Sex dependent differences in biceps brachii muscle size, overlying subcutaneous fat thickness and corticospinal excitability

By

© Olalekan B. Olarogba

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Abstract

Indirect measurement of subcutaneous fat thickness using skinfold measurement has been used as the most common method of investigating fat thickness in humans. Likewise, studies that have reported on the muscle size mostly involved cadaver studies. From this research, muscle size and subcutaneous fat thickness were measured using B-mode ultrasound and the effect of these factors on corticospinal excitability were determined. Many studies have investigated the role of the corticospinal tract in the development of voluntary force. However, the effect of muscle size and fat thickness on corticospinal excitability has not been examined. The current study was designed to assess corticospinal excitability of the biceps brachii using a transcranial magnetic stimulation (TMS) protocol and assess muscle and fat thickness via B-mode ultrasound to determine the impact of muscle and fat thickness has on corticospinal excitability and whether or not this impact was sex-dependent. The maximum stimulator output required to achieved active motor threshold was higher in female than male. Motor evoked potential (MEP) amplitudes from the biceps increased significantly ($p < .001$) and similarly ($p = .365$) for both men and women. For men there was a significant ($p < .05$, $r = 0.591$) positive relationship between muscle size and MEP amplitude, whereas for women there was a significant ($p < .05$, $r = -0.525$) negative relationship between MEP amplitude and skinfold thickness. Collectively, the data suggest that the amount of muscle size and subcutaneous fat thickness affects CSE, and these findings are sex- dependent.

Dedication

I would like to dedicate this thesis to my deceased father, Engr. Olayinka Joseph Olarogba. His greatest desire for me to achieve a graduate degree abroad has finally come alive today.

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List of Abbreviations

AMT - active motor threshold

AUC - area under curve

BMI - body mass index

CMEP - cervicomedullary motor evoked potential

CNS - central nervous system

CSE - corticospinal excitability

EMG - electromyography

MEP - motor evoked potential

M_{max} - maximum amplitude of the compound muscle action potential

MSO - maximum stimulator output

mV - millivolt

μV - microvolt

MS - muscle size

MT - motor threshold

MVC - maximum voluntary isometric contraction

Mmax - compound muscle action potential

SD - standard deviation

SEM - standard error

SF - skinfold

SFT - subcutaneous fat thickness

SRC - stimulus response curve

TES - transcranial electrical stimulation

TMS -transcranial magnetic stimulation

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Chapter 1: Introduction

1.1 Overview

Magnetic stimulation of the brain has been widely used to facilitate the production of motor evoked potentials in muscle to describe the corticospinal excitability (CSE) to that muscle during muscle contraction. However, higher or lower magnetic stimulation may be required due to the high resistivity of subcutaneous fat tissue or low resistivity of skeletal muscle, respectively (Doheny et al., 2010). To our knowledge there are no known studies that have determined the effect of changes in muscle size and subcutaneous fat thickness on corticospinal excitability. To help further understand the neurophysiological mechanisms underlying the motor evoked responses recorded from muscle, we seek to determine the effect of muscle size and subcutaneous thickness on the CSE of biceps brachii using diagnostic ultrasound. Ultrasound is a portable non-invasive imaging tool mostly used to complement or carry out diagnoses by health professionals. However, with the aid of technology, researchers have taken interest in these diagnostic tools to expand their use and application to other areas beyond the primary development area of the product (Neves et al., 2015). It could be confusing estimating the muscle size and subcutaneous fat for both clinical and physical adaptations particularly when the muscle arises from different origin or insertion points (Fischer et al., 2020). Typically, magnetic resonance imaging (MRI) and computerized tomography have been recognized for accurately assessing muscle size but are quite expensive and time consuming, which made its use limited to specific areas in the hospital. Therefore, to provide an alternative technique to overcome these limitations, ultrasound techniques were later used to measure the size of skeletal muscle as well as fat thickness (Bemben, 2002).

The use of ultrasound in measuring muscle size and subcutaneous fat thickness has been found to be a valid and reliable method of assessing muscle thickness (Andrushko, 2017). Just like muscle properties, subcutaneous fat measurement in individuals is an important body

composition for performance. This could be measured using both skinfold calipers which give an indirect measure of compressed fat between two layers of skin, and ultrasound which provides a direct measure of uncompressed fat (Giezenaar et al., 2017). When measuring the muscle size and subcutaneous fat, the involved limb must be in a rested and neutral position with the probe having the transmission gel on it placed on the surface of the skin directly on the muscle bulk. Hence, the linear distance measured on the muscle from the edge of the subcutaneous tissue to the edge of the bone will form the muscle size (Andrushko, 2017). The subcutaneous fat layer and muscle size may influence the uptake of biological signals such as electromyography, but to what extent, is unknown. Similarly, influence of body characteristics on results of excitability as may have been reported in nerve conduction studies, and sex is not of relevance in the excitability of motor nerves (Casanova et al., 2014). If the same is true for cortical excitability is yet to be explored.

