15% MVIC visually displayed on television screen.
10. Stimulate brain with 50% TMS intensity to evoke a MEP and use cap coordinates to find hotspot.
11. Once the "hotspot" is found, remove participant from Biodex.
12. Instruct the participant to perform a single leg squat to 10 to 15% MVIC with aid of the visual feedback.
13. Use TMS motor threshold assessment tool software to find AMT.
14. Repeat steps 12 and 13 until 8 MEPs at 120% intensity AMT stimulus are recorded.
15. Repeat steps 5-14 with contralateral limb.
16. Session 2 will take place approximately 14 days after session 1. Participants will complete steps 4-15 again in the same order as session 1.

IRB Protocol:

Statistical Analysis:

Intraclass Correlation Coefficients (ICC) will be used to assess intersession reliability for each dependent variable. Reliability coefficients will be interpreted as: poor (<0.7), fair (0.70-0.79), good (0.80-0.89), and high (≥0.90). Standard error of measurement (SEM) will be calculated to determine the error associated with each outcome measure. Coefficient of variation will be used to determine the relative variation. Minimal detectable change (MDC_{95}) score will be calculated to identify the 95% confidence level of a change occurring beyond that which could be associated with measurement error. Bland-Altman plots will be used to measure the agreement between sessions.

Research Hypothesis:

We hypothesize that AMT will have strong intersession reliability in the quadriceps, but MEP will have moderate intersession reliability during the performance of a functional task.

Consent Form

ICF Version Date: 8-9-21

School of Exercise and Rehabilitation Sciences
2801 W. Bancroft St. MS 119
Toledo, Ohio 43606
Phone: (419) 530-5305
Fax (419) 530-2477

ADULT RESEARCH SUBJECT INFORMATION AND CONSENT FORM

THE RELIABILITY OF FUNCTIONAL TRANSCRANIAL MAGNETIC STIMULATION AND THE INFLUENCE OF CORTICOMOTOR AND NEUROCOGNITIVE FUNCTION ON BIOMECHANICAL DUAL-TASK COST

Principal Investigator: Grant E. Norte Phd, AT, ATC, CSCS

Contact Phone number(s): (419) 530-5305

Key Study Information:
You may be eligible to take part in a research study. Take the time to carefully review this information. You should talk to the researchers about the study and ask them any questions you have. You may also wish to talk to others such as your family, friends, or doctors about joining this study. If you decide to join the study, you will be asked to sign this form before you can start study-related activities.

The purpose of this study is to determine how neurocognitive function and brain-to-muscle function influence joint biomechanics. Furthermore, the secondary purpose of this study is to determine the reliability of a novel functional brain-to-muscle testing protocol.

There can be risks associated with joining any research study. The type of risk may impact whether you decide to join the study. For this study, some of these risks may include skin irritation, muscle, discomfort and potential transient headaches from the transcranial magnetic stimulation. All forseeable risks are considered low seriousness. More detailed information will be provided later in this document.

This study may not offer any benefit to you now but may benefit others in the future by providing new information about modifiable risk factors for lower extremity injury. More information will be provided later in this document.

We expect the amount of time you will participate in the study will be 4.5 hours broken up into 3 study visits.

Your participation is voluntary. You can decide not to be in this study, or agree to take part now and change your mind later. If you decide to take part in this research or not, or if you decide to take part now but change your mind later.

Alternatives to joining this study include no participating in this study.

PURPOSE (WHY THIS RESEARCH IS BEING DONE)

301148-UT Approved
09/02/2021 - 09/01/2022

You are being asked to take part in a research study of the influence of brain function associated with reaction time and processing speed and brain-to-muscle function on landing tasks in healthy active females. We are also determining the reliability of a functional brain-to-muscle testing protocol. The purpose of the study is to:

1. investigate the associations between brain-to-muscle function and brain function related to reaction time and processing speed with the changes in mechanics during double-limb landing tasks in healthy females
2. investigate the reliability of quadriceps transcranial magnetic stimulation derived outcome measures during a single leg squat within a 14-day period in healthy females

You were selected as someone who may want to take part in this study because you are a healthy active female and we anticipate needing 25-participants for this study.

DESCRIPTION OF THE RESEARCH PROCEDURES AND DURATION OF YOUR INVOLVEMENT

If you decide to take part in this study, you will be asked to report to the Motion Analysis and Integrative Neurophysiology (MAIN) Laboratory at the University of Toledo for all testing procedures.

Visit 1:

Eligibility Screening

You will be asked several questions about your healthy status. Once your eligibility has been confirmed, your age, gender, height, and weight will be recorded.

Participant Set-up (about 5-minutes)

- You will be asked to lie on your back on a treatment table to expose the front of your thigh.
- The skin over the front of your thigh and one location on your lower leg will be shaved, cleaned with alcohol, and debrided with guaze to prepare for application of surface electromyography (EMG) recording electrodes. Cleansing these areas is necessary to remove any oils, lotions, and dry skin that may interfere with therecording of an electrical response.
- EMG electrodes will be placed over two muscles on the front of your thigh, and in one location on the front of your lower leg.
- You will then be asked to be seated in a stationary chair that will be used to test your muscle strength. Your hips will be secured, as will your shoulders, with straps. Your ankle will be secured to a padded strap below the chair. This strap is connected to a device designed to measure how much force you can produce.

Transcranial Magnetic Stimulation (about 1 hour and 15 minutes)

This test measures the quality of the signal from your brain to your thigh muscles.

