LOWER LIMB MUSCLE RESPONSES BY TRANSCRANIAL MAGNETIC STIMULATION-EVOKED KNEE EXTENSION USING MECHANOMYOGRAPHY

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ABSTRACT

Mechanomyography (MMG) and transcranial magnetic stimulation (TMS) are two non-invasive techniques that measure the mechanical responses of skeletal muscles and stimulate the motor cortex, respectively. Combining these methods can provide a more comprehensive understanding of the relationship between neural pathways and the muscular system, with potential applications in rehabilitation and the development of new treatments for muscle-related disorders. The primary objective of this study was to investigate whether specific motor control points in the motor cortex are necessary to generate a significant MMG peak amplitude (the maximum amplitude) that can produce functional muscle contractions leading to movement. Additionally, we aimed to analyze the relationship between TMS intensity and mechanical muscle output by analyzing the MMG mean peak amplitude and knee extension angle produced. Fourteen participants with no known neuromuscular or cognitive disorders underwent TMS with varying stimulation locations and intensities at a resting state throughout. This study found that specific point mapping of TMS locations did not produce significantly different muscle output compared to localization mapping, indicating that the location of motor control stimulation during TMS may not need to be specific to produce significant muscle outcomes. Despite the absence of voluntary contraction during the TMS procedure, MMG Mean Peak Amplitude increased with TMS intensity, and a specific threshold was identified at which the MMG Mean Peak Amplitude is significant and knee extension movement is present. This indicates that TMS can activate a sufficient number of motor neurons to produce a measurable mechanical response by the muscles, supporting the use of TMS for investigating the relationship between muscle output and joint angle during movement. This study also investigated whether there were differences in MMG Mean Peak Amplitude and knee angle responses to TMS-evoked contractions between active and sedentary groups, as well as between male and female participants. We found out that active participants and males had a lower threshold for TMS to produce a significant mechanomyography response, likely due to greater cortical excitability in these groups. Overall, the findings suggest that TMS with mechanomyography has potential implications for the development of innovative approaches in studying and enhancing muscle function, particularly in individuals with neuromuscular disorders. By providing a direct measure of muscle output, mechanomyography can help quantify the effectiveness of motor rehabilitation programs. This study contributes to the understanding of the underlying mechanisms of muscle contraction and the use of TMS with mechanomyography in clinical settings.

Keywords: Transcranial Magnetic Stimulation, Mechanomyography, Electromyography, Muscle output, Lower Limbs.

ABSTRAK

Mekanomiografi (MMG) dan rangsangan magnet transkranial (TMS) merupakan dua teknik mengukur respons mekanikal otot rangka dan merangsang korteks motor. Menggabungkan kedua-dua kaedah ini dapat memberikan pemahaman yang lebih komprehensif tentang hubungan antara laluan neural dan sistem otot, dengan aplikasi berpotensi dalam rehabilitasi dan pembangunan rawatan baru untuk masalah berkaitan otot. Objektif utama kajian ini adalah untuk menyiasat sama ada titik kawalan motor secara spesifik dalam korteks motor diperlukan untuk menghasilkan amplitud puncak MMG yang signifikan (amplitud maksimum) yang boleh menghasilkan kontraksi otot yang berfungsi menyebabkan pergerakan. Selain itu, kajian ini bertujuan untuk menganalisis hubungan antara intensity TMS dan hasil mekanikal otot dengan menganalisis amplitud puncak MMG dan sudut gerakan lutut yang dihasilkan. Empat belas peserta tanpa masalah neuromuskular atau kognitif menjalani TMS dengan intensiti rangsangan yang berbeza pada keadaan rehat sepanjang kajian. Kajian ini mendapati bahawa pemetaan titik spesifik lokasi TMS tidak menghasilkan hasil mekanikal otot yang berbeza secara signifikan berbanding dengan pemetaan lokal, menunjukkan bahawa lokasi rangsangan kawalan motor semasa TMS mungkin tidak perlu spesifik untuk menghasilkan hasil otot yang signifikan. Walaupun subjek tidak melakukan kontraksi otot secara sukarela semasa prosedur TMS, Amplitud Puncak MMG meningkat dengan intensiti TMS, dan had batas tertentu dikenal pasti di mana Amplitud Puncak MMG adalah signifikan dan sudut pergerakan lutut dapat dikesan. Ini menunjukkan bahawa TMS boleh mengaktifkan jumlah neuron motor yang mencukupi untuk menghasilkan respons mekanikal yang boleh diukur oleh otot, menyokong penggunaan TMS untuk menyiasat hubungan antara hasil mekanikal otot dan sudut sendi semasa pergerakan. Kajian ini juga menyiasat sama ada terdapat perbezaan dalam Amplitud Puncak Min MMG dan respons sudut lutut antara kumpulan aktif dan tidak aktif, serta antara peserta lelaki dan perempuan. Kami mendapati bahawa peserta yang aktif dan lelaki mempunyai had batas yang lebih rendah bagi TMS untuk menghasilkan respons mekanomiografi yang signifikan, mungkin disebabkan oleh kepekaan kortikal yang lebih tinggi dalam kumpulan ini. Secara keseluruhan, hasil kajian menunjukkan bahawa TMS dengan mekanomiografi mempunyai potensi untuk pembangunan inovatif dalam mengkaji dan meningkatkan fungsi otot, khususnya dalam individu yang mempunyai masalah neuromuskular. Dengan mengukur hasil mekanikal otot secara terus, mekanomiografi dapat membantu mengukur keberkesanan program rehabilitasi motor. Kajian ini menyumbang kepada pemahaman mengenai mekanisme asas kontraksi otot dan penggunaan TMS dengan mekanomiografi dalam bidang klinikal

Keywords: Stimulasi Magnet Transkranial, Meknaomiografi, Elektromiografi, Hasil Otot, Anggota Badan Bawah.

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LIST OF SYMBOLS AND ABBREVIATIONS

TMS	:	Transcranial Magnetic Stimulation
MMG	:	Mechanomyography
EMG	:	Electromyography
MEP	:	Motor Evoked Potential
MPA	:	Mean Peak Amplitude
RF	:	Rectus Femoris
VL	:	Vastus Lateralis

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Movement is crucial for maintaining neurological function and preventing deficits, particularly after an injury or illness. A comprehensive rehabilitation program is essential for promoting recovery from neurological deficits and restoring functional movement. Transcranial magnetic stimulation (TMS) is a promising intervention for neurological rehabilitation, as it can stimulate and modulate neural activity in specific brain regions involved in motor function. TMS has shown significant potential in improving motor recovery and reducing disability in individuals with neurological deficits such as stroke, traumatic brain injury, and Parkinson's disease (Demirtas-Tatlidede et al., 2012; Somaa et al., 2022). One of the aims of TMS studies is to be a part of the physical rehabilitation tool for neurophysiological disabilities. This includes stroke, which is one of the leading causes of disability worldwide that may be presented with functional impairment involving motor skills that badly affect patients' activities of daily living (Elsner et al., 2013). One intervention known as repetitive TMS could promote the excitability of motor cortex and support the recovery of motor function after stroke (Petersen et al., 2003). The year 1980 marked the application of stimulating the motor cortex stimulation in humans by using transcranial electrical stimulation (TES) by Merton and Morton (Klomjai et al., 2015). However, the uncomfortable feeling reported from high voltage TES had caused the TES use to decline (Moscatelli et al., 2021). This opened up the windows of possibilities on the use of TMS. Anthony Baker from University of Sheffield in England was the first person to discover TMS in 1985 (Basil et al., 2005). As in the early 1900s, there has been progressive studies that probe into the use of TMS for neurophysiological change in healthy adults and stroke patients (Butler & Wolf, 2007).

A common application of TMS is the mapping of the primary motor cortical system (MCS), which stimulates certain parts of the brain leading to specific muscle response (i.e., abductor pollicis brevis or tibialis anterior muscles). This stimulation response is elicited, respective to the cortical location, thereby generating an individualized functional map of the MCS (Schramm et al., 2020). Neurons in specific superficial regions of cerebral cortex were stimulated with a short lasting electric current pulse that is carried by magnetic field (Siebner et al., 2009). To be precise, a high voltage capacitor discharge system is used to provide electric current that passes through a small coil on the scalp that eventually produces magnetic field that painlessly passes through the brain tissues, over the appropriate region of motor cortex (Barker et al., 1985).

TMS has been the first tool showed that in human, there is a direct monosynaptic connection from the motor cortex to the spinal motor neurons in human, thus a good technique in the analysis of motor control (Petersen et al., 2003). This however requires necessary care during data interpretation (Petersen et al., 2003). In the pursuit of motor control study, a response known as motor evoked potential (MEP) has to be induced in the targeted muscle when the TMS induced a magnetic pulse over the contralateral primary cortex (M1) through the scalp, which is then picked up by surface EMG, which measures electrical signal via electrodes placement over the muscle of interest (Butler & Wolf, 2007). Reliable muscle sensors to record the muscle activity are said to be the EMG and MMG in order to record the muscle activity (Ibitoye, Estigoni, et al., 2014; Ibitoye et al., 2016; Ibitoye, Hamzaid, et al., 2014). EMG measures the electrical activity of the muscles using surface electrodes placed on the skin over the targeted muscles (Chowdhury et al., 2013).

Studies involving EMG had been increasing since 1950 (Vigotsky et al., 2017). EMG analysis can be carried out using visual inspection and auditory assessment, but quantitative EMG had been used extensively for research purposes (Tankisi et al., 2020).

However, muscles also generate mechanical vibrations as a result from contracting muscle fibers and the tension they produce. Unfortunately, research that focuses on these mechanical vibrations of muscles is very limited. These vibrations can provide valuable information about the contractile properties and force output of the muscle, making them an essential target for study in the field of biomechanics. Understanding the mechanical vibrations in muscles can help researchers gain insights into muscle function as well as develop new techniques for rehabilitation and training, which can be useful in clinical applications such as in the treatment of movement disorders and conditions that affect muscle function. Therefore, the study of mechanical vibrations in muscles is a crucial area of research with far-reaching implications. To achieve this, MMG can be used to measure the mechanical vibrations of muscle activities. It is a tool to record muscles surface oscillations due to the mechanical activity of the motor units (Orizio et al., 2003). However, the usage of MMG with TMS is limited as found in literature publications over the years, which brought a new insight that can be investigated.

The use of MMG to understand the mechanical vibrations of muscle fibers in response to TMS-induced MEPs is crucial as it offers valuable information on the muscle's contractile properties and force output (Ibitoye, Hamzaid, et al., 2014). MMG provides direct measurements of the mechanical vibrations and tone produced by muscle fibers, which are the primary sources of force generated by muscle contractions, resulting in forces acting upon the kinematic properties of the system, as measured by devices like force transducers (Pascal et al., 2011). Therefore, comprehending the mechanical response of muscles to TMS-induced MEPs could provide valuable insights into the clinical applications of TMS, especially for treating movement disorders and other related conditions.

In conclusion, understanding the extent and mechanical effects of MEPs using MMG via TMS on the muscles is critical to achieving functionality in neurological rehabilitation. Additionally, MMG can be a useful complementary measurement tool for studying the mechanical vibrations in muscles. Together, TMS and MMG can offer a powerful combination for developing new techniques for rehabilitation and training, enabling the restoration of functional movement in individuals with neurological deficits and advancing our understanding of the extent and effects of MEPs via TMS on muscle outcomes. This had led to three main research questions and outlined objectives to be achieved:

1.1 Research questions

1) To what extent does TMS affect the mechanical muscle output, and how is the location of motor control stimulation related to the mechanical muscle output?

2) Can MMG be used to quantify the muscle output responses evoked by TMS?

3) Does gender and activity level affect muscle output responses evoked by TMS?

1.2 Objectives

There are three main objectives of this study as represented below:

i. To identify the TMS locations to produce muscle mechanical output measured through MMG

ii. To investigate the relationship between the TMS intensity, MMG peak amplitude and knee extension angle for Rectus Femoris (RF) and Vastus Lateralis (VL) evoked by TMS

iii. To identify the differences in MMG parameters and knee extension angle produced by TMS-evoked contraction for male and female as well as between active and sedentary groups

CHAPTER 2: LITERATURE REVIEW

Complex circuits throughout the body equipped with effector muscles and glands facilitated voluntary and involuntary movements. Upper motor neuron is habitually injured due to the large areas of cortex occupied by the motor areas as well as the extensive pathways from cerebral cortex to the spinal cord (Purves D, 2001). Trauma, infections, cerebrovascular accidents, metabolic disorders and neurodegenerative disorders are one of the reasons (Emos MC, 2022). This UMN injury may lead to inputs deprivation from the brain termed as initial spinal shock but spinal cord circuits are said to regain much of the functionalities (Purves D, 2001). Besides that, stroke also had been known as one of the leading causes of disability worldwide that may be presented with functional impairment involving motor skills that badly effecting patients' activities of daily living (Elsner et al., 2013). As with stroke, brain neuroplasticity had been one of the possible explanations as such brain can heal. The ability of the undamaged areas to remap different pathways as a compensatory mechanism. In order to regain the motor functionality, neuroplasticity is the basal principle in rehabilitation (Takeuchi & Izumi, 2013). The more targeted the approach or the more specific a movement is, the stronger the brain pathways for that movement become and the easier it gets to achieve movements in the future (Perrey, 2013). The connection from the primary motor cortex to muscles of the body is so important that any damage leads to an impaired ability to move.

Impaired chronic stroke patients are the population that needs a well-equipped and refined rehabilitative measures. Furthermore, reliable measures for studying the underlying physiological mechanisms and effectiveness of interventions are crucial. In this context, cortical motor maps appear to be promising, i.e., by assessing the cortical representation of the affected muscles as measured by MEP following TMS (Trunk et al., 2022). This advancement in rehabilitation is crucial as gait and mobility impairments contributed largely to long term disability subsequent to stroke (van de Port et al., 2006). While the normal brain behavior for upper-limb motor control has been widely explored, the same is not true for lower-limb control (de Almeida et al., 2015). Related to this, one of the emerging research is in understanding the neural pathway involves TMS. TMS has also proved useful in characterizing the corticospinal correlation of behavioral deficits in several neurologic disorders (Badawy et al., 2012; Stinear et al., 2015).

This opened the windows of possibilities on the use of TMS. Since the discovery of TMS by Anthony Baker in England in 1985, psychiatric disorders had been the main interest of studies using non-invasive and painless tool to stimulate certain areas of the brain (Basil et al., 2005). TMS allows for a non-invasive investigation of corticospinal excitability. TMS applied over the primary motor cortex (M1), elicits MEPs in contralateral limb muscles, which are valuable indicators of corticospinal excitatory at the time of stimulation (Vassiliadis et al., 2018). In the early 1900s, studies progressively looked into the use of TMS for neurophysiological change in healthy adults and stroke patients (Schramm et al., 2020). Since then, efforts were made to enhance training induced cognitive and motor learning through brain stimulation (Siebner et al., 2009). TMS is also used to understand brain plasticity, which comprise of modifications in cortical properties such as representational patterns or modifications on neurons, either structurally or functionally (Barker et al., 1985).