1.2 Purpose of the study

To investigate if corticospinal excitability (CSE) of the biceps brachii is muscle and fat thickness dependent. Furthermore, we want to determine how these factors affect CSE when compared between sexes.

1.3 Research Questions

1. Does CSE of the biceps brachii change with increased muscle size and fat thickness?

2. If so, what effect do muscle size and fat thickness have on CSE between sexes.

1.4 Research Hypotheses

1. CSE of biceps brachii will increase as the muscle size of the biceps brachii increases and decrease as subcutaneous fat thickness around the biceps brachii increases.

2. The effect of muscle size and fat thickness on CSE of the biceps brachii will be sex dependent.

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Chapter 2: Literature Review

2.1 Introduction

The corticospinal tract is the part of the central nervous system that provides a pathway that connects the brain to the spinal cord to convey voluntary action signals, also known as motor evoked potentials (MEP), through the motoneurones before it gets relayed to the muscle (Taylor et al., 2002). Previous studies done in our lab have shown that voluntary contractions, chronic activity or inactivity, fatigue, muscle position and muscle state alter the corticospinal excitability (CSE) of this pathway (Collins et al., 2017; Forman et al., 2014; Pearcey et al., 2014). In all these studies other important factor(s) may have influenced CSE such as muscle size and subcutaneous fat thickness overlying the muscle. These factors could potentially affect the size of the MEP and thus alter CSE of a specific muscle. Excessive subcutaneous fat thickness affects muscle architecture which may cause CSE to vary from one person to another due to the high resistivity of fat tissue (Doheny et al., 2010) but how subcutaneous fat affect the CSE within the muscle is yet to be documented. Furthermore, the effect of muscle size and subcutaneous fat thickness on the input required to initiate MEPs in healthy subjects and sex-related characteristics has not been explored to date. The purpose of this review of literature is to provide information on some of the techniques used to assess 1) CSE; 2) muscle size and subcutaneous fat thickness and 3) discuss how muscle size and subcutaneous fat thickness may affect CSE recorded from a muscle of interest.

2.2 The Corticospinal Tract

The dominant and arguably most important descending pathway for the execution of voluntary movement is the corticospinal pathway (Almasri, 2011). A series of communication

occurs within the central nervous system (CNS) which is responsible for the production of voluntary control of movement. Such motor outputs depend on the motor commands from motor areas in the cerebral cortex and descend through the corticobulbar and corticospinal tracts. The fibers of the corticobulbar tract control the motor nuclei that innervate the facial muscles, likewise, the fibers of the corticospinal tract innervate the trunk and limb muscles through spinal motoneurones (Ghez & Krakauer, 2006). Thus, researchers have investigated this tract to gain a better understanding of how motor outputs are controlled.

Furthermore, the corticospinal tract concerned with the voluntary control of movement starts from the brain to the spinal cord in the central nervous system. Its tract fibers terminate either directly onto spinal motoneurones or indirectly via interneurons to the spinal motoneurones (Kandel et al., 2000). Derived from its name, it originates in the motor cortex as pyramidal cells located in its 5th layer and synapse onto spinal motor and interneurons located in the grey mater throughout the spinal cord. The axons of the corticospinal tract pass through white matter structures of the medulla, a pyramid-like structure from which the name 'pyramidal' arises, before they decussate or cross over (Rothwell, 1987). The axon of the corticospinal tract decussates with about 80 – 90% crossing over to the contralateral side at the medulla to branch as the lateral corticospinal tract while the remaining 10 – 20% do not cross-over and form the anterior corticospinal tract (Kaneko et al., 1997).

The descending axons from both the ipsilateral and contralateral corticospinal tracts then travel down the spinal cord and synapse directly onto spinal neurones (Eyre, 2003). Hence, the spinal neurones are influenced by both the lateral and anterior corticospinal tracts of the contralateral side, despite not following the same path. This implies that upper motoneurones originating from the left hemisphere of the motor cortex travel on the right side of the spinal cord

and innervate muscles of the right limbs, while upper motoneurones that originate from the right hemisphere travel on the left side of the spinal cord and innervate muscles of the left limbs (Nathan & Smith, 1955).