- You will be asked to remain in the chair with your knee bent to 70 a degree angle.
- You will be fitted with a Lycra swim cap and asked to sit comfortably with your hands folded in your lap. The purpose of the swim cap is to mark certain points or features of your head that will be used to determine the best areas of magnetic coil placement for stimulation.
- You will be given a pair of disposable earplugs to wear during the testing. The purpose of the earplugs is to limit the clicking sound that you hear that comes from the magnetic stimulation coil.
- A device will be placed against your head, resting against the swim cap. This device is a magnetic stimulator that is capable of delivering a safe, non-painful stimulus through the scalp and underlying skull. This magnetic response is detected by the brain as an electrical potential and will be recorded by the EMG electrodes placed on your thigh. The magnetic impulse used to stimulate the brain causes an audible "click" associated with the magnetic stimulating coil, and a brief muscle contraction (similar to a muscle "twitch") in the muscles of your thigh or leg, which will feel like what is felt during standard medical reflex testing. Otherwise there is no, or a minimal, chance of discomfort associated with the testing.

301148-UT Approved
09/02/2021 - 09/01/2022

- You will be asked to kick out between 10-15% of your maximal quadriceps' EMG activity determined from 3 maximal quadriceps contractions. Two lines that represents this amount of effort will be displayed on a TV screen, and used to help ensure the appropriate effort is given.
- There is a minimal risk for obtaining a mild headache following the application of the stimulus. The audible "click" from the magnetic stimulator will not harm your ears, but earplugs are required for your comfort.
- Once the best placement for the magnetic coil is detected, you will be removed from the chair and will stand in front of a TV screen. The EMG activity from the maximal strength testing will help determine 10-15% of the quadriceps activation during this functional task and two lines that represents this effort will be displayed across the TV screen. As you perform the squat, you will see a line representing your muscle activity that you will fit between the 10-15% lines. Once the muscle activity falls between the 10-15% lines, a single stimulus from the transcranial magnetic stimulation will be delivered.
- The intensity of the magnetic pulse will be gradually increased and decreased until a maximum electrical response is detected and recorded. You will not experience any discomfort despite the increased intensity of the stimulation. Once the best response is detected and recorded, 8 more single leg squat trials will be conducted to record the maximal electrical response of each squat
- Once this is determined on one leg we will perform the same procedures on the opposite leg.
- To determine your knee angle during the single leg squat, we will use a 2D camera to record the trials.
    - Permission to record: Will you permit the researcher to video record or audio record you during this research procedure?

YES ☐ NO ☐ Initial Here _____

Visit 2:

This is one visit that will be conducted between Visit 1 and Visit 3. This visit will be conducted between 4 to 11 days after Visit 1.

Immediate Post-Concussion Assessment & Cognitive Testing (ImPACT) (about 20 minutes)

This assessment is commonly used to determine neurocognitive function prior to and after concussion. The test evaluates reaction time, visual memory, verbal memory, and processing speed. This is a computerized test and takes about 20 minutes to complete.

NASA Task Load Index Survey (about 2 minutes for each test)

This survey is used to determine your cognitive workload and fatigue for each of the tasks to be completed. You will be asked to complete one of these surveys after each biomechanical task.

Three Dimensional (3D) Biomechanical Assessment (about 1 hour)

45 retroreflective markers will be put on your trunk and lower body to track how your joints move.
Landing Error Scoring System (LESS)

- You will be asked to stand on a raised platform (about 12 inches high).
- You will then be asked to step down and then jump straight up.
- You will be asked to complete this task until 5-successful trials are completed.
- You will then perform the same task but there will be a screen in front of you. You will be instructed to look at the screen only during the task. Once the task is started, a string of numbers will flash on the screen and you have to remember them after you complete the task and recite them to the investigation team. You will complete this task until 5-successful trials are completed.
- To clinically score the LESS, we will use 2D video to record the landing assessment.

301148-UT Approved
09/02/2021 - 09/01/2022

- Permission to record: Will you permit the researcher to video record or audio record you during this research procedure?

YES ☐ NO ☐ Initial Here _____

Single Leg Cut

- Similar to the LESS you will start on a raised platform in a single leg stance. You will hop off the box and you will have to cut either to the left or right depending on the stance limb.
- You will be asked to compelte this task until 5-successful trials are completed in each direction.
- You will then perform the same task but there will be a screen in front of you. The screen will show different colors which will determine whether you will perform a cut or if you just perform a single leg landing without a cut. You will complete this task until 5-successful trials are completed in each direction.

Visit 3:

On this final visit, you will be asked to perform the same procedures as Visit 1 to determine the inter-session reliability of the functional transcranial magnetic stimulation outcome measures.

RISKS AND DISCOMFORTS YOU MAY EXPERIENCE IF YOU TAKE PART IN THIS RESEARCH

Potential Risks/Discomforts	**Seriousness** ***Low, Moderate, High***	**Likelihood of Complications** ***Common > 15%, Occasional 5-15%, Uncommon 1-5%, Rare <1%***
Skin irritation (EMG electrodes/Reflective Markers)	Low	Occasional
Muscle Soreness (strength testing/biomechanics testing)	Low	Uncommon
Discomfort (transcranial magnetic stimulation)	Low	Uncommon
Transient Headache	Low	Rare
Lower Extremity Injury (biomechanics testing)	Low	Rare
Falling (biomechanics testing)	Low	Rare

Mild skin irritation may occur occasionally with the placement of self-adhesive EMG electrodes on your skin. If this occurs, it is temporary, and will not cause discomfort. Mild muscle soreness may occur after strength testing and after biomechanics testing as we are asking you to perform physical exercise. If this occurs, it is temporary, and commonly resolves within several days. Mild discomfort is uncommon, but may occur during the transcranial magnetic stimulation protocol. The transcranial magnetic stimulation protocol is commonly described as a feeling similar to an involuntary reflex, like when the doctor taps the tendon below you knee. Although rare, you may experience a mild, temporary headache after receiving the stimulus from the brain-to-muscle function testing. This is not common, and commonly resolves within minutes to hours if reported. No serious adverse events, including seizure, have been reported in our laboratory when using the single pulse transcranial magnetic stimulation protocol. Rare, non-serious, reports of mild transient headaches have been recorded and have fully resolved. Furthermore, published reports indicate that there are no available data to suggest that single- and paired-pulse transcranial magnetic stimulation is associated with more than minimal risk.