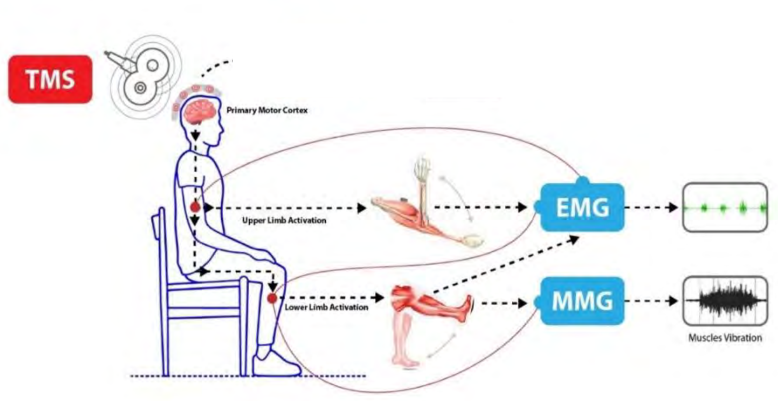


Figure 2.1: Representation of TMS experimental study and output analysis

Brain stimulation can be utilized as a rehabilitation treatment tool, which involves upper limb and lower limb. In 1997, TMS started to be used for activating the lower limbs and upper limbs but more studies reported the use of TMS on the upper limbs compared to lower limbs. Hand muscles are the main focus during gait rehabilitation experiments to study the neuroplasticity model (Butler & Wolf, 2003). In 2007, researchers compared the effectiveness of training interventions between unilateral training and bilateral training for upper limb motor function in chronic stroke patients (Summers et al., 2007). Besides training interventions, development of TMS in upper limbs have led to the establishment of rTMS to enhance the motor function recovery (Brouwer & Hopkins-Rosseel, 1997). rTMS could promote the excitability of motor cortex and supports the recovery of motor function after stroke (Petersen et al., 2003) However, its first use was only restricted to diagnosis of neuromotor disorders (Noohi & Amirsalari, 2016).

In 1998, Classen et al and colleagues had investigated the establishment of consistent thumb movements by using TMS (Classen et al., 1998). In 2004, Kim and colleagues had studied the effect of rTMS on the motor cortex involving sequential finger movements (Kim et al., 2004). Upper limbs have been the main focus on the muscles study within the primary motor cortex.

Besides upper limbs, Davies (2020) had used TMS for quantitative analysis of the cortical representation of various muscles ranging from resting tibialis anterior muscles, resting quadriceps femoris, active rectus femoris as well as vastii muscles (Davies, 2020). However, reports on TMS with hamstring and gastrocnemius muscles are still limited.

One reason of limited studies that employs TMS for the lower limbs is because of the difficulties to access and evoke responses of lower limbs motor control areas, whereby the cortical representation is located deeper from the scalp surface (Groppa et al., 2012). Very clearly, studies involving the mapping of the corticomotor pathway for lower limbs using TMS are much notably less compared to those investigations involving upper limb muscles (Kesar et al., 2018). It is worth to note that neuroplasticity of the corticospinal pathway can be impactful on the lower limb rehabilitation but more TMS studies are performed on upper limbs compared to lower limbs (Chowdhury et al., 2013). As to date, the main challenges faced in the lower extremity corticomotor pathway study is the fact that the lower extremity homunculus is located at a deeper location in the brain. Thus, it has been a challenge to accurately as well as the issue to target individual muscles of the lower extremity (Chowdhury et al., 2013).

Besides that, studies on corticospinal excitability involving lower extremities have always evolved around specific task performance, which is mostly isometric knee extensions, and lately isometric squats as it is a more similar representation of everyday joint movements. One study reported a higher reliability of corticospinal excitability measures for isometric knee extension than isometric squats, which indicates that task specificity plays a crucial role in TMS assessments (Proessl et al., 2021).

The outcome measures and data nevertheless should have good reliability as the MEP amplitudes constitute a wide variability. One of the most common outcome measurement tool for TMS is EMG. The earliest study on motor control by Johansson team involves TMS input and the use of EMG during precision grip, reported in 1994 (Johansson et al., 1994).

The EMG was used across TMS studies that shows the excitation or signals received from the brain to the muscles. EMG studies the electrical activity of the muscles during contraction at which the contraction and relaxation of the muscles are controlled by the nervous system. Surface electrodes were placed on the skin over the targeted muscles (Vigotsky et al., 2017). Anatomical and physiological properties of the muscle may influence the EMG signals obtained (Raez et al., 2006). TMS studies had extensively used EMG as the quantitative tool to analyze the outcomes of MEPs. One group of researchers were able to detect the existence of cortical silent period during a voluntary muscle contraction administered using TMS with an analysis of the pause compartment in EMG activities after the TMS stimulation (Farzan et al., 2013).

This led to the idea of using TMS to generate an inhibitory response (Farzan et al., 2013). The combination of TMS and EMG can investigate the motor system excitability at rest and during task performances by analyzing metrics that includes MEP onset latency, MEP duration as well as MEP peak to peak amplitude (Jackson & Greenhouse, 2019). To serve as a valid and meaningful assessment tool that allows for veridical inferences about corticospinal excitability, TMS-induced MEP measures need to be stable and reliable. Whether they actually are reliable, however, has been subjected for controversial debate (Schilberg et al., 2021).

However, reports about spontaneous fluctuations of MEP amplitudes causing high intra-individual variability to have led to increased concerns about the reliability of this measure. Besides that, severely impaired patients with extended damage of the corticospinal tract often show no MEP when standard TMS procedures are applied (Trunk et al., 2022).

The high variability of the MEPs and the absence of MEP with chronic patients might lead to another side of perspective. MEPs are the physiology interpretation at the muscles but the interest is shifted to the resultant physical output from the muscles produced by the physiology mechanisms elicited by TMS. The proof of the established and measurable mechanical outputs might be the onset of future research on the use of TMS as a neurorehabilitation tool to strengthen the motor control pathway for gait and motor control specializations.

While the electrical activity of the motor system has long been a focus of research in neuroscience, it is becoming increasingly clear that the mechanical counterpart of the motor system is equally important to explore to provide a complete understanding of corticomotor pathway (Alves & Chau, 2010).Muscle function and contractile properties are key determinants of motor output, and as such, an understanding of the mechanical characteristics of the motor system is necessary for a full appreciation of its function. To achieve the best outcomes for lower limb motor improvements with TMS-assisted rehabilitation, it is potential to incorporate the use of a complementary measurement tool to quantify the mechanical muscle output from the muscles elicited by TMS.

Therefore, MMG has the potential to be a complementary measurement tool to EMG, which can be used to measure the mechanical response of a muscle to TMS, providing valuable insights into the excitability of the corticomotor pathway and the underlying neural mechanisms involved in muscle function. As to date, MMG is a tool to record muscles surface oscillations due to the mechanical activity of the motor units (Talib et al., 2022; Tankisi et al., 2020) Changes in skin impedance due to sweating will not affect MMG (Fang et al., 2023). MMG can also offer more precise and reliable measurements of muscle activity, especially in situations involving changes in muscle length or joint angle. For instance, Claire et al. had employed the use of MMG and inertial measurement units (IMUs) to study the upper limb movements in stroke patients. (Meagher et al., 2020). Recent studies have used TMS with MMG to investigate the effects of high-frequency repetitive TMS on corticomotor excitability and muscle function in stroke patients, resulting in promising findings for upper limb rehabilitation.

Specifically, MMG use specific transducers such as microphones, accelerometers or piezoelectric contact sensors (Islam et al., 2013). MMG had been used as a reliable tool to study muscle function, which involves amplitude and frequency responses even if there are changes in the length of the muscles being investigated or any variability of tissue thickness between the muscle and MMG sensors (Beck et al., 2005). Besides that, muscle strength estimation could also be based on MMG (Xie et al., 2020). Not only that, TMS with different intensities had been used with the right abductor pollicis brevis muscle and EMG and MMG was used as the diagnostic tool. The study investigated the similarities of traits between the MEP parameters from MMG and EMG (Reza et al., 2005). As to compare to EMG, MMG is insensitive towards the placement of the sensors as the placement need not be accurate and it is not affected by any changes in the skin impedance caused by sweating. These plausible advantages may lead to the combination of usage of MMG and EMG in order to understand the neuromuscular function in the establishment of corticomotor pathway (Krueger E. et al., 2014).

This leads to a proposed hypothesis that the excitation received by the muscles will elicit shortening of the muscle fibers, initiating contraction and producing a targeted movement. This can be captured as muscle output in terms of vibratory measurements, sensation felt and visibility of the evoked movement. Since it has been stated that the relationship between lower limb and external environment can be studied through knee joint (Li et al., 2022), this study is interested to explore and propose new insights with a hypothesis that knee extension muscles can produce output as a result of the excitatory signals manifestation via TMS. The muscles output covers sensation provided by subjective assessment, muscles vibration measured by MMG as well as the visibility of evoked knee extension, which is manually measured by goniometer.

CHAPTER 3: METHODOLOGY

3.1 Subjects Assessment

3.1.1 Subject details

Fourteen (14) healthy male and female subjects (22.64 ± 1.15 years old; 23.8 ± 2.5 BMI; 10 males, 4 females) participated in this study. Participants footedness was evaluated using the Waterloo Footedness Questionnaire-Revised (WFQ-R) and was observed during mobilizing tasks aimed at identifying their preferred or dominant foot for activities such as kicking and balancing (van Melick, 2017). All subjects were right-hand dominant. Informed consent was obtained after the nature of the experiment had been fully explained **(Appendix D).** Subjects were excluded if they are presented with neurological deficits symptoms or any medical conditions that contraindicated with the TMS guidelines that may jeopardize the participants' safety (Keel et al., 2001). An International Physical Assessment Questionnaire (IPAQ) was given to each of the subjects to fill to determine their physical activity level, either active or sedentary. All investigations conducted with human subjects complied with the tenets of the Helsinki Declaration. This study was approved by the University Malaya Medical Centre Ethics Committee (MREC ID: 2021103-10635) **(Appendix C).**

3.1.2 Physical activity questionnaire

IPAQ was given to each of the subjects to fill **(Appendix G)**. This is to categorize the subjects as active or sedentary groups. All the physical information that includes height, weight, age, and gender were recorded.

IPAQ is a widely used survey for assessing the physical activity levels of individuals. The questionnaire contains questions about the intensity, frequency, and duration of various physical activities. By using the IPAQ, researchers can calculate the total metabolic equivalent task (MET) minutes per week for each individual, which is a measure of the total energy expended during physical activity. The MET-minutes per week can then be used to differentiate individuals into active and sedentary groups.

Active individuals are defined as those who accumulate at least 600 MET-minutes per week, while sedentary individuals are those who accumulate less than 600 MET-minutes per week. The IPAQ can also provide insights into the specific types of physical activities that active and sedentary individuals engage in. By characterizing individuals in these groups and quantifying their physical activity levels, researchers can identify the activity level implications of physical activity on muscle outcomes elicited by TMS.

3.1.3 TMS Questionnaire

TMS Questionnaire was provided for all of the subjects as part of the screening process before TMS procedure (Keel et al., 2001). Any subjects that meet with the contraindications were excluded from this study **(Appendix E)**

3.2 Experiment protocol

3.2.1 Subjects positioning at rest

Subjects were positioned on a higher platform to enable a natural reflex swing action. They were asked to sit on a chair with knees in 90º flexion corresponding to the foot placed 3 cm above the ground (Figure 3.1). The arms are relaxed on the thigh with 90º elbow flexion. Any metal-based jewelry or accessories are removed from the subjects.

Distance of foot to ground

Figure 3.1: Distance of foot to ground

3.2.2 Electromyography

Electromyographic (EMG) (Nicolet EDX EMG System, Natus Medical Incorporated, USA) responses were recorded from bipolar Ag/AgCl surface electrodes with of diameter of 10mm filled with conducting jelly placed over the RF and VL of the right leg. Two electrodes were placed at the motor and insertion point of the muscle belly and one electrode was placed at the patella as a reference point. Electrode placement was preceded by abrasion of skin surface to reduce the impedance to less than 5kΩ. The EMG signals were sampled at a rate of 5kHz using EMG software analysis (Natus Elite Software, Natus

Medical Incorporated, USA). EMG signals were recorded at rest when TMS was given to the subjects. Data were stored on a hard drive for off-line analysis.

3.2.3 Mechanomyography (MMG)

The MMG response was detected by two acquisition units, round-shaped accelerometer (BP150 andnHLT100C, BIOPAC System Inc., Goleta, CA, USA) and one software (Acqknowledge 4.3.1, BIOPAC System Inc., CA, USA) were used to record and store the raw MMG data on a computer for offline analyses.

The accelerometers were attached by medical adhesive tapes at the intermediate point of EMG electrodes (Figure 3.2). BIOPAC recorded the MMG signal at a sampling rate of 2kHz. The MMG signal was connected to an amplifier built in a low and high bandpass of 20Hz to 20kHz for filtering and amplifying. The raw data were relayed and transformed by a finite impulse response band-pass filter between 20Hz and 200Hz, which was recommended by BIOPAC for the assessment of muscle effort (Abd Aziz et al., 2020).

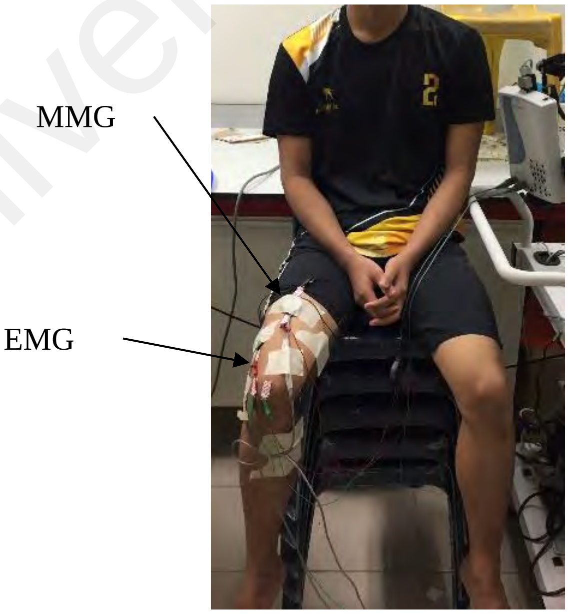


Figure 3.2: EMG and MMG electrodes placement

3.2.4 Goniometer

Goniometer was placed at the medial of the right leg with the center fixed at the knee joint. Cameras were used to record the knee angle shown on the goniometer. This refers to the angle formed between the femur and tibia bones at the knee joint when it is flexed or bent. This angle (˚) is commonly regarded as the knee's resting position when seated and is used as a uniform baseline for evaluating joint movement and muscle activation during different activities. The knee angle displayed on the goniometer was recorded using cameras. If the angle increases, it indicates that the knee joint has extended further from its original seated resting position.

3.3 Response Acquisition

3.3.1 Maximum Voluntary Contraction (MVC)

Subjects were seated comfortably with 90º knee flexion. Both legs were tied to the chair and the subjects were asked to resist as in trying to perform full right knee extension (Hamzaid et al., 2013). They had to sustain the contraction for three seconds. MMG signals were recorded for five attempts. Subjects were asked to take 20 seconds of rest for each attempt to avoid fatigue.

3.3.2 Locations Mapping

Subjects were asked to don a lycra swimming cap with a middle reference line. The nasion and inion were marked and vertex is drawn (Temesi et al., 2014). A cartesian plan of x-axis and y-axis were drawn with 1cm in gap for each. Three main locations were marked as A1, A2, and A3. The point A1 is in the (1,0) coordinates, A2 is in the (1, -1) and A3 is in the (2, -2) coordinates (Figure 3.3).