In addition, the ratio of polysynaptic and monosynaptic connections, however, depends on the motoneurone pool being examined and monosynaptic connections is most dense for muscles of the distal arm, hand, and digits (Palmer & Ashby, 1992). For instance, the biceps brachii and most muscles of the upper limbs possess a large monosynaptic connection with spinal motoneurones, and their axons exit the spinal cord forming part of the brachial plexus (Rothwell, 1987). Therefore, an increase or decrease in corticospinal excitability of biceps brachii depends on the excitability of cortical/spinal neurones involved (Power et al., 2018).

2.3 Assessing Corticospinal Excitability (CSE)

The corticospinal tract is responsible for conveying voluntary movement commands from the motor cortex in the brain to the spinal cord which then gets relayed to the muscle through motoneurones. The excitability of the corticospinal tract can affect how much input from the higher command centers is required to cause a MEP response in the specified muscle. These responses can be recorded using electromyography (EMG) (Palmer & Ashby, 1992). The goal is to identify not only how CSE changes but also where the source of this change originates (i.e., supraspinal and/or spinal), and it could be influenced by peripheral excitability (Lockyer et al., 2021). Hence, the use of transcranial magnetic stimulation (TMS) and transmastoid electrical stimulation (TMES) as the main techniques for assessing corticospinal excitability and spinal excitability, respectively while electrical stimulation (ES) is applied to the innervating nerve root to the muscle.

2.3.1 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive technique that is widely used to investigate corticospinal excitability (Petersen et al., 2003). The transcranial magnetic stimulator induces a magnetic pulse which bypasses the skull and excites the neural cells in a given area of the brain (Barker et al., 1985). This stimulation generates excitatory or inhibitory neural responses that create an electrical current and depolarizes cerebral neurons in the cortex (Terao & Ugawa, 2002). TMS activates cortical motoneurones directly or through trans-synaptic activation to produce D-waves (direct waves) and I-waves (indirect waves), respectively (Burke et al., 1993; Taylor et al., 2002). The D-waves which are produced when the cortical neurones are activated directly have a shorter latency within a few millimetres of the cell body I-waves. I-waves on the other hand, have a longer latency (~1-1.4 ms) and are produced when the cortical neurones are stimulated indirectly, via synaptic inputs. These D-waves and I-waves all induce postsynaptic potentials which summate at the motoneurone (Forman et al., 2014). If the volley summation is excitatory, a response will be induced (or multiple responses) in the muscle, which is called a motor evoked potential (MEP).

The ability of TMS to stimulate deep neural structures, such as the motor cortex, has enabled researchers to assess the integrity of the brain to muscle pathway and the functionality of cortical networks (Goodall et al., 2014). By using surface EMG recording electrodes, TMS-evoked responses are usually recorded from the target muscle group as compound muscle action potentials in the EMG trace and are referred to MEPs (Rossini et al., 2015; Taylor et al., 2002). There are different characteristics of MEPs that could be used to examine changes in corticospinal excitability across different experimental conditions although, the methods are either the peak-to-peak amplitude or area of the MEP (i.e., MEP amplitude). The amplitude and area of a MEP are

essential indexes because they provide a direct measure of excitability of the corticospinal pathway when we account for peripheral excitability (Goodall et al., 2014). Hence, a corresponding increase or decrease in MEP amplitude or area represents an increase or a decrease in corticospinal excitability, respectively.

Using TMS, motor threshold (MT), MEP latency and silent period, and recruitment curves are other measures for examining changes in corticospinal excitability apart from MEP amplitude. MT can be described as the minimum required stimulation intensity to the motor cortex to elicit a reliable and discernable MEP in the target muscle (i.e., the lowest TMS intensity or magnetic stimulator output (MSO) that can evoke an MEP). The motor threshold is determined by increasing the intensity of the stimulator by small increments until an MEP is elicited reliably. Motor threshold can be measured either at rest (resting motor threshold, RMT) or with minimal tonic contraction (active motor threshold, AMT) (Rossini et al., 2015). Motor threshold is usually lower at rest and in distal muscles compared to an active state (i.e. muscle contraction) and in proximal muscles (Collins, 2017; Rothwell, 1987).