301148-UT Approved
09/02/2021 - 09/01/2022

RISKS TO UNBORN CHILDREN
This research represents a significant risk to unborn children. Therefore, if you are a female of childbearing potential, you will be given a pregnancy test prior to the start of this research. If this test is positive, you will not be able to take part in this research. If your pregnancy test is negative at present and you choose to take part in this research, you will be given information on birth control procedures that must be used while you are taking part in this research so that you can avoid getting pregnant. You also will be told about the danger to the fetus (unborn child) should you become pregnant.

Please be sure to ask the researcher any questions that you may have about acceptable methods of birth control and the risk to you, your partner or your unborn child at any time before or, if you decide to enroll, while you are taking part in this research.

POSSIBLE BENEFIT TO YOU IF YOU DECIDE TO TAKE PART IN THIS RESEARCH
The information from this study will help clinicians understand further modifiable risk factors for lower extremity injury and potentially improve patient care. We cannot and do not guarantee or promise that you will receive any benefits from this research.

COST TO YOU FOR TAKING PART IN THIS STUDY
All procedures in this study will be provided at no cost to you or your health insurance. You will be responsible for the cost of travel to come to any study visit and for any parking costs.

PAYMENT OR OTHER COMPENSATION TO YOU FOR TAKING PART IN THIS RESEARCH
For completing all of the procedures of this study, you will receive a $30 gift card.

ALTERNATIVE(S) TO TAKING PART IN THIS RESEARCH
The only alternative to taking part in this research is not to participate.

CONFIDENTIALITY
Participation in research involves collecting and using your personal information to conduct research. The members of the research team will use this information, together with the data or other information collected from you as described above in the Description of Research Procedures section, for the purpose of conducting this research study. While very unlikely, there is always a risk of breach in confidentiality. All appropriate measures to protect your private information will be taken. Research records will be stored in a locked cabinet (HHS 1412) and electronically on a password protected server. All data will be given a letter and number that is uniquely associated with you. This code will not contain any partial identifiers (i.e. last four digits of your SSN, etc.) and will be stored in a separate secure location. No identifiers will be stored with the research data. Only those individuals with an active role in this study will have access to the research data have access to identifying information. The signed documents will be kept indefinitely. When all participants' have completed active participation in the study and data collection is completed, the code will be destroyed.

We will use your information for the purpose of conducting the research study as described in the research consent form, and it will remain confidential. Your information will be stored in a locked filing cabinet and electronically on a password protected computer. Only members of the research team will have access to your information.

With your permission, the identifiable information that are collected from your participation in this research may be used in future research studies without your consent, but only after your identifying information has been removed from the information. If you do not grant permission for your data to be de-identified and used for future research purposes, you **can** still participate in the research described in

301148-UT Approved
09/02/2021 - 09/01/2022

this document. Your agreement to this is voluntary and there are no consequences should you decline to allow your data to be used for future research purposes.

If you agree to allow us to use and/or share your de-identified information for future research purposes, please place your initials here: ____ (opt-in)

We may also use your information to contact you after this study is closed to update your contact information should we decide it is important to continue following your progress, or to open a new study to follow-up on people who take part in this study. To authorize research staff from The University of Toledo to contact you to update your information or invite you to participate in a new follow-up study, place your initials here: _____(opt-in).

The results of this study could be published in an article, but the publication would not include any information that would let others know who you are.

Under some circumstances, the Institutional Review Board, or the Research and Sponsored Programs of the University of Toledo or their designees may review your information for compliance audits. If you receive any payments for taking part in this study, your personal information and limited information about this study will be given to The University of Toledo's accounts payable department as necessary to process payment to you. We may also disclose your personal information when required by law, such as in response to judicial orders.

You have the right to revoke (cancel) the permission you have given use to use your personal information at any time by giving written notice to the principal investigator. However, the cancelation will not prevent us from using information obtained prior to the cancelation as necessary to maintain the integrity of the research study.

IN THE EVENT OF A RESEARCH-RELATED INJURY

If you suffer a research-related injury, medical treatment is available but you can choose where to go for treatment.

The University of Toledo and The University of Toledo Medical Center do not offer reimbursement for medical expenses or other compensation for research-related injuries. In the event that any medical expenses are not reimbursed by the Sponsor, they will be billed to you or your insurance.

By signing this form you do not give up any of your legal rights if you are injured.

In the event of a research-related injury, contact:

Grant Norte, PhD, AT, ATC, CSCS at (951) 529-6923.

VOLUNTARY PARTICIPATION

Taking part in this study is voluntary. You may refuse to participate or discontinue participation at any time without penalty or a loss of benefits to which you are otherwise entitled. If you decide not to participate or to discontinue participation, your decision will not affect your future relations with the University of Toledo or The University of Toledo Medical Center.

NEW FINDINGS

You will be notified of new information that might change your decision to be in this study if any becomes available.

CONTINUED NEXT PAGE

OFFER TO ANSWER QUESTIONS

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over. If you have questions regarding the research at any time before, during or after the study, you may contact Grant Norte, PhD, AT, ATC, CSCS at (419) 530-5305 or grant.norte@utoledo.edu.

If you have questions beyond those answered by the research team or your rights as a research subject or research-related injuries, please feel free to contact the Chairperson of the University of Toledo Biomedical Institutional Review Board at 419-383-6796.