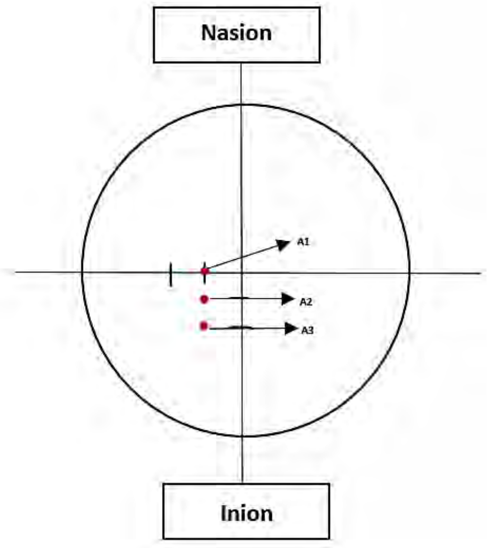


Figure 3.3: Mapped locations on the primary motor cortex

3.3.3 Familiarization session

Subjects were given a brief on the TMS procedure. This experiment started with one trial for each of the stimulus intensity starting from 30%, 40%, 50%, 60%, 70% and 80%. Range of motion (ROM) test was conducted as a baseline to measure the full ROM is achievable by each subject. Before starting the TMS procedure, the subjects were asked to perform voluntary full knee extension with three attempts. This is a priming attempt giving exposure to one stimulus, which can influence a response without conscious guidance.

3.3.4 TMS procedure

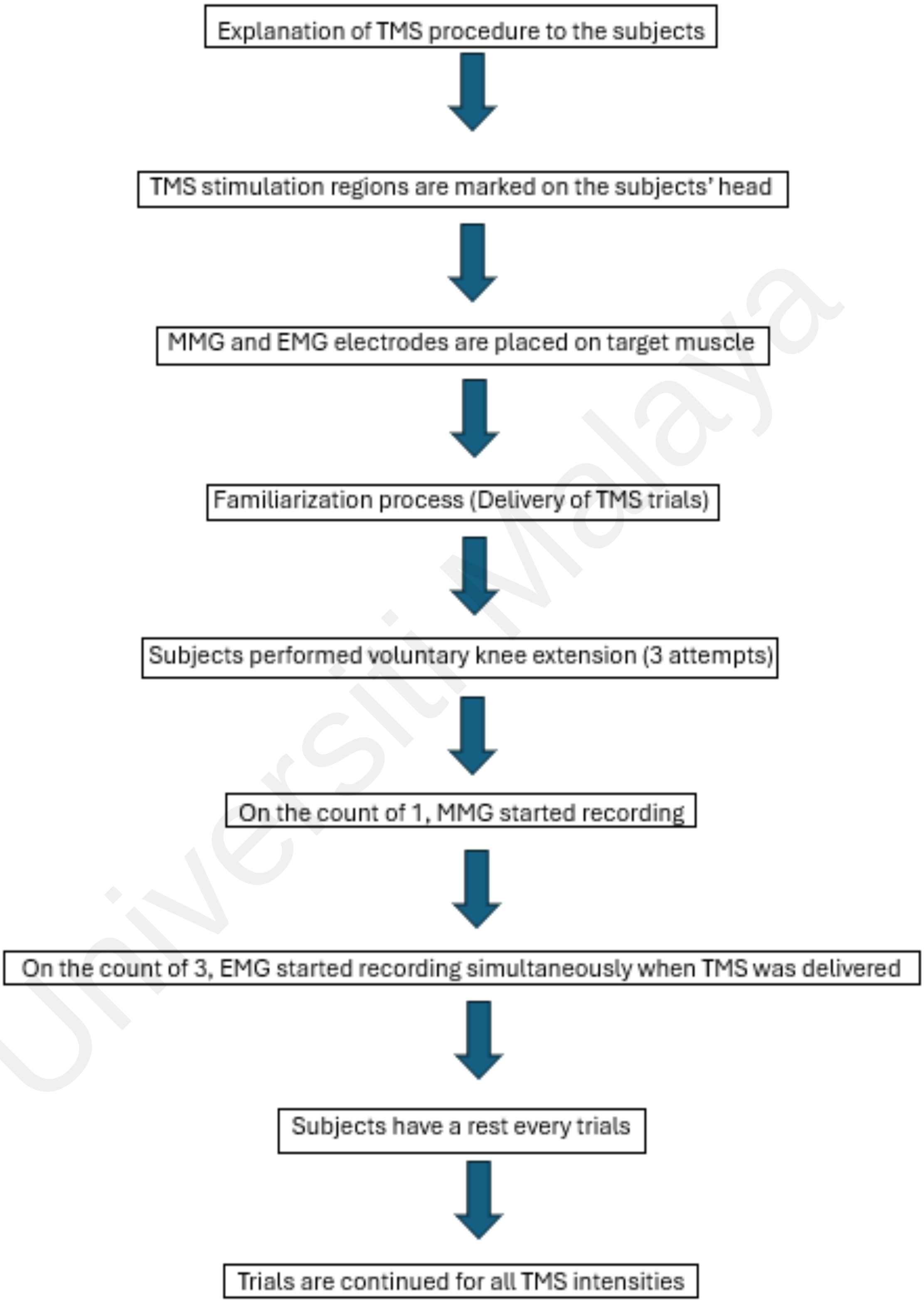


Figure 3.4 Flowchart of TMS procedure

Magnetic stimulation was carried out using a double cone coil with two large adjacent circular wings at an angle of 95º connected (Magstim Bistim^2, The Magstim Company Limited, Whittaker, UK) was placed according to the mapped locations to elicit responses from the RF and VL (Temesi et al., 2014). The stimulus intensity was set at 30% and increasing at a manner of 40%, 50%, 60%, 70% and 80%. TMS intensity was decided to disallow beyond 80% as higher intensities used for the deep penetration of primary motor cortex would induce some discomfort (Rossi et al., 2009). Responses were recorded with five trials at each level of stimulus intensity. The subjects were seated in a relaxed position with the legs hanging at perpendicular to the floor (90º in flexion).

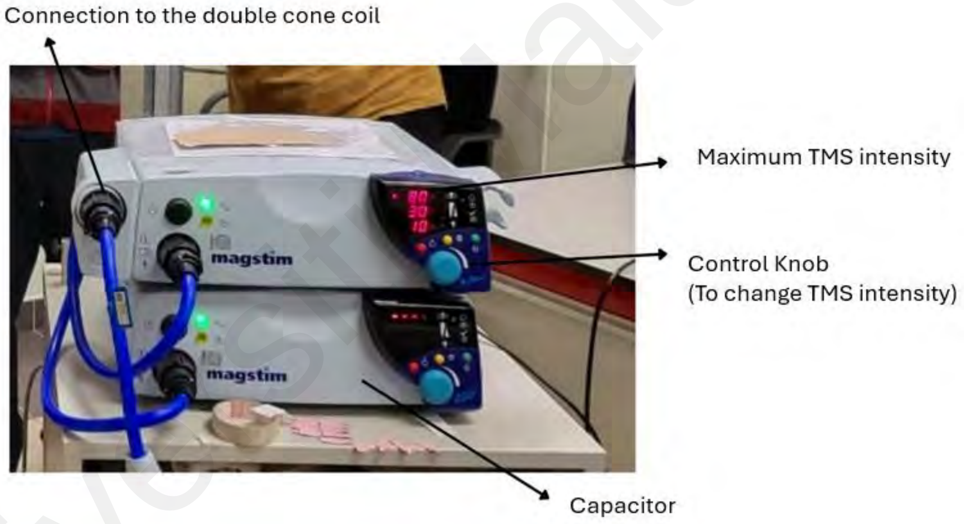
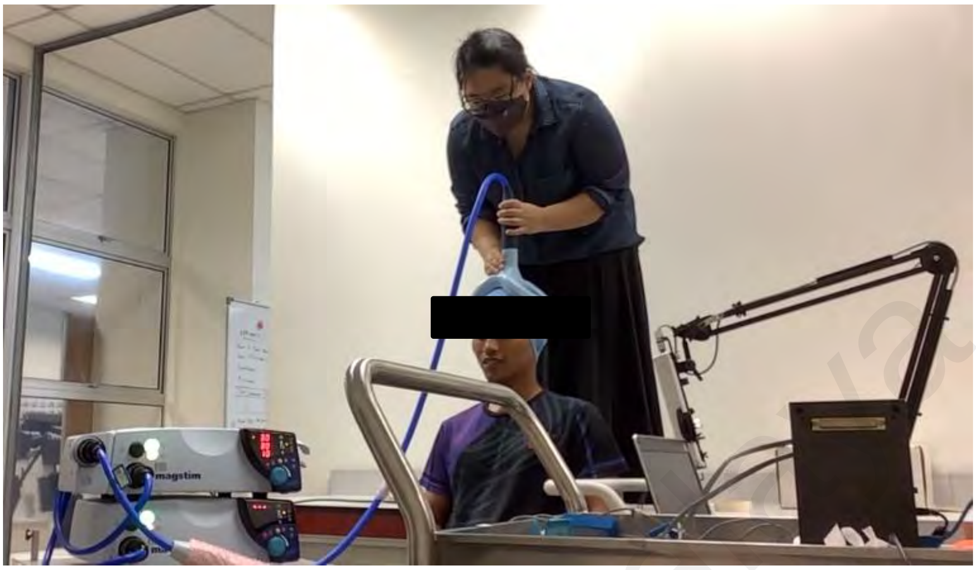


Figure 3.5: TMS machine

Subjects were given five minutes rest after performing a maximum voluntary contraction (MVC). The TMS intensity was delivered to the subjects starting at 30% stimulus intensity (Figure 3.5). EMG and MMG are used to record the signals. Cameras captured the knee angle manifested on the goniometer. Subjects were asked regarding the sensation felt for each trial using the sensation score as in Table 3.1. The procedures were recorded for further screening of the authenticity of the subjective assessment. There was a 20s rest between each attempt. Subjects that had any discomfort are asked to stop and only resume with further consent.



[redacted]

Figure 3.6: Placement of the double cone coil on the locations mapped on the primary motor cortex

Table 3.1: Sensation score during the TMS procedure

Scores	Sensation
1	Has no sensation
2	Has a little sensation
3	Medium sensation with indefinite location of sensation
4	Strong sensation with indefinite location of sensation
5	Strong sensation with definite location of sensation

3.3.5 Statistics

Descriptive values include mean and standard deviation. The bars in the graphs are expressed in mean and standard error. For all 14 subjects, normality was confirmed for all variables via the Shapiro-Wilk test (p>0.05). Thus, parametric tests were conducted for the statistical analysis. Pearson correlation, Chi square test, independent samples T-test and Analysis of Variance (ANOVA) was used for analysis. The data of EMG MEPs, MMG MPA and knee extension angle were analyzed using IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA). Analysis of Variance (ANOVA) was used to determine the statistical significance between variables for both active and sedentary as well as male and female groups. The correlation between the EMG MEP and MMG MPA were carried out using Pearson correlation based on the criteria $0.3 < r < 0.5$ indicates low correlation, $0.5 < r < 0.7$ is moderately correlated and $0.8 < r < 1.0$ indicates strong correlation (Mukaka, 2012). Relationship and accountability of variance in the predicted outcome between the MMG MPA and knee extension angle was obtained by using regression analysis.

3.3.6 Data analysis

The data of the five repetitions of each mapped location at each TMS intensity were averaged for each subject. The peak-to-peak amplitudes of the motor evoked potentials (MEP) were calculated. The MEP amplitudes were normalized from scale 0 to 100% (100% referring to the highest MEPs). The dataset processed from the MMG signal could be in the time or the frequency domain. In the time domain, the amplitude was identified as voltage values.

EMG MEPs and MMG MPA data of five repetitions of each mapped locations for applied TMS intensity were averaged for each subject for MEPs and MMG MPA. The dataset processed from the MMG signal was based on the time domain and the amplitude was identified as voltage value. The data for MMG MPA and knee extension angle were plotted at each increment of TMS intensity from 30% to 80% at 10% increment. The area estimation under the graph for MMG MPA was calculated using the time taken from the start of the MMG MPA to the end of the MMG MPA on area under the graph as the formula below:

$$Area\ under\ the\ graph(a\ triangle) = \frac{1}{2}\ MMG\ MPA(ms^2)\ x\ time(s)$$

All data were plotted using Microsoft Excel 2014 (Microsoft, Redmond, WA, USA).

3.3.7 Minimization Crosstalk

It is true that MMG and EMG signals are generated by different physiological processes and should not crosstalk, there is still a possibility of crosstalk occurring if the recording electrodes are not carefully placed or if the signals are not properly processed. Thus, some ways have been used to ensure crosstalk is at its minimum.

However, it is generally accepted that MMG signals have a different frequency range than EMG signals, which can help to minimize crosstalk. EMG signals typically range from 10 to 500 Hz, while MMG signals are typically in the range of 20 to 2000 Hz. Therefore, by filtering the signals in the appropriate frequency range, it is possible to separate the two signals and reduce the risk of crosstalk. Additionally, MMG signals are generated by mechanical vibrations caused by muscle contraction, while EMG signals are generated by the electrical activity of the muscle fibers. Therefore, MMG signals are less susceptible to interference from external sources such as power lines or other electrical equipment.

These are some of the ways adopted in this study:

1. Multi-channel recording techniques are used to differentiate between EMG and MMG signals and improve the accuracy of recordings.

2. High-pass filters for MMG and EMG are used to remove low-frequency noise that may be related to movement artifacts or other sources of interference.

3. The recording electrodes for both EMG and MMG are carefully positioned to reduce the risk of signal overlap or interference.

4. Appropriate frequencies are chosen for recording EMG and MMG signals, such as using a higher frequency range for MMG recordings to minimize overlap with lower frequency EMG signals.

3.3.8 Active and Sedentary based on IPAQ Questionnaire

IPAQ is a widely used questionnaire for measuring physical activity levels. The questionnaire asks individuals about their level of physical activity in various domains, including leisure time, work, and transportation.

To calculate the MET score using the IPAQ questionnaire, the total amount of physical activity in each domain is calculated. The MET score is then calculated by multiplying the total amount of physical activity in each domain by the MET value assigned to that activity. The method is as follows:

Table 3.2: Leisure time physical activity

Questions	**Time**
How many days per week do you engage in moderate-intensity physical activity (such as brisk walking, bicycling at a regular pace or double tennis)?	3 days
How much time do you usually spend doing this activity on one of those days (in minutes)?	120 minutes
Total time spent	5 days x 120 minutes/day=600 minutes/week
MET value for moderate-intensity physical activity	4.0
MET score	600 minutes/week x 4.0 METs=2400 MET-minutes/week

Table 3.3: Work-related physical activity

Questions	Time
During the last 7 days, on how many days did you do any physical activity or tasks as part of your job that were at least as intense as brisk walking (carrying heavy loads, digging, construction work, or fast bicycling)?	2 days
How much time do you usually spend doing this activity on one of those days (in minutes)?	45 minutes
Total time spent	4 days x 45 minutes/day=180 minutes/week
MET value for moderate-intensity physical activity	4.0
MET score	180 minutes/week x 4.0 METs =720 MET-minutes/week

Table 3.4: Transportation-related physical activity

Questions	Time
During the last 7 days, how many days did you walk or use a bicycle for at least 10 minutes continuously to get to and from places?	5 days
How much time do you usually spend doing this activity on one of those days (in minutes)?	20 minutes
Total time spent	5 days x 30 minutes/day=150 minutes/week
MET value for moderate-intensity physical activity	3.3 MET
MET score	150 minutes/week x 3.3 METs =495 MET-minutes/week

Total MET score

$$= \boldsymbol{MET\ score\ for\ leisure\ time\ physical\ activity} + \boldsymbol{MET\ score\ for\ workrelated\ physical\ activity} + \boldsymbol{MET\ score\ for\ transportation - related\ physical\ activity}$$

$$= \mathbf{2400}\ \boldsymbol{MET}\,\frac{\boldsymbol{minute}}{\boldsymbol{week}} + \mathbf{720}\ \boldsymbol{MET}\,\frac{\boldsymbol{minutes}}{\boldsymbol{week}} + \mathbf{495}\ \boldsymbol{MET}\,\frac{\boldsymbol{minutes}}{\boldsymbol{week}}$$

$$= \mathbf{3165}\ \boldsymbol{MET}\,\frac{\boldsymbol{minutes}}{\boldsymbol{week}}$$

In this example, the individual has a total MET score of 3,615 MET-minutes/week, which falls into the category of high physical activity according to the IPAQ guidelines.