Changes in resting threshold can result from a multitude of reasons such as the structure and number of excitatory projections onto the primary motor cortex, the neurone membrane, axonal electrical properties, or upregulation of receptors of this region. Hence, at rest MT represents an assessment of the excitability of inactive pyramidal neurones, thus it requires a higher TMS output to generate a MEP in the muscle of interest. However, during a slight muscle contraction, the inactive pyramid neurons are now active or at a subliminal fringe, and results in a significant reduction of MT (Collins, 2017). Another measure of CSE is latency, which is expressed in milliseconds and defined as the time interval between the TMS delivery and onset of the MEP. It indicates the time taken by the activation of neural cells and descending impulses to

reach the target muscle and produce MEP response. The time to MEP onset is affected by conduction velocities in their axons to the target muscle. A shorter latency to a given muscle from pre- to post of an experimental condition would indicate increased efficiency of the corticospinal pathway (Abbruzzese, 2010). Another way to assess CSE is through a recruitment curve. The recruitment curve measures the excitability of the CSE by evaluating the slope of the input-output curve (Valls-Solé et al., 1994). The steeper the curve, the greater the output from the corticospinal pathway for a given input. This could be related to the strength of the corticospinal projections (Chen et al., 1999).

The corticospinal silent period occurs during a period of interruption in voluntary activation after TMS has been delivered. A period of EMG 'silence' will follow the MEP whenever a strong TMS stimulus is delivered during an intense voluntary contraction (Goodall et al., 2014). It depends mainly on the stimulus intensity and could last from 200 to 300 msec on the average. The initial and later parts of the silent period are generally accepted to be of spinal and cortical origin respectively, with the first part of the silent period (50-60ms) attributed to the spinal cord (activation of Renshaw cells), and then the latter portion to the cortex (y-aminobutyric acid (GABA) type B receptor-mediated inhibition) but the mechanisms are still not fully understood (Chen et al., 1999; Goodall et al., 2014).

The size of the MEP depends on the excitability of cortical and spinal motoneurones and innervated muscle fibers since the MEP response is recorded from surface EMG. Because the MEP is representative of the whole corticospinal pathway, a change in its size may occur anywhere along that pathway. (Lockyer et al., 2021; Rossini et al., 2015). Thus, TMS is used alongside with other modes of stimulation to determine whether the change in the MEP response occurs supraspinally, spinally, or peripherally.

2.3.2 Stimulus Response Curve with Transcranial Magnetic Stimulation

The use of TMS as a technique to provide measure of the corticospinal system in human using stimulus response curve (SRC) is gradually becoming popular. It is explained by plotting the size of the EMG potential or muscle twitched evoked by magnetic stimulation of a fixed site on the scalp at a range of different intensities (Ridding & Rothwell, 1997). The change in corticospinal excitability in addition to the more usual measure of absolute threshold is determined by number of factors. Among them are, the amplitude of the MEP and duration of the silent period (SP), which are dependent on the stimulation intensity of the TMS are used in plotting the stimulus response curve based on the relationship that helps in determining the motor threshold (Pearce et al., 2013). The motor threshold is the lowest stimulation intensity to generate a MEP and which investigators use varying the stimulation intensities between participants.

The characteristics of the SRC could be explained as such that the initial segment of the region is flat and deviates from zero, corresponding to the motor threshold. The ascending portion of the curve represents a linear increase in MEP amplitude as stimulus intensity increases (Rossini et al., 2015). At greater stimulus intensities, the SRC will plateau with no further increase in MEP amplitude despite the increased stimulus intensity. The plateau in amplitude potential corresponds with a cancellation of the descending train of motor unit action potentials (Rossini et al., 2015). MEP amplitude may also no longer increase in amplitude due to previously stimulated neurons being maximally active, and subsequent stimulation may drive recruited units into a refractory period, causing MEP amplitude to decrease. Therefore, the resultant SRC is often sigmoidal in shape partly due to cortical pathways recruited by TMS, motoneurone recruitment, descending aspects of the CST, and increased synchronization of discharged motor units at higher stimulus intensities (Forman et al., 2019). Another method used to characterize stimulus response

relationship is determining the area under the curve (AUC). It is seldom used with respect to the input-output relationship between magnetic stimulation intensity but has high face validity, as it is derived from all elements of the stimulus response curve (Carroll et al., 2001; Carson et al., 2013). Summarily, the descending excitability spikes and the progression of recruited corticospinal fibres establish the SRC.

2.3.3 Maximum Amplitude of the Compound Muscle Action Potential (Mmax)

The Mmax is a measure of excitability of the peripheral nerve, neuromuscular junction, and the muscle itself. It can be used to normalize evoked responses from the CNS. By normalizing these central responses, it eliminates the effect of change in peripheral excitability on CSE during a given protocol (Forman et al., 2014). Bearing in mind that TMS induced MEPs may be impacted by peripheral transmission, therefore when assessing MEPs, it is important to consider the peripheral aspects of the system to isolate the changes within the CNS. This is made possible by normalizing a MEP to a maximal muscle compound action potential (M-max). To elicit an M-max in the muscle of interest, a maximal electrical stimulation is applied to the innervating nerve which causes a maximal response in the muscle as measured by EMG (Rodriguez-Falces et al., 2013). Since EMG is measured at the muscle, MEPs pass through both the central and peripheral systems to evoke a potential at the muscle.