SIGNATURE SECTION (Please read carefully)

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDICATES THAT YOU HAVE READ THE INFORMATION PROVIDED ABOVE, YOU HAVE HAD ALL YOUR QUESTIONS ANSWERED, AND YOU HAVE DECIDED TO TAKE PART IN THIS RESEARCH.

The date you sign this document to enroll in this study, that is, today's date, MUST fall between the dates indicated on the approval stamp affixed to the bottom of each page. These dates indicate that this form is valid when you enroll in the study but do not reflect how long you may participate in the study. Each page of this Consent Form is stamped to indicate the form's validity as approved by the UT Biomedical Institutional Review Board (IRB).

Name of Subject (please print)	Signature of Subject or Person Authorized to Consent	Date
Relationship to the Subject (Healthcare Power of Attorney authority or Legal Guardian)		Time a.m. p.m.
Name of Person Obtaining Consent (please print)	Signature of Person Obtaining Consent	Date
Name of Witness to Consent Process (when required by ICH Guidelines) (please print)	Signature of Witness to Consent Process (when required by ICH Guidelines)	Date

YOU WILL BE GIVEN A SIGNED COPY OF THIS FORM TO KEEP.

301148-UT Approved
09/02/2021 - 09/01/2022

Questionnaires

Eligibility Checklist

IRB #:

MAIN LAB ELIGIBILITY CHECKLIST

Subject ID: _____ Investigator Initials: _____

Age: _____ Height (in): _____ Weight (lbs): _____

Date of Visit: _____ Time of Day: _____

Dominant Limb: R L

INCLUSION CRITERIA

Yes	No	
☐	☐	Age of 18 – 30 years
☐	☐	No history of lower extremity surgery
☐	☐	No history of lower extremity injury in the last 12 months
☐	☐	Tegner Activity Scale rating of 5 or greater

EXCLUSION CRITERIA

Yes	No	
☐	☐	Lower extremity joint injury or surgery within previous 12 months
☐	☐	History of brain surgery, stroke, migranes, and/or severe head injury
☐	☐	History of concussion within the last 12 months
☐	☐	Known history of neurological or psychiatric disorders (poorly controlled migraines, seizure disorder, history or immediate family history of seizures and/or epilepsy)
☐	☐	Current form of neuropathy (numbness and tingling)
☐	☐	Implanted biomedical device (active or inactive) implants (including device leads, deep brain stimulators, cochlear implants, and vagus nerve stimulators)
☐	☐	Known history of cardiopulmonary disorder
☐	☐	Pregnancy
☐	☐	Currently taking any medications which may influence objective clinical data (e.g. antispastics, anxiolytics, hypnotics, ant-epileptics)

Have you consumed and of the following stimulants or depressants within the last 24 hours?

☐ Caffeine ☐ Alcohol ☐ Tobacco

Comments:

_____

_____

_____

_____

_____

_____

_____

_____

_____

301148-UT Approved
09/02/2021 - 09/01/2022

Tegner Activity Scale

Tegner Activity Scale

Please indicate in the spaces below the HIGHEST level of activity that you participated in BEFORE YOUR INJURY and the highest level you are able to participate in CURRENTLY.

BEFORE INJURY: Level _____ CURRENT: Level _____

Level 10	Competitive sports – soccer, football, rugby (national elite)
Level 9	Competitive sports – soccer, football, rugby (lower divisions), ice hockey, wrestling, gymnastics, basketball
Level 8	Competitive sports – racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing
Level 7	Competitive sports – tennis, running, motorcars speedway, handball Recreational sports – soccer, football, rugby, bandy, ice hockey, basketball, squash, racquetball, running
Level 6	Recreational sports – tennis and badminton, handball, racquetball, down-hill skiing, jogging at least 5 times per week
Level 5	Work – heavy labor (construction, etc.) Competitive sports – cycling, cross-country skiing Recreational sports – jogging on uneven ground at least twice weekly
Level 4	Work – moderately heavy labor (e.g. truck driving, etc.)
Level 3	Work – light labor (nursing, etc.)
Level 2	Work – light labor Walking on uneven ground possible, but impossible to back pack or hike
Level 1	Work – sedentary (secretarial, etc.)
Level 0	Sick leave or disability pension because of knee problems

301148-UT Approved
09/02/2021 -
09/01/2022

Rate of Perceived Exertion

#	Level of Exertion
6	No exertion at all
7	
7.5	Extremely light (7.5)
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Sample Size Estimation

Intraclass Correlation Coefficient (ICC) - Hypothesis Testing[1]

Minimum acceptable reliability (ICC) (ϱ_0):	0.50	
Expected reliability (ICC) (ϱ_1):	0.85	
Significance level (α):	0.05	Two-tailed
Power (1 - β):	80	%
Number of raters/repetitions per subject (k):	2	
Expected dropout rate:	15	%

Calculate Reset

Sample size, n =	17
Sample size (with 15% dropout), n_{drop} =	20

Appendix D

Additional Results

Table D.1: Comparison of measures over a 14-day period

Measure	Dominant VM		Non-Dominant VM	
	Day 1	Day 14	Day 1	Day 14
Normalized MVIC (Nm/kg)	2.51 ± 0.86	2.30 ± 0.78	2.36 ± 0.94	2.41 ± 0.74
AMT 120%	32.53 ± 5.83	31.67 ± 5.72	34.20 ± 5.72	31.40 ± 7.69
Normalized MEP	13.18 ± 10.30	12.72 ± 8.61	12.93 ± 6.64	11.67 ± 9.06
Knee Flexion Angle (°)	28.61 ± 10.42	27.68 ± 8.33	27.42 ± 9.67	29.47 ± 8.28
RPE	8.36 ± 1.43	7.33 ± 1.29	8.08 ± 1.41	7.33 ± 1.16

Abbreviations: MVIC, maximum voluntary isometric contraction; AMT, active motor threshold; MEP, motor evoked potential; RPE, rate of perceived exertion