The calculated MET score is used to categorize an individual's physical activity level into one of three categories:

1. Low physical activity: Less than 600 MET-minutes per week
2. Moderate physical activity: Between 600 to 3000 MET-minutes per week
3. High physical activity: Greater than 3000 MET-minutes per week

For this study, the subjects who were presented with low and moderate physical activity were categorized in sedentary group and high physical activity were in active group.

CHAPTER 4: RESULTS

4.1 MMG Mean Peak Amplitude at mapped locations

MMG MPA for RF [F (2, 249) =1.109] at Location A2 and A3 is not significant as compared to the MMG MPA at A1, p=0.227 and p=0.177 respectively. MMG MPA RF [F (2, 249) =1.109] at location A2 is not significant to MMG MPA at A3, p=0.886. Table 4.2 shows the MMG MPA RF for each location at each TMS intensity from 30% to 80%.

Table 4.1: MMG MPA of RF at different mapped locations

TMS intensity (%)	MMG Mean Peak Amplitude for RF		
	Location A1	**Location A2**	**Location A3**
30	0.10±0.36	0.06±0.24	0.00±0.00
40	0.06±0.23	0.10±0.00	0.00±0.00
50	0.72±1.16	0.18±1.43	0.13±0.26
60	2.95±2.90	1.37±4.34	1.46±1.98
70	4.57±3.87	3.87±5.56	3.39±3.24
80	7.45±6.25	5.97±3.65	5.97±7.01

4.2 MMG Mean Peak Amplitude and TMS Intensity

A total of five trials at each stimulus intensity for each location mapped A1, A2, and A3 were completed by all subjects. The magnetic pulse intensity of the TMS was expressed as a percentage of the maximum stimulator output (100%). Figure 4.1 shows a representation of the relationship between MMG MPA at each TMS intensity from 30% to 80%. The maximum MMG MPA RF at 80% intensity was the highest. The MMG MPA for both RF and VL increased with the TMS intensity starting at 30% to 80%.

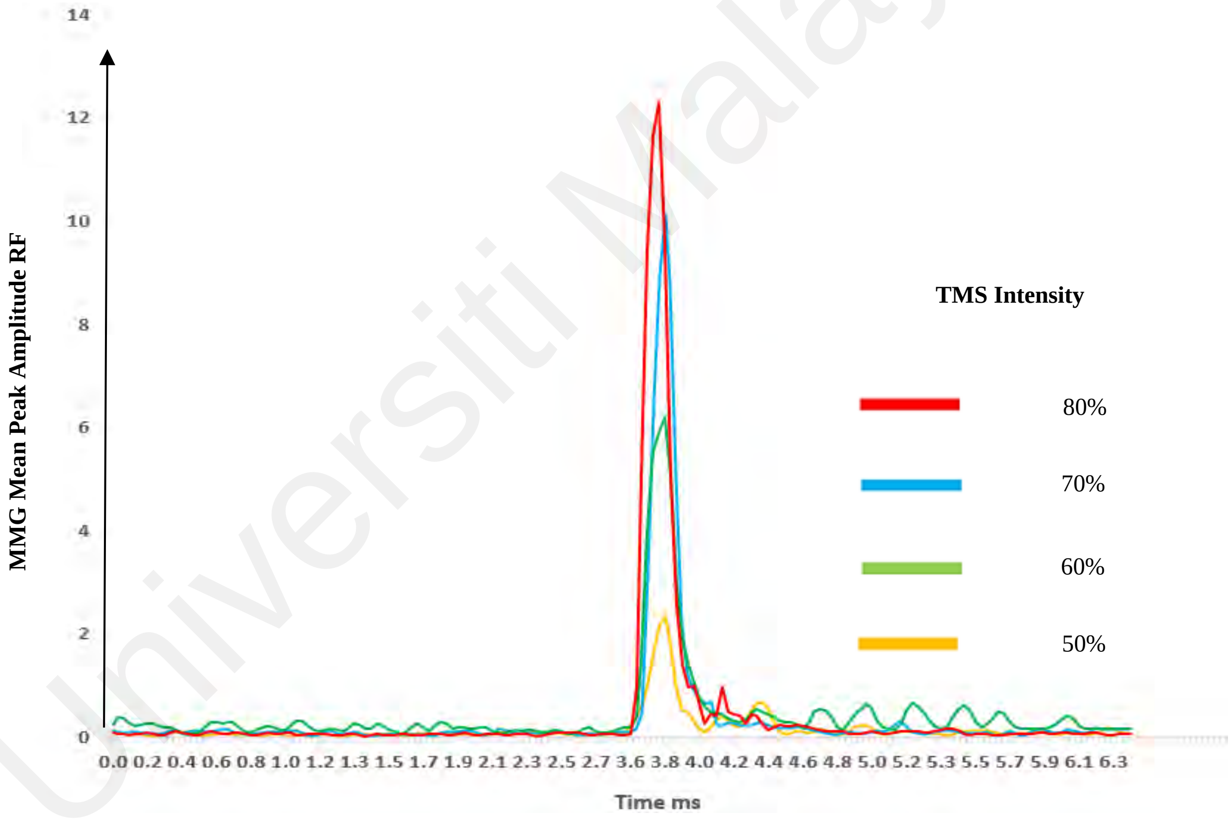


Figure 4.1: A representation of MMG peak amplitude at each TMS intensity (subjects)

An Analysis of Variance test (ANOVA) was conducted to compare the MMG MPA at different TMS intensity for both RF and VL muscles. There was a significant difference on the MMG MPA for RF [F (5,246) =29.48)] and VL [F (5, 246) =37.64)] at the $p<0.05$ level for the TMS intensity level. Post hoc comparisons using Least Significance Variance

(LSD) and Bonferroni following ANOVA test comparing at 6 different levels of TMS intensity, i.e., from 30% to 80% (Table 4.1). This is to determine the TMS intensity that was the most significant in producing MMG MPA. Both tests showed the significance of MMG MPA of RF (M=1.93, SD=2.256) at 60% TMS intensity, 70% TMS intensity (M=3.94, SD=3.780) and at 80% TMS intensity (M=6.47, SD=6.187) compared to MMG MPA of RF at 30% TMS intensity. MMG MPA for VL at 60% (M=2.06, SD=2.229), 70% (M=4.44, SD=4.018), and 80% (M=6.64, SD=5.95) are significant compared to MMG MPA of VL at 30% TMS intensity.

MEPs recorded by EMG and MPA by MMG at 30% to 80% is tabulated a in Table 4.1. MEP values increased as the TMS intensity increased, which is correlated with MMG parameters, knee extension angle.

Table 4.2: MEP by EMG and MPA by MMG for RF and VL

TMS intensity (%)	**EMG MEP (x 10^{-6} V)**		**MMG MPA (mV)**		**Maximum Knee Angle (°)**	**Sensation**
	RF	**VL**	**RF**	**VL**		
30	1.00 ± 0.08	1.00 ± 0.00	0.05 ± 0.25	1.04 ± 0.83	90.00 ± 0.00 (No change)	0
40	1.00 ± 0.18	1.00 ± 0.50	0.02 ± 0.13	2.29 ± 1.82	90.00 ± 0.00 (No change)	0
50	2.00 ± 0.06	2.00 ± 0.50	0.34 ± 0.75	1.65 ± 1.41	91.67 ± 3.69	1
60	3.00 ± 0.08	2.00 ± 0.40	1.93 ± 2.26[a]	2.98 ± 2.11[a]	94.87 ± 7.12[a]	2
70	3.00 ± 0.18	3.00 ± 0.50	3.94 ± 3.78[b]	5.04 ± 3.91[b]	101.03 ± 9.05[b]	3
80	4.00 ± 0.71	3.00 ± 0.50	6.47 ± 6.19[b]	6.97 ± 5.10[b]	106.79 ± 10.97[b]	4

[a]Statistical significance at the 0.05 level; [b]Statistical significance at the 0.01 level. TMS, Transcranial Magnetic Stimulation; MMG MPA, Mechanomyography Mean Peak Amplitude; MEP, Motor Evoked Potential; RF, Rectus Femoris and VL, Vastus Lateral

4.3 Area under the Curve Estimation for MMG responses at different TMS Intensity

The area estimation of RF [F (5,246) =28.16] at 60% (M=0.492, SD=0.601) is significantly different than at 30% (M=0.006, SD=0.038). However, the area estimation of VL [F (5,246) =9.54] is statistically significant at 70% (M=1.755, SD=4.280) compared to 30% intensity (M=0.010, SD=0.068). A Pearson Correlation coefficient was computed to assess the linear relationship between the MMG MPA and area estimation under the MMG response graph for both RF and VL muscles. There was a positive correlation between the two variables for RF, r (252) = 0.957, p<.001 and VL, r (252) = 0.603, p<.001. Putting it together, these results suggested that the level of TMS intensity must be at 60% and above to obtain a significant area estimation under the graph for RF and VL (Figure 4.2 and Figure 4.3)

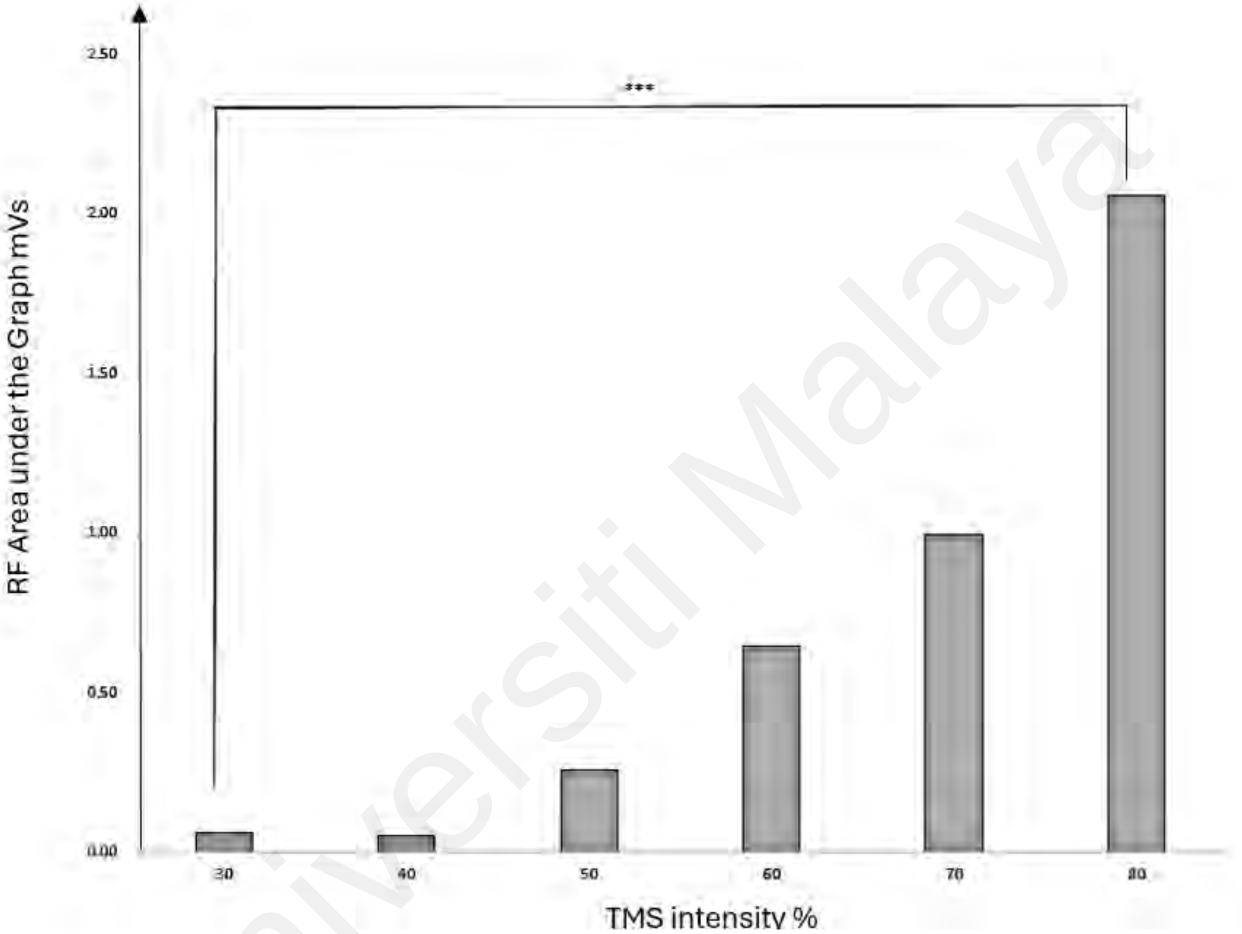


Figure 4.2: Area Estimation vs TMS Intensity for RF

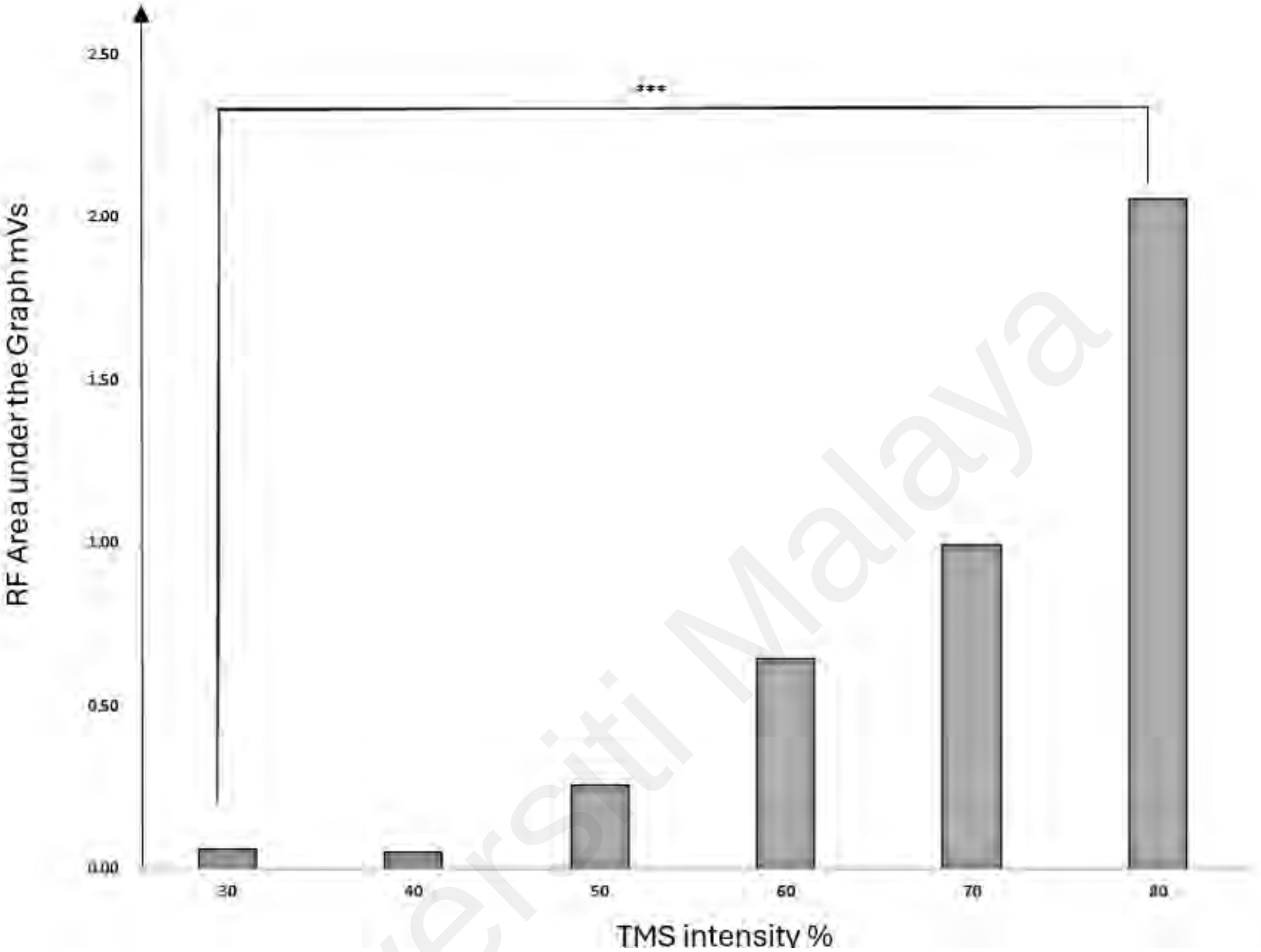


Figure 4.3: Area Estimation vs TMS Intensity for VL

4.4 Knee Extension Angle at different TMS Intensity

A one-way ANOVA was conducted to compare the effect of TMS intensity on the knee extension angle produced. There was a significant effect of the TMS intensity on the knee extension angle produced at the p<.0.05 level [F (5,228) = 40.996]. Post-hoc comparisons using LSD and Bonferroni testes indicated that the mean knee extension angle produced (M=94.87°, SD=7.116°) at 60% TMS intensity was significantly different than mean knee extension angle at 30% TMS intensity (M=90°, SD=0.00°). However, the mean knee extension angle at 40% (M=90°, SD=0.00°) and 50% (M=91.67°, SD=3.687°) did not significantly differ than mean knee extension angle at 30% TMS intensity (M=90°, SD=0.00°). Figure 4.4 shows the mean knee extension angle at different TMS intensity.