2.4 Principles of Ultrasound

The use of ultrasound for accurate and reliable measurement of muscle thickness as well as non-contractile tissue is important for researchers and clinicians. However most clinicians investigating peripheral nervous system (PNS) diseases still rely on blood tests, electrophysiology (nerve conduction study and electromyography EMG) and muscle or nerve biopsies to confirm

their diagnosis (van Alfen & Mah, 2018). Ultrasound measurement of human muscle characteristics longitudinally may also be useful to monitor neurophysiological changes (van Alfen & Mah, 2018) such as the influence of muscle and fat thickness on CSE.

Although sound travels in the form of a cyclical wave, and humans can detect sound with a frequency in the range of about 20 to 20,000 Hz. Ultrasound operates at a frequency >20 KHz, and frequencies >2 MHz are used for ultrasonic imaging. This occurs when piezo- electric crystals in the transducer of the scan head produce pulses of ultrasound (Wagner, 2013). The ultrasound beam is then transmitted through the skin. When the beam encounters a tissue interface (e.g., skin-subcutaneous fat, fat muscle, and muscle bone), it is partially reflected to the transducer as an echo. Thus, the transducer has a dual function of transmitting the ultrasound and receiving it. The echoes are converted into signals for processing by the transducer. The strength of each reflected wave is represented by a dot, and the position of the dot represents the depth from which the echo was received. These dots then combine to form an image. Overall, the fundamental principle of ultrasound imaging is reflection of ultrasound waves from tissue in the path of the beam. The amount of sound reflected is dependent on the changes in acoustic impedance between two tissue interfaces. Hence, acoustic impedance is the product of tissue density and acoustic velocity (Wagner, 2013).

2.4.1 Ultrasound Characteristics

Fat tissue consists mainly of lipids with sparse connective tissues. Using ultrasound, human subcutaneous fat is found separated from the muscle by a fascia which is seen as a continuous white layer on the scan image. This layer usually generates a strong reflection of the ultrasound pulse and is referred to as the fat boundary (Carovac et al., 2011). Also, there are several factors affecting the visual interpretation of the fat boundary due to the presence of smaller amounts of

intermuscular fat tissues between the subcutaneous fat and muscle tissue. The usual practice for a sonographer to determine the subcutaneous fat thickness is to draw a vertical line from the surface of skin to the fascia (Ng et al., 2009). In addition, fibrous membranes of connective tissue (whose length, thickness and density vary between people and body sites), can be found within the layer of fat. As a result, the layer of subcutaneous fat appears to be more echogenic in the presence of thicker connective tissue. For example, dense connective tissues may appear near the fascia and make the fat-muscle boundary less clear, and a long connective tissue layer may also be wrongly interpreted as the fascia. Moreover, it is harder to define the boundary between subcutaneous fat and muscle because of the smaller amounts of intermuscular fat tissues (Ng et al., 2009).

2.4.2 Ultrasound of Human Muscle and Subcutaneous Fat

Air has almost no impedance, while fat and muscle have impedances of 0.138 $g \cdot cm^{-1} \cdot s^{-1}$ and 0.170 $g \cdot cm^{-1} \cdot s^{-1}$, respectively, and bone has a relatively high impedance of 0.78 $g \cdot cm^{-1} \cdot s^{-1}$. Because the acoustic impedances of fat and muscle are similar, there is a weaker echo for the fat-muscle interface than for the muscle-bone interface (Kang et al., 2020; Wagner, 2013). The relative strength, or amplitude, of echoes is represented by the brightness of the image on the computer screen. Strong reflections (hyperechoic) appear white; weaker reflections (hypoechoic) appear grey, and no echoes (anechoic) are black. This produces a two-dimensional grey-scale image with white borders for the skin-subcutaneous fat and muscle-bone interfaces and a visible, but less distinct, border for the fat-muscle interface (Wagner et al., 2020).

Also, normal human muscle tissue has relatively few transitions, which provides a relatively black appearance on the screen. This has been recorded as a “starry night” appearance: a black night sky (the muscle fiber tissue) with speckles that look like stars in transverse images. Longitudinally, the muscle fascicle architecture become visible, which shows as a linear, bi-

pennate or triangular structure in the ultrasound image while peripheral nerves look like round, oval, flat or triangular structures with a white edge (the epineural rim), filled with black dots of varying sizes (the nerve fascicles) (van Alfen & Mah, 2018).