Table D.2: Bland-Altman Plot Vastus Medialis AMT and MEP Data

Outcome Measure	Mean Difference	SD	Lower Limit of Agreement	Upper Limit of Agreement	Range	% of Data Points Outside of LOA
Dominant AMT	0.722	3.511	-6.301	7.745	14.046	0.00
Non-Dominant AMT	2.556	6.280	-10.004	15.115	25.120	5.56
Dominant MEP	-0.684	10.022	-20.728	19.361	40.089	5.88
Non-Dominant MEP	2.831	8.262	-13.692	19.355	33.047	11.76

Abbreviations: AMT, active motor threshold; MEP, motor evoked potential; SD, standard deviation; LOA, limit of agreement

Table D.3: Comparison of descriptive data using independent t-test

Knee Joint Angle	t-score	p-value
Dominant	0.505	0.62
Non-Dominant	-1.208	0.244

Appendix E

Back Matter

Recommendations for future research

For future research, I would recommend that more research is conducted with control and pathological populations. We can potentially strengthen the reliability of TMS-derived outcome measures. If the active motor threshold is reliable in both limbs during functional testing, we can utilize it in further outcome measure research. Then, we can use AMT to determine risk factors for lower extremity injuries. Additionally, clinicians can evidently use dominant AMT measures to target neuromuscular control post injury.

NATA Conference Abstract

Context: Altered corticospinal pathways contribute to quadriceps dysfunction following anterior cruciate ligament reconstruction (ACLR). To date, very few studies have explored the reliability of corticospinal excitability, as measured by transcranial magnetic stimulation (TMS), during functional tasks. Understanding the reliability of closed kinetic chain TMS measures can expand our knowledge of brain-to-muscle communication during activities of daily living by serving as a dynamic assessment tool to identify neuromuscular deficiencies that may relate to lower extremity injury in healthy, active individuals. **Objective:** To investigate the intersession reliability of TMS-derived outcomes of quadriceps corticomotor function during a single leg squat in healthy, active individuals. **Design:** A descriptive laboratory study with a test-retest design. **Subjects:** 18 healthy active females (21.83 $\pm$ 2.57 years, 166.40 ± 6.43 cm, 66.26 $\pm$ 14.38 kg). **Independent variable(s):** Time (day 1 and 14) and limb (dominant and non-dominant) were assessed. **Main Outcome Measure(s):** Active motor threshold (AMT) and normalized motor evoked potentials (MEPs) were assessed in the vastus medialis (VM). Two-way mixed effects intraclass correlation coefficients were used to assess intersession reliability for internal consistency ($ICC_{Consistency}$) and absolute agreement ($ICC_{Absolute}$). Bland-Altman plots were used to further describe agreement between sessions. Minimal detectable differences with 95% confidence intervals (MDC_{95}) were calculated for significant findings. **Results:** The dominant AMT produced the highest reliability ($ICC_{Consistency}$ = 0.734, $ICC_{Absolute}$ = 0.737, $p < .011$, MDC_{95} = 6.77) with moderate agreement between sessions and acceptable degree of agreement through Bland-Altman plot analysis. However, the non-dominant AMT produced the lowest reliability ($ICC_{Consistency}$ = 0.179, $ICC_{Absolute}$ =0.188, MDC_{95}=18.39). Dominant

($ICC_{Consistency}$ = 0.179, $ICC_{Absolute}$ =0.188, MDC_{95}=18.39) and non-dominant ($ICC_{Consistency}$ = 0.249, $ICC_{Absolute}$ =0.238, MDC_{95}=15.53) MEPs had poor reliability. **Conclusion:** Although previous literature established that TMS is reliable when performing open kinetic chain tasks in the lower extremity, only the dominant limb AMT was moderately reliable in our study. We found that non-dominant AMT and bilateral MEP measurements have poor intersession reliability over a two-week period. Future research is likely warranted to improve the standardization of this technique prior to incorporating in outcomes research. **Word Count:** 336

NATA Poster

Intersession Reliability of Quadriceps Corticospinal Excitability: A Functional TMS Study

Kiana Young, AT, ATC, Grant Norte, PhD, AT, ATC, CSCS, Justin Rush, MS, AT, ATC , David Bazett-Jones, PhD, AT, ATC, CSCS, Adam Lepley, PhD, ATC
Motion Analysis & Integrative Neurophysiology Laboratory , University of Toledo

THE UNIVERSITY OF TOLEDO

INTRODUCTION

Altered corticospinal pathways contribute to quadriceps dysfunction following a knee injury. To date, very few studies explore the reliability of corticospinal excitability, as measured by transcranial magnetic stimulation (TMS), during functional tasks. Understanding the reliability of closed kinetic chain TMS measures can expand our knowledge of brain and muscle communication during activities of daily living by serving as a dynamic assessment tool to identify neuromuscular deficiencies that may relate to lower extremity injury in healthy, active individuals.

Purpose: to investigate the intersession reliability of quadriceps corticospinal excitability outcome measures (AMT and MEP amplitude) in the dominant and nondominant limbs during a single leg squat in healthy, active females over 14 days.