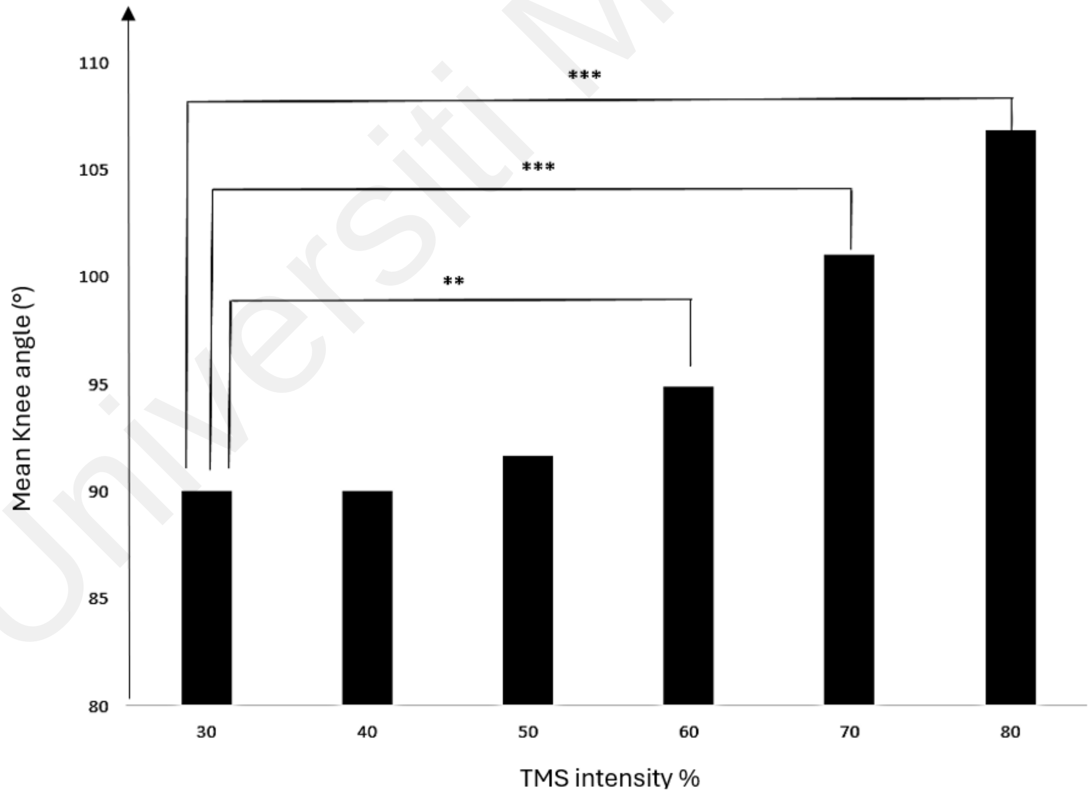


Figure 4.4: Mean Knee Extension Angle vs TMS Intensity

4.5 Relationship between MMG Mean Peak Amplitude and Knee Extension Angle

A Chi-square test of Independence was performed to assess the relationship between MMG MPA RF and VL with the Mean knee extension angle produced. There was a significant relationship between the two variables, X^2 (840, N=234) =1625.00, p<.001. Subsequently, A Pearson Correlation coefficient was computed to assess the linear relationship between the MMG MPA and mean knee extension angle for both RF and VL muscles. There was a positive correlation between the two variables for RF, r (252) =0.596, p<.001 and VL, r (252) =0.675, p<.001. The relationship can be seen in

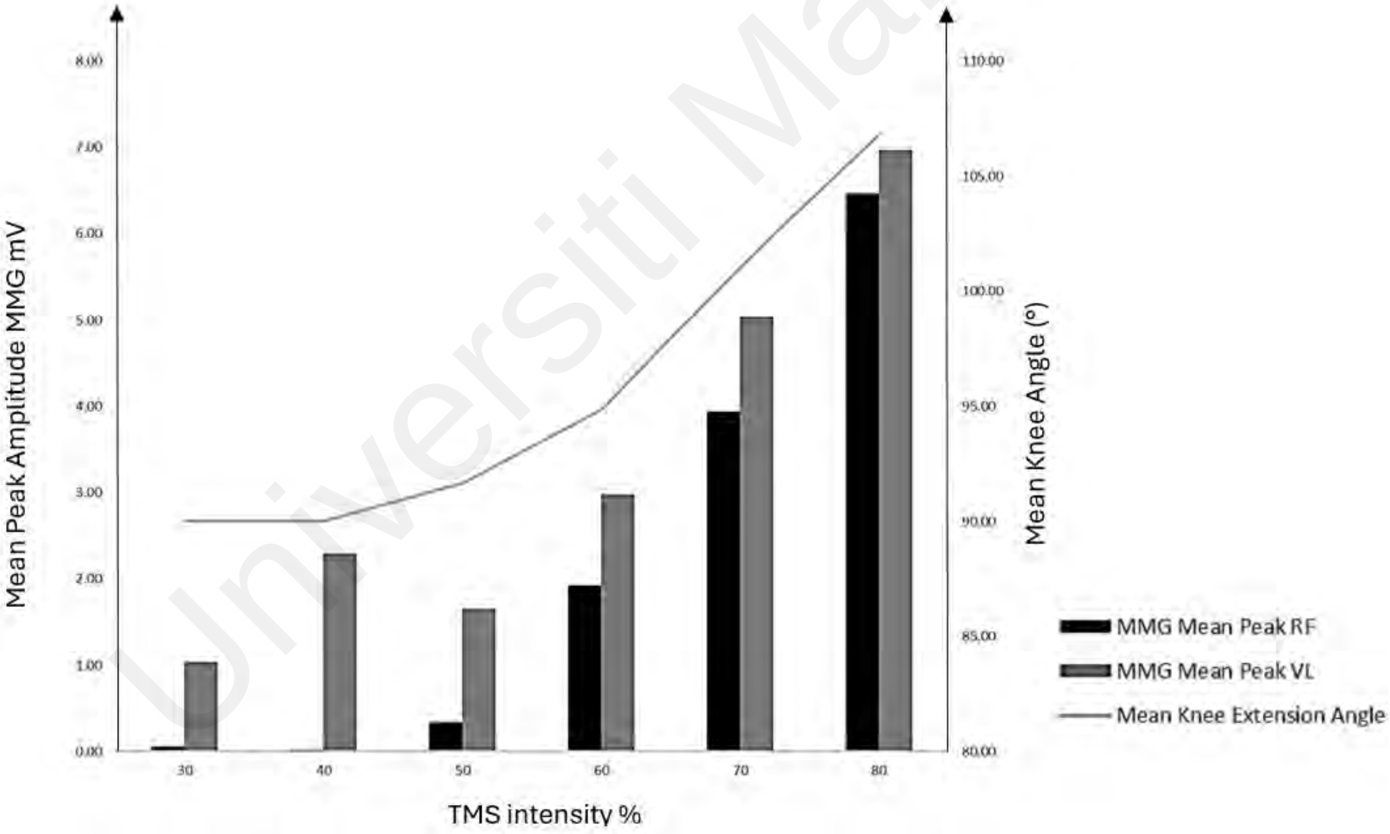


Figure 4.5: Relationship between MMG Peak amplitude for RF and VL with Knee Extension Angle

4.6 Difference in MMG responses to TMS in Active vs Sedentary group

MMG MPA at different TMS intensity was compared between two groups, active and sedentary groups. Further ANOVA test results presented that active group [F (5,102) =31.88] was statistically significant in terms of MMG MPA of RF at 60% (M=2.82, SD=2.474) compared to MMG MPA of RF at 30%. However, for sedentary group [F (5, 138) =9.49], the MMG MPA RF was significant at 70% (M=2.01, SD=2.457) compared to 30% TMS intensity. In addition to this, MMG peak amplitude of VL for active group [F (5,102) =52.17] also showed significance at 60% (M=2.86, SD=2.547) compared to at 30% TMS intensity. Similarly, the sedentary group [F (5, 138) =12.178] showed significance of MMG peak amplitude of VL at 70% (M=2.05, SD=2.108) compared to 30% TMS intensity.

Besides that, mean knee extension angle at 30% to 80% TMS intensity was also compared between active and sedentary groups as shown in Figure 4.6. ANOVA test showed mean knee extension angle for active group [F (5, 102) =35.61] was statistically significant at 60% TMS intensity compared to 30% and mean knee extension angle for sedentary group [F (5, 120) =23.82] was statistically significant at 70% compared to 30% TMS intensity.

4.7 Difference in MMG responses to TMS in Male vs Female

Figure 4.6 showed the MMG MPA of RF between male and female. Regardless the stimulus intensity, the MMG MPA RF for male (M=2.46, SD=4.40) is significantly higher compared to female (M=1.30, SD=2.02), conditions; t (250) =2.138, p=0.033. Consecutively, the ANOVA results presented that male [F (5,174) =25.27, p<.001] showed bigger increment of MMG MPA for RF from 0.08±0.292mV at 30% to 7.72±6.805mV at 80% meanwhile for female [F (5,66) =8.44] the MMG MPA RF is less than 0.001mV at 30% and increased to only 3.33±2.348mV at 80%. For male [F (5,174) =25.27, p<.001], the MMG MPA for RF at 50% is highly significant compared to MMG MPA RF at 70% and 80%. For females [F (5,66) =8.44], the MMG MPA RF at 50% is significantly different than 70% and 80%.

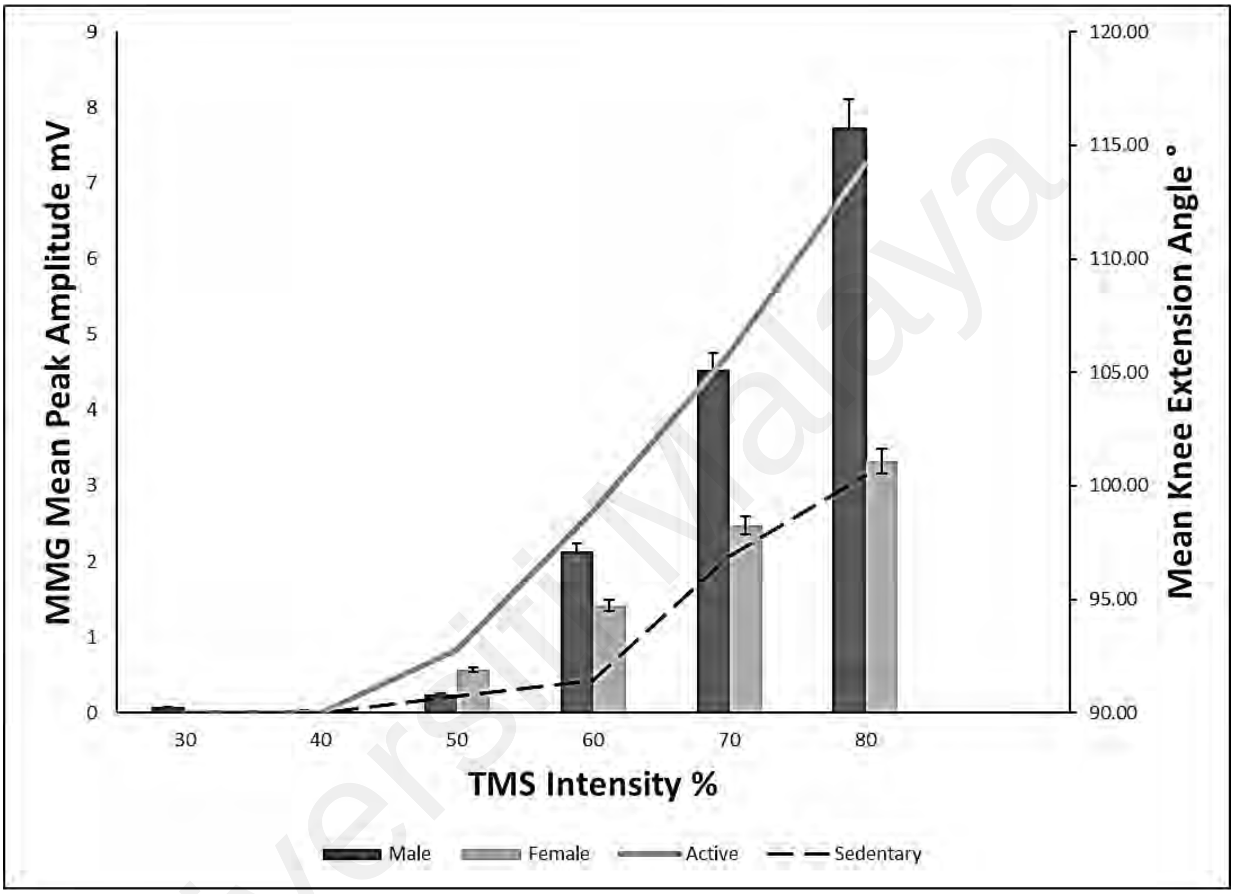


Figure 4.6: Mean Knee Extension Angle (Active vs Sedentary) and MMG Mean Peak Amplitude (Male vs Female) at different TMS Intensity

In terms of mean knee extension angle, male [F (5,174) =31.153] showed significance compared to female [F (5,48) =10.964]. The mean knee extension angle for male and female at 60% (M=94.33°, SD=6.121°, M=96.67°, SD=10.00°) respectively is significant compared to the mean knee extension angle at 30% TMS intensity (Table 4.4.). Females showed a higher mean knee extension angle at 50% TMS intensity (M=93.33°, SD=5.00°), 60% TMS intensity (M=96.67°, SD=10.00°) 70% (M=105.40°, SD= 9.682°) and 80% TMS intensity (M=111.67°, SD=12.748°) compared to males which are at 50%

(M=91.17°, SD=3.130°), 60% (M=94.33°, SD=6.121°), 70% (M=99.83°, SD=8.659°) and 80% (105.33°, SD=10.165°) TMS intensities.