2.4.3 Measuring Muscle Size and Subcutaneous Fat Thickness Using B-Mode Ultrasound

There are several modes of Ultrasound that could be used for diagnostic purposes but the one that is best used for taking muscle and fat measurements is the B-mode. There is 1) B-mode which produces a two-dimensional image on screen, and 2) M-mode, where M stands for motion and the Doppler mode, which makes use of the Doppler effect in measuring and visualizing blood flow. (Carovac et al., 2011). B-Mode ultrasound relies on the transmission of echo pulses from an ultrasound transducer. The capacity of ultrasound waves to penetrate the body is directly proportional to their wavelength, whereas image spatial resolution is inversely proportional to it. For the study of skeletal muscles, frequencies of 510 MHz (and, on rare occasions, up to 17.5 MHz for superficial muscles) are mostly used. As ultrasound waves penetrate the body, they pass areas distinguished by different acoustic impedance lowest for air and highest for bone and are partly reflected to the transducer (Müller et al., 2013). The reflection coefficient (and thus the amount of echo returned to the transducer) depends primarily on the ratio of the acoustic impedances at the interface between two tissues and the angle at which the ultrasound beam hits the structure of interest (angle of incidence equals angle of reflection). An improper transducer orientation, steering angle (i.e., the angle at which ultrasound waves are emitted from the transducer) or rotation of muscle fascicles during contraction may therefore lead to echoes not being detected by the transducer (Franchi et al., 2018).

One key limitation of standard B-mode ultrasound is its relatively small field of view, which is determined by the size of the (linear array) transducer. The field of view typically ranges

from 4 to 6 cm, although it may be as large as 10 cm in some instances. Hence, one way to overcome the limitation of a small field of view is to choose a longer transducer (Wakeling et al., 2013). Although this limitation may have a negligible influence on scans performed at rest or during slow passive joint rotations, it may impede the study of muscular contractions involved in faster movements. Furthermore, body surface contours are rarely straight, so the usage of longer transducers may lead to uneven compression of underlying tissues and, consequently, muscle deformations. However, to obtain ultrasound image for the measurement of subcutaneous fat and muscle thickness, A-mode ultrasound have been an emerging device of interest using a 2.25 MHz linear array probe, but body composition researchers and clinicians are yet to obtain its reliability (Wakeling et al., 2013).

2.5 Skinfold Caliper and Assessment of Subcutaneous Fat

The use of skinfold calipers (SC) have been commonly used to determine subcutaneous fat thicknesses although identifying the exact separation of muscle and fat can complicate measurements and affects its accuracy particularly in individuals whose adipose tissue does not separate well from underlying tissue. (Selkow et al., 2011). Skinfold caliper is acclaimed as one of the most commonly used field methods of body composition assessment which involves pinching a fold of skin and using the calipers to measure the thickness of the fold (Wagner & Teramoto, 2020). Although, this technique is relatively cheap non-invasive method used mostly in the clinical settings, achieving accurate measurement is important in research. This is because it provides an indirect estimate of subcutaneous fat and the skinfold measurement technique requires considerable practice to become proficient (Wagner & Teramoto, 2020). Hence, seeking a measure to provide the direct estimate of subcutaneous fat became more apparent.

Similarly, as found in a laboratory study, it was suggested that subcutaneous fat might affect the conduction of electric stimulation by forming a dielectric with high resistance between the skin and muscle layer (Petrofsky, 2008). Therefore, ultrasound was recommended as a probable alternative technique to overcome the resistance and offer a direct measure of uncompressed fat thickness without pinching the skin which can be used in all individuals of various sizes (Müller et al., 2013; Selkow et al., 2011).

Overall, SC and ultrasound assessments have been found to be highly correlated despite the discrepancies (Selkow et al., 2011) and that skinfold versus ultrasound measures of fat thickness showed that compressibility of subcutaneous adipose tissue depends largely on the site and the person (Müller et al., 2013)