METHODS

Study Design: Descriptive laboratory study with a test-retest design
Subjects: 18 participants (age: 21.83 ± 2.57 years, height: 166.40 ± 6.43cm, weight: 66.26 ± 14.38kg, dominant limb: 18 Right/0 Left)

Inclusion Criteria: 18-30 years old healthy active females with a Tegner Activity Scale rating ≥ 5
Exclusion Criteria: lower extremity injury/surgery, history of severe head injury/illness, neurological or muscular disorder, medication that alters neural excitability

Independent Variables:
Time at 2 levels: day 1 & day 14
Limb at 2 levels: dominant & non-dominant
Dependent Variables:
Active motor threshold (AMT, %)
Normalized motor evoked potential (MEP)

Procedures:
MVIC - 3 trials
Hotspot Mapping
Functional TMS
– 8 single leg squats to 10-15% MVIC at 120% AMT for each limb

Statistical Analysis: Intraclass correlation coefficient, standard error of measurement, minimal detectable change, Bland-Altman plots

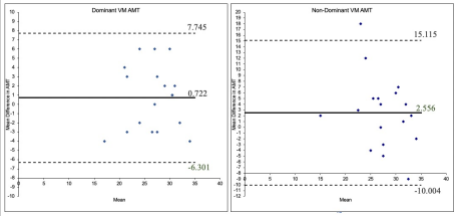
RESULTS

Measure	n	ICC (Consistency)	ICC (Absolute)	p-value	SEM	MDC
Dom AMT	18	0.734	0.737	< 0.001	2.44	6.77
Dom MEP	17	0.179	0.188	0.349	6.63	18.39
Non AMT	18	0.382	0.357	0.053	4.58	12.70
Non MEP	17	0.249	0.238	0.287	5.57	15.43

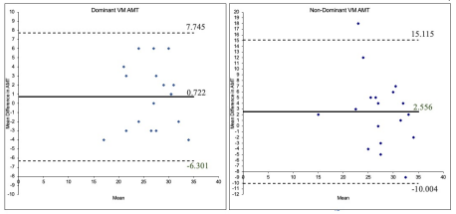
Dominant Vastus Medialis Active Motor Threshold is **moderately** reliable during a single leg squat over a 14-day period

Good
Moderate
Poor

Active Motor Threshold Bland-Altman Plots:



Motor Evoked Potential Bland-Altman Plots:



CONCLUSIONS

Although previous literature established that TMS is reliable when performing open kinetic chain tasks in the lower extremity, only the dominant limb AMT was moderately reliable in our study. We found that non-dominant AMT and bilateral MEP measurements have poor intersession reliability over a 14-day period. Future research would need to improve the methods such as including large control and pathological populations to enhance the reliability of fTMS in outcome measure research.

REFERENCES

1. Luc BA, Lepley AS, Tevald MA, Gribble PA, White DB, Pietrosimone BG. Reliability of corticomotor excitability in leg and thigh musculature at 14 and 28 days. *J Sport Rehabil*. Nov 2014;23(4):330-8. doi:10.1123/jsr.2013-0069
2. Pietrosimone BG, Lepley AS, Ericksen HM, Clements A, Sohn DH, Gribble PA. Neural Excitability Alterations After Anterior Cruciate Ligament Reconstruction. *J Athl Train*. Jun 2015;50(6):665-74. doi:10.4085/1062-6050-50.1.11
3. Proessl F, Beckner ME, Sinnott AM, et al. Reliability of corticospinal excitability estimates for the vastus lateralis: Practical considerations for lower limb TMS task selection. *Brain Res*. Mar 2 2021;1761:147395. doi:10.1016/j.brainres.2021.147395
4. Scheurer SA, Sherman DA, Glaviano NR, Ingersoll CD, Norte GE. Corticomotor function is associated with quadriceps rate of torque development in individuals with ACL surgery. *Exp Brain Res*. Feb 2020;238(2):283-294. doi:10.1007/s00221-019-05713-w

FUELING TOMORROWS

@UToledo_MAINLab

Appendix F

Bibliography

1. Hootman JM, Macera CA, Ainsworth BE, Addy CL, Martin M, Blair SN. Epidemiology of musculoskeletal injuries among sedentary and physically active adults. *Med Sci Sports Exerc*. May 2002;34(5):838-44. doi:10.1097/00005768-200205000-00017
2. Griffin LY, Albohm MJ, Arendt EA, et al. Understanding and preventing noncontact anterior cruciate ligament injuries: a review of the Hunt Valley II meeting, January 2005. *Am J Sports Med*. Sep 2006;34(9):1512-32. doi:10.1177/0363546506286866
3. Pietrosimone BG, McLeod MM, Lepley AS. A theoretical framework for understanding neuromuscular response to lower extremity joint injury. *Sports Health*. Jan 2012;4(1):31-5. doi:10.1177/1941738111428251
4. Ingersoll CD, Grindstaff TL, Pietrosimone BG, Hart JM. Neuromuscular consequences of anterior cruciate ligament injury. *Clin Sports Med*. Jul 2008;27(3):383-404, vii. doi:10.1016/j.csm.2008.03.004
5. Pamukoff DN, Pietrosimone BG, Ryan ED, Lee DR, Blackburn JT. Quadriceps Function and Hamstrings Co-Activation After Anterior Cruciate Ligament Reconstruction. *J Athl Train*. May 2017;52(5):422-428. doi:10.4085/1062-6050-52.3.05
6. Pietrosimone BG, Lepley AS, Ericksen HM, Gribble PA, Levine J. Quadriceps strength and corticospinal excitability as predictors of disability after anterior cruciate ligament reconstruction. *J Sport Rehabil*. Feb 2013;22(1):1-6. doi:10.1123/jsr.22.1.1
7. Blackburn JT, Pietrosimone B, Harkey MS, Luc BA, Pamukoff DN. Quadriceps Function and Gait Kinetics after Anterior Cruciate Ligament Reconstruction. *Med Sci Sports Exerc*. Sep 2016;48(9):1664-70. doi:10.1249/MSS.0000000000000963

8. Pietrosimone BG, Gribble PA. Chronic ankle instability and corticomotor excitability of the fibularis longus muscle. *J Athl Train*. Nov-Dec 2012;47(6):621-6. doi:10.4085/1062-6050-47.6.11