Table 4.3: Mean Knee Extension Angle at different TMS Intensity in Males and Females

TMS Intensity (%)	Mean Knee Extension Angle (°)	
	Male	**Female**
30	90.00±0.00	90.00±0.00
40	90.00±0.00	90.00±0.00
50	91.17±3.13	93.33±5.00
60	94.33±6.12[a]	96.67±10.00[a]
70	99.83±8.66[b]	105.00±9.68[b]
80	105.33±10.165[b]	111.67±12.75[b]

[a]Statistical significance at the 0.05 level; [b]Statistical significance at the 0.01 level.

4.8 Sensation Score

This study used a subjective assessment tabulated using the sensation score. Table 4.5 shows the summary of number of participants choosing the sensation score at each TMS intensity. At 30% TMS intensity, all the participants chose sensation score 1, which means they have no sensation felt during the TMS procedure. As the TMS intensity increases, the sensation score chosen by the participants increases indicating more sensation can be felt. Starting at 50% TMS intensity, the sensation score chosen by the participants is between 3, 4 and 5. However, only three participants chose sensation score 5 at 80% TMS intensity at which they felt strong sensation and can point a definite location of that sensation.

Table 4.4: Sensation score at each TMS Intensity

TMS Intensity (%)	**Number of subjects**				
Sensation Score	**1**	**2**	**3**	**4**	**5**
30	14				
40	12	2			
50		10	4		
60			11		
70			5	9	
80			4	7	3

CHAPTER 5: DISCUSSION

Most TMS studies are within the scope to understand the physiology of the muscles which involves the corticospinal excitability at the muscles. Thus, EMG was used as the modality to measure the motor evoked potentials produced by TMS. However, this present study targets the mechanical output of the muscles during contraction evoked by TMS using MMG. Specifically, this study presented the analysis on whether specific coordinates for lower limbs stimulation is imperative to obtain significant muscle responses, analysis on resultant MMG MPA over different level of TMS intensity as well as how sex differences and physical activity may influence the TMS evoked muscle mechanical output.

5.1 Pinpoint mapping of TMS locations to produce MMG muscle response

Earlier studies had adopted different methodologies to identify the optimum locations on primary motor cortex for lower limbs stimulation. Gz coordinate which refers to the location on the scalp corresponding to the vertex of head is used. The International 10-20 system is commonly used to locate the Gz coordinate. Based on this reference point, researchers would normally try to find the exact location that produces the most significant results or what is called, hotspots. Using the International 10-20 system, this study used pinpoint locations with specific coordinates around the proposed area of motor control. However, comparing between each coordinate, MMG output in terms of the peak amplitude was insignificant as shown in Table 4.1. Coordinates A1, A2 and A3 produced very minor differences of the MMG output data. Since lower limbs are difficult to stimulate, instead of using Figure-of-eight coil which is common for upper limbs stimulation, this study used a double cone coil. It has more elongated shape and is suitable for deeper stimulation of cortical regions. Temesi et al (2014) reported that although double cone coils cannot achieve pinpoint locations on the primary motor cortex, they can still allow for precise localization of specific brain areas (Temesi, 2014). This means that the insignificant MMG results between three specific coordinates had proved that finding the hotspots using exact specific points are not critical as long as it is in the localized region for lower limbs motor control. Based on this study, this localized area have been found to produce measurable MMG signals and visible muscle movements without the need to find the exact hotspot points without any voluntary contraction from subjects. Clinicians require to find the hotspots but instead provide stimulation within the localization area on the primary motor cortex to accurately interpret the mechanical output of the lower limb muscles.

5.2 Relationship between the TMS intensity, MMG peak amplitude and knee extension angle for both RF and VL muscles.

MMG MPA shows an increase as TMS intensity increases. The highest MMG MPA was identified at the maximum TMS intensity, 80%. Physiologically, when the motor cortex was stimulated using an intensity, the excitatory signals travel through the neural pathway and reach the junction between the neural and muscular system, known as neuromuscular junction. The muscle can produce response (vibrations and contractions) when excitatory signals reach the junction and meet the threshold. Muscle contraction, whether voluntary or stimulated, results from this electro-mechanical coupling that causes the shortening of sarcomeres and whole muscle fibers (Macgregor et al., 2018). In this study, the MEP values obtained from EMG is to verify the existence of TMS excitatory signals at the muscles. The vibrations of muscles fibers are represented by MMG MPA that showed vibrations from muscle fibers are greater at higher MMG MPA. This may be proposed that as TMS intensity increases, it produces larger excitatory signals that lead to significant mechanical muscle response, in this case muscle fiber vibrations. However, this relationship between TMS intensity and the magnitude of response can depend on other several mechanical factors such as muscle fiber compositions, tensile strength and others. These factors can be investigated in future TMS research.

Studies have shown that increasing the strength of TMS leads to an increased response probability of motor units firing as well as recruitment of new units that discharge during the response peak (Bawa & Lemon, 1993). During evoked 'single motor unit activity', a linear relationship exists between signal amplitude and the specific rate and amount of force produced (Macgregor et al., 2018). Any change in force, no matter how small, is reflected in the amplitude of the MMG signal (Beck et al., 2005). Takamori et al proposed that the MMG peak amplitude is linked to the release of ca^{2+} during the active state's intensity (Takamori et al., 1971). Orizio and coresearchers suggested that the single twitch MMG peak-to-peak at rest is primarily affected by peripheral mechanisms of muscle activation such as excitation-contraction coupling (Orizio, 1993; Orizio et al., 2003). The MMG signal sums up the activity from individual motor units and has been suggested to provide information regarding motor control strategies of various muscles during isometric and dynamic muscle actions, as it is sensitive to time and frequencies of the MMG signals (Orizio, 1993). Reza et al also supported the idea that during a voluntary contraction, the MMG is related to muscle force and interpreted in respect to increasing firing rates and motor unit recruitment (Reza et al., 2005).

While there was an increasing trend in MMG MPA with higher TMS intensities, MMG MPA between 30% and 50% TMS intensity were found to be inconsequential. This suggests that at lower intensities, the excitatory input may increase firing rates, but the number of motor units recruited may be insufficient to elicit excitation-contraction coupling, preventing muscle fiber shortening and transfer of vibrations to the tendon insertion point for recordable peak amplitude and visible knee extension. Therefore, it is reasonable to propose that the excitatory signals received by the muscles via TMS activated the recruited motor units to generate a contraction. This contraction was captured as the MMG peak amplitude, indicating the extent of mechanical output of the muscle.

Following this, results showed at 60% of TMS intensity, MMG MPA was substantial to produce a measurable knee joint extension angle (Figure 4.4). In 2004, Ochala and colleagues proposed that torque and joint’s angular position (joint dynamics) can be used to describe the mechanical properties of muscles and joints (Ochala et al., 2004). Petitjean et al (1992) had also suggested a linear relationship between MMG amplitude and forearm flexion torque for upper limb muscles (biceps brachii and brachioradialis) (Petitjean et al., 1992). Coburn et al investigated the relationship between MMG amplitude and torque for the vastus medialis during submaximal to maximal concentric isokinetic muscle actions of the leg extensors (Coburn et al., 2004). They reported a linear relationship between MMG amplitude and vastus medialis extension torque from 10 to 100%. Beck and team suggested that the reason of the increment of MMG amplitude with leg extension torque was because of the firing rates and the pattern change of active motor units (Beck et al., 2005).

This study demonstrated that visible knee extension as an evidence that, at an appropriate TMS intensity which is in this case, 60%. Additionally, within the localized area of motor control, motor evoked potentials can reach firing threshold and activate sufficient motor units to induce muscle fiber vibrations. At a certain threshold, getting a functional movement resulting from TMS activation would be challenging. Thus, when the knee extension angle produced did not reach full range of motion, MMG can still verify the presence of muscle responses to TMS activation by capturing the vibrations of muscle fibers. A key finding of this study is that these results can serve as a guide for clinicians in determining the appropriate stimulus intensity to produce visible motor output beyond a simple twitch response. This study was carried out with the absence of voluntary contraction during TMS procedure. This could be a baseline condition for patients with neuromuscular deficits with zero lower limb motor function. In the context of neurorehabilitation, TMS is a potential intervention for improving lower limb motor control, but obtaining a visible evoked movement may be difficult for patients with no motor function. Instead, this study proposes the use of MMG to capture even the smallest vibrations at the muscle fibers level as a response to the TMS intervention. This approach could help evaluate the efficacy of TMS interventions for patients with neuromuscular deficits who are unable to produce visible movements.

5.3 Differences in MMG parameters and knee extension angle produced by TMS-evoked contraction between active versus sedentary groups and males versus females

The relationship between TMS intensity and the magnitude of response (MMG MPA and knee extension angle) can depend on other several factors such as the characteristics of the individual receiving the stimulation. This leads into another novel insight for this study where the subjects were divided into active and sedentary groups. This is to investigate how the activity level can influence the muscle output by the TMS. Hypothetically, an active subject would require lower TMS intensity to evoke a more substantial contraction comparable to sedentary subjects. This study showed that active groups produced bigger MMG MPA and bigger knee angle compared to sedentary groups.

One of the underlying reasons might be related to the responsiveness of the muscles towards a stimulus. A more active person would have a higher responsiveness level of muscles in response to a stimulation that indirectly affects the contraction force produced. Contractile properties are dependent on many factors such as the muscle fiber-type composition. The responsiveness of muscles are said to be affected by the fiber-type composition changes (Thompson, 2002). For instance, antigravity muscles and muscles that provide posture are more likely to have atrophy due to less activity. Besides that, comparing between fast twitch type II muscles and slow twitch type I muscles, it shows that slow twitch type I fibers atrophy more. This was the same for extensor muscles which atrophy more than flexor muscles (Thompson, 2002). Thus, active people are not affected much by the atrophy mechanisms because muscles are actively used but the sedentary groups defined as lower probability of actively using the muscles might be affected by the muscle atrophy. This might be deduced as the reason of why sedentary groups produced smaller MMG MPA because of the inactivity of the muscles that can affect the fiber type composition as well as the contractile properties.

Ikai and Funaga (1968) suggested that human skeletal muscle of normal healthy subjects produce the maximum voluntary isometric force which is proportional to the muscle cross sectional area (Ikai & Fukunaga, 1968). In 1970, the same authors had reported that the muscle experienced a 92% and 23% increment in strength and cross sectional area respectively as a result of 100 days strength training program (Ikai & Fukunaga, 1970). Apparently, it can dictate that suitable training can produce an increment of muscle strength per cross sectional area unit.

Vikne et al (2006) also made a comparison between once a week training for each muscle group and twice or thrice training weekly. They found that the more training can produce up to twice in increase of the quadriceps and elbow flexors cross sectional area (Vikne et al., 2006). This might also explain the higher force production produced by the active groups compared to sedentary groups.

Some studies found that excitability of corticospinal pathway can be improved by short term motor skill training. This can increase the cortical muscles region and create changes in the motor cortex. Jensen et al (2005) found that TMS-evoked MEPs can be increased with the three to four times per week skill training. The minimum stimulus intensity to elicit MEPs at rest and during contraction also can be lower (Jensen et al., 2005). An increase number of action potentials for the muscle fibers are translated as EMG amplitude (Enoka & Duchateau, 2017) and, hence, more synaptic input to the motor neuron pool to achieve the required discharge rate of the identified motor unit. Todd et al (2016) had proposed that the discharge rate of action potentials and how many motor units recruited can affect the force exertion during a voluntary contraction (Todd et al., 2016). This can be seen by the MMG output data representing the active group.

Sex differences have always been a factor of comparison in most fields of research. Males and females are said to be different commonly in terms of muscle mass, muscle strength as well as muscle fibers compositions. Women are said to have two thirds of muscle mass and physically built in general as men according to the National Strength and Conditioning Assessment Association. Activities such as lunges and squats are easy to perform in women when compared to pullups and pushups (Mohan et al., 2017).

Pauhl and coworkers (2022) had highlighted the importance of including analyses of sex differences in neurophysiology research (Pauhl et al., 2022). Supporting this, this study compared the differences in the muscle output between males and females. Figure 4.6 shows that males were capable of producing bigger MMG MPA at each of the TMS intensity. This is correlated with bigger knee joint angle recorded in males than in females. Why? Muscle fascicle length has been suggested to play a key role in determining the maximum contraction velocity of the muscle and the range of active force production. Longer fascicle length can produce higher contraction velocity through sarcomeres (Bartolomei et al., 2021). For instance, fascicle length of VL were significantly longer in males compared to females, thus producing bigger contraction of force and velocity.

Research has shown that the strength of muscle contractions is closely tied to the strength of the muscles themselves. For example, Miller et al (1993) suggested that men tend to have larger muscle fibers, which gives them a greater overall strength (Miller et al., 1993). Mohan et al (2017) also noted that factors such as muscles size and shape, body size differences and other physiological factors can be related to the muscle strength differences between males and females (Mohan et al., 2017). Muscle characteristics, the specificity of task carried out by the muscles and anthropometry can lead to the contrast in muscle power between sex (Jaworowski et al., 2002; Nindl et al., 1995). In general, male skeletal muscles tend to be faster and capable of generating more power than female muscles (Glenmark et al., 2004) and Lang (2011) had proposed that testosterone production especially during puberty which was said to cause muscle mass and strength increment had contributed to the sex differences (Lang, 2011). During this period, there will be a dramatic escalation of testosterone levels in males which influences the strength, muscle mass and hemoglobin levels (Handelsman et al., 2018). This can also help to explain why males are typically larger and stronger than females (Hilton & Lundberg, 2021).

Although males produced a larger MMG MPA overall, this study shows that females produced a larger knee extension angle compared to males. Human muscles contain both fast and slow twitch fibers, which can range from 15 to 85% fast twitch fibers based on Gaussian distribution of composition (Saltin et al., 1977). High power generation in a short time can be seen in human fast-twitch muscle fibers but are easily fatigued, whereas slow-twitch fibers are more resistant to fatigue (Lievens et al., 2020). Studies have reported women have more slow twitch fibers but men have more fast twitch fibers (Aniansson et al., 1986; Essén-Gustavsson & Borges, 1986) proposing the differences to be at muscle fibres. Nindl et al (1995) also supported this notion and added that glycolytic capacity also to be greater in males than in females (Nindl et al., 1995). The amount of each fiber type influences muscle performance. Conversely, female muscles are generally more resistant to fatigue and recover faster during repeated contractions (Glenmark et al., 2004) which may explain why females produced a larger knee extension angle than males at an increasing TMS intensity. Further research could investigate fatigue in TMS-evoked contractions.

5.4 Movement-based Priming prior to TMS

Another interesting perspective in this study, knee extension movement was used as a movement-based priming method prior to TMS procedure. Priming is a pre-stimulation technique that involves the use of a sub-threshold stimulus to modulate the excitability of the motor cortex and enhance the subsequent response to a stimulus. Previous studies have demonstrated that priming can be an effective tool for modulating the cortical excitability and enhancing the MEP amplitudes in healthy individuals and in individuals with neurological disorders (Stoykov et al., 2017). However, it is not clear whether movement-based priming can also affect the muscle mechanical output measured by MMG signals. Although the results of this study did not provide any comparison of significant increase in MMG signals following movement-based priming, this method could still be useful in future research to investigate the effects of priming on muscle mechanical output and to optimize the parameters of priming for clinical applications. Furthermore, the findings of this study may contribute to a better understanding of the underlying mechanisms of TMS and muscle activation and provide insights for clinicians and researchers to develop new and effective interventions for individuals with neuromuscular disorders.

5. 5 Sensation during TMS procedure

Besides using objective measures such as EMG, MMG and goniometer, subjective assessment may provide some different insights. The subjects in this study reported different levels of sensation based on the study’s sensation score **(Appendix F).** Some would not feel any kind of sensation but some can precisely determine the location of where the sensation felt.