2.6 The Effect of Muscle Size on CSE

The neural processes in the modulation of motor output have been credited to both cortical and spinal pathways (Forman et al., 2019). In this review, muscle size, muscle thickness and muscle length will be used interchangeably. Primarily, the cross-sectional area of the whole muscle and individual muscle fibres, are due to an increase in myofibrillar size and number (Folland & Williams, 2007). According to Collins et al., (2017), limb posture and not muscle length have been found to alter corticospinal excitability of skeletal muscles which also form an integral factor in influencing both the planning and execution of motor outputs. Additionally, it has been shown that voluntary muscle contraction increases the motor cortex and motoneurone pool excitability (Martin et al., 2006), and MEPs, evoked via transcranial magnetic stimulation of the motor cortex, are smaller during lengthening muscle actions than during constant-length and shortening contractions. Thus, the responsiveness of the corticospinal tract is reduced during lengthening muscle actions (Behrens, 2017). With the muscle characteristics, it is intuitive that different muscle

condition has different evoked patterns to cortical stimulation, but no known study has documented the direct evidence of muscle length on corticospinal excitability. However, MEP response to TMS stimulation recorded from the biceps brachii muscle to investigate the differences in supraspinal and spinal excitability during various force outputs between chronic and non-resistance trained individuals show that MEP amplitudes were smaller in the chronic resistance trained group during elbow flexion forces > 50% of MVC when compared to the non-resistance trained group (Pearcey et al., 2014). The chronically resistance trained group had larger bicep brachii muscles than the non-resistance trained group and the data could not decipher whether or not CSE was different between groups due to enhanced nervous system output, increased muscle mass, decreased subcutaneous thickness over the biceps brachii or a combination thereof.

2.7 The Effect of Subcutaneous Fat Thickness on CSE

The interaction between subcutaneous fat thickness (SFT) and TMS in explaining the excitation of corticospinal tract is rudimentary. However, the relationship between SFT and electrical stimulation have been well documented. For example, Petrofsky et al., (2008) performed an experiment using 2D ultrasound to assess the interrelationships between body fat and skin blood flow and the current required for electrical stimulation of human muscle. When electrical stimulation was applied above the motor point of the quadriceps, biceps, and lateral gastrocnemius muscles, they found that the greater the thickness of the fat layer the greater the current necessary to elicit a contraction in the muscle (Petrofsky et al., 2008).

In another study the effect of SFT, and EMG electrode placement on stimulus intensity needed for nerve fiber excitation was examined (Doheny et al., 2010). The experiment had an idealized finite element model of the human thigh developed and coupled to a model of neural activation. Seventeen subjects were then asked to be seated in a Cybex Dynamometer with their

right knee held at 60◦ of flexion before electrical stimulation was applied and M-wave data were recorded from the rectus femoris muscle. Using both a theoretical model and experimental model, the authors showed that stimulus current required to reach the threshold for muscle activation increased with fat thickness, electrode size, and inter-electrode distance (Doheny et al., 2010).

Also, in an experiment conducted by Baars et al., (2006) to identify the attenuating influence of skin and subcutaneous fat on surface-EMG amplitude and frequency parameters They used to ultrasound to determine tissue thickness by quantifying the electrode-muscle-distance of the biceps brachii of twenty-seven subjects (12 females and 15 males) six times each during a 43-week period. They also measured the surface-EMG amplitude and frequency parameters during isometric MVC to quantify the neuromuscular activity and fatigue. The result of the study showed that electrode-muscle-distance explained up to 31% of the variance of EMG-amplitude during MVC, i.e., interindividual variation of surface-EMG amplitude can be explained by tissue thickness (Baars et al., 2006).

To the best of my knowledge, no known study has delved into explaining the effect of SFT on CSE. Therefore, while it is clear the electrical stimulation could be altered by the thickness of subcutaneous fat, it is still unclear how SFT can change the CNS excitability at the supraspinal level.

2.8 The Effect of Sex on CSE

The commonly used indices for assessing changes in corticospinal excitability in human muscle include position relative to the body (Collins et al., 2017b), state of the muscle (Stefanelli et al., 2019), activation level of the target muscle (Kujirai et al., 2006) and the time of the day (Sale et al., 2007). Most of these studies on the evaluation of CSE using TMS have focused on comparing neurophysiological variables and their association with cortical responses. Although research on

the effect of non-physiological or non-modifiable human factors on CSE has not been well documented, few studies have compared the impact of sex on cortical function (Shibuya et al., 2016). Shibuya and colleagues (2016) determined if there existed age and gender differences in short interval intracortical inhibition and intracortical facilitation as biomarkers of corticomotoneuronal function. In this study, they used paired-pulse transcranial magnetic stimulation to measure changes in cortical excitability and results showed male demonstrated prolongation of MEP, Mmax and F-wave latencies compared to female. However, there was a non-significant difference in MEP and CMAP amplitudes based on sex (Shibuya et al., 2016).