9. Ward SH, Pearce A, Bennell KL, Pietrosimone B, Bryant AL. Quadriceps cortical adaptations in individuals with an anterior cruciate ligament injury. *Knee*. Aug 2016;23(4):582-7. doi:10.1016/j.knee.2016.04.001

10. Pietrosimone BG, Lepley AS, Ericksen HM, Clements A, Sohn DH, Gribble PA. Neural Excitability Alterations After Anterior Cruciate Ligament Reconstruction. *J Athl Train*. Jun 2015;50(6):665-74. doi:10.4085/1062-6050-50.1.11

11. Bryant AL, Kelly J, Hohmann E. Neuromuscular adaptations and correlates of knee functionality following ACL reconstruction. *J Orthop Res*. Jan 2008;26(1):126-35. doi:10.1002/jor.20472

12. Lepley AS, Ericksen HM, Sohn DH, Pietrosimone BG. Contributions of neural excitability and voluntary activation to quadriceps muscle strength following anterior cruciate ligament reconstruction. *Knee*. Jun 2014;21(3):736-42. doi:10.1016/j.knee.2014.02.008

13. Needle AR, Lepley AS, Grooms DR. Central Nervous System Adaptation After Ligamentous Injury: a Summary of Theories, Evidence, and Clinical Interpretation. *Sports Med*. Jul 2017;47(7):1271-1288. doi:10.1007/s40279-016-0666-y

14. Anand S, Hotson J. Transcranial magnetic stimulation: neurophysiological applications and safety. *Brain Cogn*. Dec 2002;50(3):366-86. doi:10.1016/s0278-2626(02)00512-2

15. Luc BA, Lepley AS, Tevald MA, Gribble PA, White DB, Pietrosimone BG. Reliability of corticomotor excitability in leg and thigh musculature at 14 and 28 days. *J Sport Rehabil*. Nov 2014;23(4):330-8. doi:10.1123/jsr.2013-0069

16. Weavil JC, Amann M. Corticospinal excitability during fatiguing whole body exercise. *Prog Brain Res*. 2018;240:219-246. doi:10.1016/bs.pbr.2018.07.011

17. Van Wittenberghe IC, Peterson DC. Corticospinal Tract Lesion. *StatPearls*. 2020.

18. Rossini PM, Rossi S, Babiloni C, Polich J. Clinical neurophysiology of aging brain: from normal aging to neurodegeneration. *Prog Neurobiol*. Dec 2007;83(6):375-400. doi:10.1016/j.pneurobio.2007.07.010

19. Groppa S, Oliviero A, Eisen A, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol*. May 2012;123(5):858-82. doi:10.1016/j.clinph.2012.01.010

20. Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. *Lancet Neurol*. Mar 2003;2(3):145-56. doi:10.1016/s1474-4422(03)00321-1

21. Fisher BE, Wu AD, Salem GJ, et al. The effect of exercise training in improving motor performance and corticomotor excitability in people with early Parkinson's disease. *Arch Phys Med Rehabil*. Jul 2008;89(7):1221-9. doi:10.1016/j.apmr.2008.01.013

22. Kline PW, Morgan KD, Johnson DL, Ireland ML, Noehren B. Impaired Quadriceps Rate of Torque Development and Knee Mechanics After Anterior Cruciate Ligament Reconstruction With Patellar Tendon Autograft. *Am J Sports Med*. Oct 2015;43(10):2553-8. doi:10.1177/0363546515595834

23. van Hedel HJ, Murer C, Dietz V, Curt A. The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for torque and motor task. *J Neurol*. Aug 2007;254(8):1089-98. doi:10.1007/s00415-006-0493-4

24. Cacchio A, Cimini N, Alosi P, Santilli V, Marrelli A. Reliability of transcranial magnetic stimulation-related measurements of tibialis anterior muscle in healthy subjects. *Clin Neurophysiol*. Feb 2009;120(2):414-9. doi:10.1016/j.clinph.2008.11.019

25. Temesi J, Ly SN, Millet GY. Reliability of single- and paired-pulse transcranial magnetic stimulation for the assessment of knee extensor muscle function. *J Neurol Sci*. Apr 15 2017;375:442-449. doi:10.1016/j.jns.2017.02.037

26. O'Leary TJ, Morris MG, Collett J, Howells K. Reliability of single and paired-pulse transcranial magnetic stimulation in the vastus lateralis muscle. *Muscle Nerve*. Oct 2015;52(4):605-15. doi:10.1002/mus.24584

27. Scheurer SA, Sherman DA, Glaviano NR, Ingersoll CD, Norte GE. Corticomotor function is associated with quadriceps rate of torque development in individuals with ACL surgery. *Exp Brain Res*. Feb 2020;238(2):283-294. doi:10.1007/s00221-019-05713-w

28. Seo HY, Kim GW, Won YH, Park SH, Seo JH, Ko MH. Changes in Intracortical Excitability of Affected and Unaffected Hemispheres After Stroke Evaluated by Paired-Pulse Transcranial Magnetic Stimulation. *Ann Rehabil Med*. Aug 2018;42(4):495-501. doi:10.5535/arm.2018.42.4.495

29. Masse-Alarie H, Elgueta Cancino E, Schneider C, Hodges P. Paired-Pulse TMS and Fine-Wire Recordings Reveal Short-Interval Intracortical Inhibition and Facilitation of

Deep Multifidus Muscle Fascicles. *PLoS One*. 2016;11(8):e0159391. doi:10.1371/journal.pone.0159391

30. Annavarapu RN, Kathi S, Vadla VK. Non-invasive imaging modalities to study neurodegenerative diseases of aging brain. *J Chem Neuroanat*. Jan 2019;95:54-69. doi:10.1016/j.jchemneu.2018.02.006