Sensation is a complex and subjective experience that can provide valuable insights into how individuals perceive the world around them. This can include questions about the intensity, location, and quality of the sensation, as well as any associated feelings or emotions. The subjective nature of sensation means that individuals may have different experiences of the same stimulus, which can have important implications for research studies. Understanding how people experience different sensations can help researchers to design better studies and to interpret their findings more accurately.

The relationship between TMS intensity and the sensation experienced by participants is not always straightforward. While it is generally true that higher TMS intensities can result in a more definite sensation as presented in this study, there are many factors that can affect the relationship between TMS intensity and sensation. For example, the location of the TMS stimulation can have a significant impact on the sensation experienced by participants. Stimulation of certain areas of the brain may result in a more pronounced or uncomfortable sensation, while stimulation of other areas may be less noticeable or even imperceptible. Additionally, individual differences in sensory perception can also affect the relationship between TMS intensity and sensation. Some individuals may be more sensitive to TMS stimulation and may experience a more intense sensation at lower TMS intensities, while others may require higher intensities to experience a similar sensation.

It is also important to note that the sensation experienced during TMS can vary greatly between individuals and can also depend on the location and intensity of the TMS stimulation. Some individuals may experience a sensation of tingling or warmth, while others may experience a sensation of pressure or movement. Additionally, the intensity of the sensation can vary from mild to intense, and the duration of the sensation can also vary. Overall, while it is generally true that higher TMS intensities can result in a more definite sensation, the relationship between TMS intensity and sensation is complex and can be affected by many factors. By carefully assessing and controlling for sensation, researchers can improve the accuracy and reliability of their findings in TMS studies, ultimately leading to a deeper understanding of the complex relationship between the brain and the body.

CHAPTER 6: CONCLUSION

This TMS study with MMG highlighted several key findings in relation to the research questions and objectives.

The first objective of this study which is to identify the need of using pinpoint locations to obtain significant mechanical muscle responses has been achieved. The insignificant MMG MPA between three pinpoint locations on the motor cortex indicates that stimulation can be carried out in the localized region instead of using specific coordinates to quantify the muscle response evoked by TMS. This can be vital as clinicians can quantify the functional outcome from the muscles via TMS without the need of finding hotspots but instead using localized motor control areas.

The second objective aimed to investigate the relationship between TMS intensity, MMG MPA and knee extension angle. At an increasing TMS intensity, MMG MPA showed an increase as well as the knee extension angle. 60% TMS intensity was found as the specific threshold that can produce a functional mechanical response for the lower limb muscles. This shows that at 60% TMS intensity, significant MPA was quantified from the vibrations from the muscle fibers and a visible knee extension can be obtained. Since this was carried out without any voluntary contraction from the subjects during TMS procedure, clinicians could quantify the efficacy of neurorehabilitation intervention with patients of zero motor function by using this threshold as the baseline to obtain mechanical muscle responses. However, further research need to be done as there are other cofounding factors that might contribute to the evoked muscle responses.

The third objective was to identify the MMG MPA and knee extension angle produced between active and sedentary groups as well as between males and females. The results of this study suggest that there are differences in MMG parameters and knee extension angle produced by TMS-evoked contraction between active and sedentary groups. Active groups and males require a lower TMS intensity to produce significant MMG MPA and knee extension angle. These results are vital to prove that sex differences and physical activity level may influence the mechanical muscle responses evoked via TMS. Eventhough as stated above, 60% TMS intensity was the threshold to evoke significant mechanical responses from the lower limbs, clinicians need to consider the sex difference and physical activity as the threshold may change. This requires more extensive research by using bigger population to verify the hypothesis.

Overall, this study has contributed important insights into the effects of TMS on mechanical muscle output, how MMG can quantify the mechanical responses of the muscles without voluntary contraction and even without the visible knee extension. The level of physical activity as well as sex differences can impact the muscle responses evoked by TMS. These findings have implications for the development of innovative approaches to studying and enhancing muscle function and may have important implications for sports science and rehabilitation medicine.

6.1 Limitations

MMG has demonstrated that excitatory signals in the muscles via TMS can translate into mechanical vibrations that may lead to knee extension. However, several confounding factors must be considered to produce visible knee extension, including the precision of the placement of the TMS coil, the anatomy of the agonist and antagonist muscles that act on the target joint, the linearity of the voluntary torque and superimposed twitch relation, and intra- and interindividual variability in the TMS-evoked EMG and force responses (Todd et al., 2016). The technical challenges associated with measuring voluntary activation with TMS over the motor cortex, such as the poor precision of TMS that activates the cortical representation of many muscles in the target limb, including antagonist muscles, can result in insignificant MMG peak amplitude and invisible knee extensions due to too many antagonist muscles being elicited compared to agonist muscles during the delivery of TMS. Besides that, the sample size for active and sedentary as well as males and females are small but the parametric tests were chosen for data analysis are parametric tests. This was considered based on the factor of power consideration. Parametric tests were supposed to be more powerful than non-parametric tests, at which this study aims to highlight the likelihood of detecting significant differences. However, more extensive research with bigger sample size is crucial to prove this study’s results on the MMG MPA and knee extension angle for all the groups.

6.2 Future work

This research can be extended with the use of MMG in TMS studies with stroke patients or individuals with zero motor function to help to develop different motor rehabilitation avenues. However, there are several important directions for future investigation. One key area of focus will be on developing effective strategies for priming the muscles to produce measurable MMG signals. This may involve developing targeted interventions such as electrical stimulation or vibration to improve muscle excitability and facilitate the generation of MMG signals or a less invasive priming such as movement-based priming can be adopted. Additionally, it will be important to further explore the relationship between MMG and EMG signals to better understand the underlying mechanisms of muscle activation and control. This may involve identifying specific synchronization signals between the two signals to better interpret the data obtained from MMG recordings. In addition, future research may also explore the use of advanced imaging techniques such as functional MRI or diffusion tensor imaging to better understand the neural circuits and pathways involved in the generation of MMG signals. Overall, the development of more reliable and effective methods for detecting and interpreting MMG signals in stroke patients or individuals with zero motor function will be critical for advancing our understanding of motor function and developing more targeted interventions to improve motor outcomes in these populations.

REFERENCES

Abd Aziz, M., Hamzaid, N. A., Hasnan, N., & Dzulkifli, M. A. (2020). Mechanomyography-based assessment during repetitive sit-to-stand and stand-to-sit in two incomplete spinal cord-injured individuals. *Biomed Tech (Berl), 65*(2), 175-181. https://doi.org/10.1515/bmt-2018-0200

Alves, N., & Chau, T. (2010). Uncovering patterns of forearm muscle activity using multi-channel mechanomyography. *Journal of Electromyography and Kinesiology*, *20*(5), 777-786. https://doi.org/https://doi.org/10.1016/j.jelekin.2009.09.003

Aniansson, A., Hedberg, M., Henning, G. B., & Grimby, G. (1986). Muscle morphology, enzymatic activity, and muscle strength in elderly men: a follow-up study. *Muscle Nerve, 9*(7), 585-591. https://doi.org/10.1002/mus.880090702

Badawy, R. A., Loetscher, T., Macdonell, R. A., & Brodtmann, A. (2012). Cortical excitability and neurology: insights into the pathophysiology. *Funct Neurol*, *27*(3), 131-145.

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet*, *1*(8437), 1106-1107. https://doi.org/10.1016/s0140-6736(85)92413-4

Bartolomei, S., Grillone, G., Di Michele, R., & Cortesi, M. (2021). A Comparison between Male and Female Athletes in Relative Strength and Power Performances. *J Funct Morphol Kinesiol*, *6*(1). https://doi.org/10.3390/jfmk6010017

Basil, B., Mahmud, J., Mathews, M., Rodriguez, C., & Adetunji, B. (2005). Is there evidence for effectiveness of transcranial magnetic stimulation in the treatment of psychiatric disorders? *Psychiatry (Edgmont), 2*(11), 64-69.

Bawa, P., & Lemon, R. N. (1993). Recruitment of motor units in response to transcranial magnetic stimulation in man. *J Physiol*, *471*, 445-464. https://doi.org/10.1113/jphysiol.1993.sp019909

Beck, T. W., Housh, T. J., Cramer, J. T., Weir, J. P., Johnson, G. O., Coburn, J. W., Malek, M. H., & Mielke, M. (2005). Mechanomyographic amplitude and frequency responses during dynamic muscle actions: a comprehensive review. *Biomed Eng Online*, *4*, 67. https://doi.org/10.1186/1475-925x-4-67

Brouwer, B., & Hopkins-Rosseel, D. H. (1997). Motor cortical mapping of proximal upper extremity muscles following spinal cord injury. *Spinal Cord*, *35*(4), 205-212. https://doi.org/10.1038/sj.sc.3100384

Butler, A. J., & Wolf, S. L. (2003). Transcranial magnetic stimulation to assess cortical plasticity: a critical perspective for stroke rehabilitation. *J Rehabil Med*(41 Suppl), 20-26. https://doi.org/10.1080/16501960310010106

Butler, A. J., & Wolf, S. L. (2007). Putting the brain on the map: use of transcranial magnetic stimulation to assess and induce cortical plasticity of upper-extremity movement. *Phys Ther*, *87*(6), 719-736. https://doi.org/10.2522/ptj.20060274

Chowdhury, R. H., Reaz, M. B., Ali, M. A., Bakar, A. A., Chellappan, K., & Chang, T. G. (2013). Surface electromyography signal processing and classification techniques. *Sensors (Basel)*, *13*(9), 12431-12466. https://doi.org/10.3390/s130912431

Classen, J., Liepert, J., Wise, S. P., Hallett, M., & Cohen, L. G. (1998). Rapid plasticity of human cortical movement representation induced by practice. *J Neurophysiol*, *79*(2), 1117-1123. https://doi.org/10.1152/jn.1998.79.2.1117

Coburn, J. W., Housh, T. J., Weir, J. P., Malek, M. H., Cramer, J. T., Beck, T. W., & Johnson, G. O. (2004). Mechanomyographic responses of the vastus medialis to isometric and eccentric muscle actions. *Med Sci Sports Exerc*, *36*(11), 1916-1922. https://doi.org/10.1249/01.mss.0000145449.14799.5f

Davies, J. L. (2020). Using transcranial magnetic stimulation to map the cortical representation of lower-limb muscles. *Clin Neurophysiol Pract*, *5*, 87-99. https://doi.org/10.1016/j.cnp.2020.04.001

de Almeida, P. M. D., Vieira, A. I. C. M. d. F., Canário, N. I. S., Castelo-Branco, M., & de Castro Caldas, A. L. (2015). Brain Activity during Lower-Limb Movement with Manual Facilitation: An fMRI Study. *Neurology Research International*, *2015*, 701452. https://doi.org/10.1155/2015/701452

Demirtas-Tatlidede, A., Vahabzadeh-Hagh, A. M., Bernabeu, M., Tormos, J. M., & Pascual-Leone, A. (2012). Noninvasive brain stimulation in traumatic brain injury. *J Head Trauma Rehabil*, *27*(4), 274-292. https://doi.org/10.1097/HTR.0b013e318217df55

Elsner, B., Kugler, J., Pohl, M., & Mehrholz, J. (2013). Transcranial direct current stimulation (tDCS) for improving function and activities of daily living in patients after stroke. *Cochrane Database Syst Rev*(11), Cd009645. https://doi.org/10.1002/14651858.CD009645.pub2

Emos MC, R. J. (2022). *Neuroanatomy, Upper Motor Nerve Signs.* . StatPearls Publishing. https://doi.org/https://www.ncbi.nlm.nih.gov/books/NBK541082/

Enoka, R. M., & Duchateau, J. (2017). Rate Coding and the Control of Muscle Force. *Cold Spring Harb Perspect Med*, *7*(10). https://doi.org/10.1101/cshperspect.a029702

Essén-Gustavsson, B., & Borges, O. (1986). Histochemical and metabolic characteristics of human skeletal muscle in relation to age. *Acta Physiol Scand*, *126*(1), 107-114. https://doi.org/10.1111/j.1748-1716.1986.tb07793.x

Fang, Q., Cao, S., Qin, H., Yin, R., Zhang, W., & Zhang, H. (2023). A Novel Mechanomyography (MMG) Sensor Based on Piezo-Resistance Principle and with a Pyramidic Microarray. *Micromachines*, *14*(10), 1859. https://www.mdpi.com/2072-666X/14/10/1859

Farzan, F., Barr, M. S., Hoppenbrouwers, S. S., Fitzgerald, P. B., Chen, R., Pascual-Leone, A., & Daskalakis, Z. J. (2013). The EEG correlates of the TMS-induced EMG silent period in humans. *Neuroimage*, *83*, 120-134. https://doi.org/10.1016/j.neuroimage.2013.06.059

Glenmark, B., Nilsson, M., Gao, H., Gustafsson, J. A., Dahlman-Wright, K., & Westerblad, H. (2004). Difference in skeletal muscle function in males vs. females: role of estrogen receptor-beta. *Am J Physiol Endocrinol Metab*, *287*(6), E1125-1131. https://doi.org/10.1152/ajpendo.00098.2004

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S., Thickbroom, G. W., Rossini, P. M., Ziemann, U., Valls-Solé, J., & Siebner, H. R. (2012). A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol*, *123*(5), 858-882. https://doi.org/10.1016/j.clinph.2012.01.010

Hamzaid, N. A., Smith, R. M., & Davis, G. M. (2013). Isokinetic Cycling and Elliptical Stepping: A Kinematic and Muscle Activation Analysis. *Clinical Research on Foot & Ankle*, *2013*, 1-7.