31. Liu S, Cai W, Liu S, et al. Multimodal neuroimaging computing: a review of the applications in neuropsychiatric disorders. *Brain Inform*. Sep 2015;2(3):167-180. doi:10.1007/s40708-015-0019-x

32. Rossini PM, Burke D, Chen R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol*. Jun 2015;126(6):1071-1107. doi:10.1016/j.clinph.2015.02.001

33. Rossini PM, Rossi S. Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential. *Neurology*. Feb 13 2007;68(7):484-8. doi:10.1212/01.wnl.0000250268.13789.b2

34. Rossini PM, Rossini L, Ferreri F. Brain-behavior relations: transcranial magnetic stimulation: a review. *IEEE Eng Med Biol Mag*. Jan-Feb 2010;29(1):84-95. doi:10.1109/MEMB.2009.935474

35. Brownstein CG, Ansdell P, Skarabot J, Howatson G, Goodall S, Thomas K. An optimal protocol for measurement of corticospinal excitability, short intracortical inhibition and intracortical facilitation in the rectus femoris. *J Neurol Sci*. Nov 15 2018;394:45-56. doi:10.1016/j.jns.2018.09.001

36. Talelli P, Greenwood RJ, Rothwell JC. Arm function after stroke: neurophysiological correlates and recovery mechanisms assessed by transcranial magnetic stimulation. *Clin Neurophysiol*. Aug 2006;117(8):1641-59. doi:10.1016/j.clinph.2006.01.016

37. Wang J, Meng HJ, Ji GJ, et al. Finger Tapping Task Activation vs. TMS Hotspot: Different Locations and Networks. *Brain Topogr*. Jan 2020;33(1):123-134. doi:10.1007/s10548-019-00741-9

38. Wagle-Shukla A, Ni Z, Gunraj CA, Bahl N, Chen R. Effects of short interval intracortical inhibition and intracortical facilitation on short interval intracortical facilitation in human primary motor cortex. *J Physiol*. Dec 1 2009;587(Pt 23):5665-78. doi:10.1113/jphysiol.2009.181446

39. Neto T, Sayer T, Theisen D, Mierau A. Functional Brain Plasticity Associated with ACL Injury: A Scoping Review of Current Evidence. *Neural Plast*. 2019;2019:3480512. doi:10.1155/2019/3480512

40. Kaneko F, Onari K, Kawaguchi K, Tsukisaka K. The main factor causing prolonged reaction time on force producing process following anterior cruciate ligament reconstruction. *Hiroshima J Med Sci*. Dec 2000;49(4):145-51.

41. Maffiuletti NA, Aagaard P, Blazevich AJ, Folland J, Tillin N, Duchateau J. Rate of force development: physiological and methodological considerations. *Eur J Appl Physiol*. Jun 2016;116(6):1091-116. doi:10.1007/s00421-016-3346-6

42. Andersen LL, Aagaard P. Influence of maximal muscle strength and intrinsic muscle contractile properties on contractile rate of force development. *Eur J Appl Physiol*. Jan 2006;96(1):46-52. doi:10.1007/s00421-005-0070-z

43. Folland JP, Buckthorpe MW, Hannah R. Human capacity for explosive force production: neural and contractile determinants. *Scand J Med Sci Sports*. Dec 2014;24(6):894-906. doi:10.1111/sms.12131

44. Morel B, Rouffet DM, Saboul D, Rota S, Clemencon M, Hautier CA. Peak torque and rate of torque development influence on repeated maximal exercise performance: contractile and neural contributions. *PLoS One*. 2015;10(4):e0119719. doi:10.1371/journal.pone.0119719

45. Cossich V, Maffiuletti NA. Early vs. late rate of torque development: Relation with maximal strength and influencing factors. *J Electromyogr Kinesiol*. Dec 2020;55:102486. doi:10.1016/j.jelekin.2020.102486

46. Conchola EC, Thiele RM, Palmer TB, Smith DB, Thompson BJ. Effects of neuromuscular fatigue on electromechanical delay of the leg extensors and flexors in young men and women. *Muscle Nerve*. Nov 2015;52(5):844-51. doi:10.1002/mus.24598

47. Sonnery-Cottet B, Saithna A, Quelard B, et al. Arthrogenic muscle inhibition after ACL reconstruction: a scoping review of the efficacy of interventions. *Br J Sports Med*. Mar 2019;53(5):289-298. doi:10.1136/bjsports-2017-098401

48. Palmieri RM, Weltman A, Edwards JE, et al. Pre-synaptic modulation of quadriceps arthrogenic muscle inhibition. *Knee Surg Sports Traumatol Arthrosc*. Jul 2005;13(5):370-6. doi:10.1007/s00167-004-0547-z

49. Kuenze CM, Hertel J, Weltman A, Diduch D, Saliba SA, Hart JM. Persistent neuromuscular and corticomotor quadriceps asymmetry after anterior cruciate ligament reconstruction. *J Athl Train*. Mar 2015;50(3):303-12. doi:10.4085/1062-6050-49.5.06

50. Ward SH, Blackburn JT, Padua DA, et al. Quadriceps Neuromuscular Function and Jump-Landing Sagittal-Plane Knee Biomechanics After Anterior Cruciate Ligament Reconstruction. *J Athl Train*. Feb 2018;53(2):135-143. doi:10.4085/1062-6050-306-16

51. Terada M, Kosik KB, McCann RS, Drinkard C, Gribble PA. Corticospinal activity during a single-leg stance in people with chronic ankle instability. *J Sport Health Sci*. Aug 28 2020;doi:10.1016/j.jshs.2020.08.008

52. Proessl F, Beckner ME, Sinnott AM, et al. Reliability of corticospinal excitability estimates for the vastus lateralis: Practical considerations for lower limb TMS task selection. *Brain Res*. Mar 2 2021;1761:147395. doi:10.1016/j.brainres.2021.147395