Handelsman, D. J., Hirschberg, A. L., & Bermon, S. (2018). Circulating Testosterone as the Hormonal Basis of Sex Differences in Athletic Performance. *Endocr Rev*, *39*(5), 803-829. https://doi.org/10.1210/er.2018-00020

Hilton, E. N., & Lundberg, T. R. (2021). Transgender Women in the Female Category of Sport: Perspectives on Testosterone Suppression and Performance Advantage. *Sports Medicine*, *51*(2), 199-214. https://doi.org/10.1007/s40279-020-01389-3

Ibitoye, M. O., Estigoni, E. H., Hamzaid, N. A., Wahab, A. K., & Davis, G. M. (2014). The effectiveness of FES-evoked EMG potentials to assess muscle force and fatigue in individuals with spinal cord injury. *Sensors (Basel)*, *14*(7), 12598-12622. https://doi.org/10.3390/s140712598

Ibitoye, M. O., Hamzaid, N. A., Hasnan, N., Abdul Wahab, A. K., Islam, M. A., Kean, V. S., & Davis, G. M. (2016). Torque and mechanomyogram relationships during electrically-evoked isometric quadriceps contractions in persons with spinal cord injury. *Med Eng Phys*, *38*(8), 767-775. https://doi.org/10.1016/j.medengphy.2016.05.012

Ibitoye, M. O., Hamzaid, N. A., Zuniga, J. M., Hasnan, N., & Wahab, A. K. (2014). Mechanomyographic parameter extraction methods: an appraisal for clinical applications. *Sensors (Basel)*, *14*(12), 22940-22970. https://doi.org/10.3390/s141222940

Ikai, M., & Fukunaga, T. (1968). Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. *Int Z Angew Physiol*, *26*(1), 26-32. https://doi.org/10.1007/bf00696087

Ikai, M., & Fukunaga, T. (1970). A study on training effect on strength per unit cross-sectional area of muscle by means of ultrasonic measurement. *Int Z Angew Physiol*, *28*(3), 173-180. https://doi.org/10.1007/bf00696025

Islam, M. A., Sundaraj, K., Ahmad, R. B., & Ahamed, N. U. (2013). Mechanomyogram for muscle function assessment: a review. *PLoS One*, *8*(3), e58902. https://doi.org/10.1371/journal.pone.0058902

Jackson, N., & Greenhouse, I. (2019). VETA: An Open-Source Matlab-Based Toolbox for the Collection and Analysis of Electromyography Combined With Transcranial Magnetic Stimulation. *Front Neurosci*, *13*, 975. https://doi.org/10.3389/fnins.2019.00975

Jaworowski, A., Porter, M. M., Holmbäck, A. M., Downham, D., & Lexell, J. (2002). Enzyme activities in the tibialis anterior muscle of young moderately active men and women: relationship with body composition, muscle cross-sectional area and fibre type composition. *Acta Physiol Scand*, *176*(3), 215-225. https://doi.org/10.1046/j.1365-201X.2002.t01-2-01004.x

Jensen, J. L., Marstrand, P. C., & Nielsen, J. B. (2005). Motor skill training and strength training are associated with different plastic changes in the central nervous system. *J Appl Physiol (1985)*, *99*(4), 1558-1568. https://doi.org/10.1152/japplphysiol.01408.2004

Johansson, R. S., Lemon, R. N., & Westling, G. (1994). Time-varying enhancement of human cortical excitability mediated by cutaneous inputs during precision grip. *J Physiol*, *481 (Pt 3)*(Pt 3), 761-775. https://doi.org/10.1113/jphysiol.1994.sp020480

Keel, J. C., Smith, M. J., & Wassermann, E. M. (2001). A safety screening questionnaire for transcranial magnetic stimulation. *Clin Neurophysiol*, *112*(4), 720. https://doi.org/10.1016/s1388-2457(00)00518-6

Kesar, T. M., Stinear, J. W., & Wolf, S. L. (2018). The use of transcranial magnetic stimulation to evaluate cortical excitability of lower limb musculature: Challenges and opportunities. *Restor Neurol Neurosci*, *36*(3), 333-348. https://doi.org/10.3233/rnn-170801

Kim, Y. H., Park, J. W., Ko, M. H., Jang, S. H., & Lee, P. K. (2004). Facilitative effect of high frequency subthreshold repetitive transcranial magnetic stimulation on complex sequential motor learning in humans. *Neurosci Lett*, *367*(2), 181-185. https://doi.org/10.1016/j.neulet.2004.05.113

Klomjai, W., Katz, R., & Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Ann Phys Rehabil Med*, *58*(4), 208-213. https://doi.org/10.1016/j.rehab.2015.05.005

Krueger E., E. M. Scheeren, G. Nogueira-Neto, V. L. S. N. Button, & Nohama, P. (2014). Advances and perspectives of mechanomyography.

Lang, T. F. (2011). The bone-muscle relationship in men and women. *J Osteoporos, 2011*, 702735. https://doi.org/10.4061/2011/702735

Li, Z., Gao, L., Lu, W., Wang, D., Cao, H., & Zhang, G. (2022). Estimation of Knee Extension Force Using Mechanomyography Signals Based on GRA and ICS-SVR. *Sensors (Basel)*, *22*(12). https://doi.org/10.3390/s22124651

Lievens, E., Klass, M., Bex, T., & Derave, W. (2020). Muscle fiber typology substantially influences time to recover from high-intensity exercise. *Journal of Applied Physiology*, *128*(3), 648-659. https://doi.org/10.1152/japplphysiol.00636.2019

Macgregor, L. J., Hunter, A. M., Orizio, C., Fairweather, M. M., & Ditroilo, M. (2018). Assessment of Skeletal Muscle Contractile Properties by Radial Displacement: The Case for Tensiomyography. *Sports Med*, *48*(7), 1607-1620. https://doi.org/10.1007/s40279-018-0912-6

Meagher, C., Franco, E., Turk, R., Wilson, S., Steadman, N., McNicholas, L., Vaidyanathan, R., Burridge, J., & Stokes, M. (2020). New advances in mechanomyography sensor technology and signal processing: Validity and intrarater reliability of recordings from muscle. *Journal of Rehabilitation and Assistive Technologies Engineering*, *7*, 2055668320916116. https://doi.org/10.1177/2055668320916116

Miller, A. E. J., MacDougall, J. D., Tarnopolsky, M. A., & Sale, D. G. (1993). Gender differences in strength and muscle fiber characteristics. *European Journal of*

Applied Physiology and Occupational Physiology, *66*(3), 254-262. https://doi.org/10.1007/BF00235103

Mohan, S., Mariappan, B., & Ramaswamy, P. (2017). Study of gender variation in muscle function among young adults. *National Journal of Physiology, Pharmacy and Pharmacology*, *7*, 1. https://doi.org/10.5455/njppp.2017.7.0308402042017

Moscatelli, F., Messina, A., Valenzano, A., Monda, V., Salerno, M., Sessa, F., La Torre, E., Tafuri, D., Scarinci, A., Perrella, M., Marsala, G., Monda, M., Cibelli, G., Porro, C., & Messina, G. (2021). Transcranial Magnetic Stimulation as a Tool to Investigate Motor Cortex Excitability in Sport. *Brain Sci*, *11*(4). https://doi.org/10.3390/brainsci11040432

Mukaka, M. M. (2012). Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J*, *24*(3), 69-71.

Nindl, B. C., Mahar, M. T., Harman, E. A., & Patton, J. F. (1995). Lower and upper body anaerobic performance in male and female adolescent athletes. *Med Sci Sports Exerc*, *27*(2), 235-241.

Noohi, S., & Amirsalari, S. (2016). History, Studies and Specific Uses of Repetitive Transcranial Magnetic Stimulation (rTMS) in Treating Epilepsy. *Iran J Child Neurol*, *10*(1), 1-8.

Ochala, J., Valour, D., Pousson, M., Lambertz, D., & Van Hoecke, J. (2004). Gender Differences in Human Muscle and Joint Mechanical Properties During Plantar Flexion in Old Age. *The Journals of Gerontology: Series A*, *59*(5), B441-B448. https://doi.org/10.1093/gerona/59.5.B441

Orizio, C. (1993). Muscle sound: bases for the introduction of a mechanomyographic signal in muscle studies. *Crit Rev Biomed Eng*, *21*(3), 201-243.

Orizio, C., Gobbo, M., Diemont, B., Esposito, F., & Veicsteinas, A. (2003). The surface mechanomyogram as a tool to describe the influence of fatigue on biceps brachii motor unit activation strategy. Historical basis and novel evidence. *Eur J Appl Physiol*, *90*(3-4), 326-336. https://doi.org/10.1007/s00421-003-0924-1

Pascal, M., Afshin, S., Mark de, Z., & Uwe, K. (2011). Biomechanical Assessments in Sports and Ergonomics. In K. Vaclav (Ed.), *Theoretical Biomechanics* (pp. Ch. 16). IntechOpen. https://doi.org/10.5772/21255

Pauhl, A., Yasen, A., & Christie, A. (2022). Corticospinal Excitability and Inhibition Are Not Different between Concussed Males and Females. *Brain Sci*, *12*(7). https://doi.org/10.3390/brainsci12070824

Perrey, S. (2013). Promoting motor function by exercising the brain. *Brain Sci*, *3*(1), 101-122. https://doi.org/10.3390/brainsci3010101

Petersen, N. T., Pyndt, H. S., & Nielsen, J. B. (2003). Investigating human motor control by transcranial magnetic stimulation. *Exp Brain Res*, *152*(1), 1-16. https://doi.org/10.1007/s00221-003-1537-y

Petitjean, M., Maton, B., & Cnockaert, J. C. (1992). Evaluation of human dynamic contraction by phonomyography. *J Appl Physiol (1985)*, *73*(6), 2567-2573. https://doi.org/10.1152/jappl.1992.73.6.2567

Proessl, F., Beckner, M. E., Sinnott, A. M., Eagle, S. R., LaGoy, A. D., Conkright, W. R., Canino, M. C., Sterczala, A. J., Midhe Ramkumar, P. P., Sciavolino, B. M., Connaboy, C., Ferrarelli, F., Germain, A., Nindl, B. C., & Flanagan, S. D. (2021). Reliability of corticospinal excitability estimates for the vastus lateralis: Practical considerations for lower limb TMS task selection. *Brain Res*, *1761*, 147395. https://doi.org/10.1016/j.brainres.2021.147395

Purves D, A. G., Fitzpatrick D, et al., editors. . (2001). *Damage to Descending Motor Pathways: the Upper Motor Neurone*. https://doi.org/https://www.ncbi.nlm.nih.gov/books/NBK10898/

Raez, M. B., Hussain, M. S., & Mohd-Yasin, F. (2006). Techniques of EMG signal analysis: detection, processing, classification and applications. *Biol Proced Online*, *8*, 11-35. https://doi.org/10.1251/bpo115

Reza, M. F., Ikoma, K., Chuma, T., & Mano, Y. (2005). Mechanomyographic response to transcranial magnetic stimulation from biceps brachii and during transcutaneous electrical nerve stimulation on extensor carpi radialis. *J Neurosci Methods*, *149*(2), 164-171. https://doi.org/10.1016/j.jneumeth.2005.05.013

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol*, *120*(12), 2008-2039. https://doi.org/10.1016/j.clinph.2009.08.016

Saltin, B., Henriksson, J., Nygaard, E., Andersen, P., & Jansson, E. (1977). Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. *Ann N Y Acad Sci*, *301*, 3-29. https://doi.org/10.1111/j.1749-6632.1977.tb38182.x

Schilberg, L., Ten Oever, S., Schuhmann, T., & Sack, A. T. (2021). Phase and power modulations on the amplitude of TMS-induced motor evoked potentials. *PLoS One*, *16*(9), e0255815. https://doi.org/10.1371/journal.pone.0255815

Schramm, S., Haddad, A. F., Chyall, L., Krieg, S. M., Sollmann, N., & Tarapore, P. E. (2020). Navigated TMS in the ICU: Introducing Motor Mapping to the Critical Care Setting. *Brain Sci*, *10*(12). https://doi.org/10.3390/brainsci10121005

Siebner, H. R., Hartwigsen, G., Kassuba, T., & Rothwell, J. C. (2009). How does transcranial magnetic stimulation modify neuronal activity in the brain? Implications for studies of cognition. *Cortex*, *45*(9), 1035-1042. https://doi.org/10.1016/j.cortex.2009.02.007

Somaa, F. A., de Graaf, T. A., & Sack, A. T. (2022). Transcranial Magnetic Stimulation in the Treatment of Neurological Diseases. *Front Neurol*, *13*, 793253. https://doi.org/10.3389/fneur.2022.793253

Stinear, C. M., Petoe, M. A., & Byblow, W. D. (2015). Primary Motor Cortex Excitability During Recovery After Stroke: Implications for Neuromodulation. *Brain Stimul*, *8*(6), 1183-1190. https://doi.org/10.1016/j.brs.2015.06.015

Stoykov, M. E., Corcos, D. M., & Madhavan, S. (2017). Movement-Based Priming: Clinical Applications and Neural Mechanisms. *J Mot Behav*, *49*(1), 88-97. https://doi.org/10.1080/00222895.2016.1250716

Summers, J. J., Kagerer, F. A., Garry, M. I., Hiraga, C. Y., Loftus, A., & Cauraugh, J. H. (2007). Bilateral and unilateral movement training on upper limb function in chronic stroke patients: A TMS study. *J Neurol Sci*, *252*(1), 76-82. https://doi.org/10.1016/j.jns.2006.10.011

Takamori, M., Gutmann, L., & Shane, S. R. (1971). Contractile properties of human skeletal muscle. Normal and thyroid disease. *Arch Neurol*, *25*(6), 535-546. https://doi.org/10.1001/archneur.1971.00490060069007

Takeuchi, N., & Izumi, S. (2013). Rehabilitation with poststroke motor recovery: a review with a focus on neural plasticity. *Stroke Res Treat*, *2013*, 128641. https://doi.org/10.1155/2013/128641

Talib, I., Sundaraj, K., Hussain, J., Lam, C. K., & Ahmad, Z. (2022). Analysis of anthropometrics and mechanomyography signals as forearm flexion, pronation and supination torque predictors. *Scientific Reports, 12*(1), 16086. https://doi.org/10.1038/s41598-022-20223-6

Tankisi, H., Burke, D., Cui, L., de Carvalho, M., Kuwabara, S., Nandedkar, S. D., Rutkove, S., Stålberg, E., van Putten, M., & Fuglsang-Frederiksen, A. (2020).

Standards of instrumentation of EMG. *Clin Neurophysiol*, *131*(1), 243-258. https://doi.org/10.1016/j.clinph.2019.07.025

Temesi, J., Gruet, M., Rupp, T., Verges, S., & Millet, G. Y. (2014). Resting and active motor thresholds versus stimulus–response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. *Journal of neuroengineering and rehabilitation*, *11*(1), 40. https://doi.org/10.1186/1743-0003-11-40

Temesi, J., Gruet, M., Rupp, T., Verges, S., & Millet, G. Y. . (2014). Resting and active motor thresholds versus stimulus-response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. *Journal of neuroengineering and rehabilitation*, *11*, *40*. https://doi.org/https://doi.org/10.1186/1743-0003-11-40

Thompson, L. V. (2002). Skeletal muscle adaptations with age, inactivity, and therapeutic exercise. *J Orthop Sports Phys Ther*, *32*(2), 44-57. https://doi.org/10.2519/jospt.2002.32.2.44

Todd, G., Taylor, J. L., & Gandevia, S. C. (2016). Measurement of voluntary activation based on transcranial magnetic stimulation over the motor cortex. *J Appl Physiol (1985)*, *121*(3), 678-686. https://doi.org/10.1152/japplphysiol.00293.2016

Trunk, B. H., Ziegler, L., & Gharabaghi, A. (2022). Ipsilateral corticospinal maps correspond to severe poststroke motor impairment. *Brain Stimul*, *15*(3), 758-760. https://doi.org/10.1016/j.brs.2022.05.007

van de Port, I. G., Kwakkel, G., van Wijk, I., & Lindeman, E. (2006). Susceptibility to deterioration of mobility long-term after stroke: a prospective cohort study. *Stroke*, *37*(1), 167-171. https://doi.org/10.1161/01.STR.0000195180.69904.f2

van Melick, N., Meddeler, B. M., Hoogeboom, T. J., Nijhuis-van der Sanden, M., & van Cingel, R. (2017). https://doi.org/10.1371/journal.pone.0189876. (2017). How to determine leg dominance: The agreement between self-reported and observed performance in healthy adults. *PloS one, 12(12),*. https://doi.org/https://doi.org/10.1371/journal.pone.0189876

Vassiliadis, P., Grandjean, J., Derosiere, G., de Wilde, Y., Quemener, L., & Duque, J. (2018). Using a Double-Coil TMS Protocol to Assess Preparatory Inhibition Bilaterally. *Front Neurosci*, *12*, 139. https://doi.org/10.3389/fnins.2018.00139

Vigotsky, A. D., Halperin, I., Lehman, G. J., Trajano, G. S., & Vieira, T. M. (2017). Interpreting Signal Amplitudes in Surface Electromyography Studies in Sport and Rehabilitation Sciences. *Front Physiol*, *8*, 985. https://doi.org/10.3389/fphys.2017.00985

Vikne, H., Refsnes, P. E., Ekmark, M., Medbø, J. I., Gundersen, V., & Gundersen, K. (2006). Muscular performance after concentric and eccentric exercise in trained men. *Med Sci Sports Exerc*, *38*(10), 1770-1781. https://doi.org/10.1249/01.mss.0000229568.17284.ab

Xie, J., Zhang, Y., Yang, K., & Xia, C. (2020). Estimation of triceps muscle strength based on Mechanomyography. *Journal of Physics: Conference Series*, *1544*(1), 012055. https://doi.org/10.1088/1742-6596/1544/1/012055