Furthermore, it should be noted that as found in nerve conduction studies, research suggest that sex may influence cortical excitability via modulation of GABA-ergic interneurons, the main inhibitory neurotransmitter of the CNS (Kokate et al., 1994). The claim found on the basis that, progesterone enhances the activity of GABA-ergic system by increasing GABA receptor activity, hence, has the capacity difference for influencing cortical excitability. This was further strengthened in a study by Smith et al. (1999), where TMS was used to determine menstrual cycle effects on cortical excitability. They tested the effect of a subthreshold conditioning pulse on the cortex by measuring the response to a second suprathreshold test pulse and comparing it with the response elicited by the test pulse administered alone among thirteen healthy females during the follicular (low-progesterone) and luteal (high-progesterone) phases of the menstrual cycle. They reported that conditioning TMS produced more inhibition in the luteal phase than in the follicular phase, of a similar magnitude as benzodiazepine drugs. Their results showed direct evidence may exist with changes in cortical excitability (Smith et al., 1999).

Later, Casanova et al. (2014) sought to investigate if variability existed between male and female measures of motor excitability in a study involving healthy controls to confirm results

earlier reported in prior peripheral nerve conduction studies. Their report revealed no significant difference between sex in all excitability parameters evaluated, concluding that sex is not a major relevance to axonal threshold findings in motor nerves (Casanova et al., 2014).

Li and colleagues (2020) used ultrasound for measuring the cross-sectional area of the biceps brachii muscle to study the prevalence of sarcopenia in male and female. They found that males were significantly higher in the measure for muscle thickness while females recorded a significantly higher value for fat thickness. Using either ultrasound or skinfold calipers, other studies have supported the evidence that muscle thickness is higher in male (Abe et al., 2015; Ichinose et al., 1998), and that female had higher subcutaneous fat thickness (Kavak, 2006) but the influences of sex differences in muscle and subcutaneous fat thickness on cortical function have not been assessed.

2.9 Conclusion

In conclusion, measuring muscle thickness and subcutaneous fat may provide important information of their effect on CSE responses. In humans, the central control of voluntary movement is typically measured through the corticospinal tract via indirect stimulation techniques. Hence, by utilizing TMS, and peripheral nerve stimulation, the cortical connection to the muscles can be segmented into corticospinal and peripheral aspects which allows researchers to determine the level at which the muscle size and SFT can influence the motor system response of a given muscle. However, no study to date has looked at how muscle size and SFT influences CSE of the biceps brachii during contraction.

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Chapter 3: Statement of Contributions

3.1 Co-authorship Statement

My contributions to this thesis are outlined below:

i. The research idea was conceived by Dr. Button with a view to provide template for clinical research. Dr. Button encouraged me to develop expertise in the use of diagnostic ultrasound.

ii. I reviewed the literature and then, Dr. Button and I together wrote the project outline and planed the experiment.

iii. I recruited all participants and analyzed all the experimental data collected required for this study.

iv. With the help of fellow master students, Jirho Ogolo and Angie Katherin Antolinez Romero, I collected all experimental data for this thesis

v. I prepared the manuscript and thesis with the help and guidance of my supervisor, Dr. Duane Button

vi. Dr. Duane Button provided constructive feedback on the manuscript and thesis.

Chapter 4: Sex dependent differences in biceps brachii muscle size, overlying subcutaneous fat thickness and corticospinal excitability

Olalekan B. Olarogba

School of Human Kinetics and Recreation, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Running Head: Muscle size, subcutaneous fat and corticospinal excitability

Key words: active motor threshold, ultrasound, transcranial magnetic stimulation, motor evoked potential

4.1 Abstract

The aim of this study was to examine the effect of muscle size and subcutaneous fat thickness on CSE of biceps brachii and whether or not this effect was sex- dependent. Eighteen participants comprising of ten (10) males and eight (8) females had their biceps brachii muscle thickness and subcutaneous fat (SCF) thickness over the bicep brachii assessed using ultrasound. These were measured before completing a single experimental session in which corticospinal excitability of the biceps brachii was assessed using transcranial magnetic stimulation (TMS) of the motor cortex. Motor evoked potentials (MEPs), elicited by TMS, were recorded at eight different TMS mean stimulator output (MSO) intensities from 90 -160% of active motor threshold (AMT) from the biceps brachii during a 90° elbow flexor isometric contraction at 10% maximum voluntary contraction (MVC). Before and after the stimulations, maximal compound muscle action potential (Mmax) during 10% MVC was also recorded. Males had significantly higher (p = .003, d = 7.27) and lower (p = .003, d = 2.20) muscle size and SCF thickness, respectively compared to females. The MSO required to achieved AMT was higher in women than men. MEP amplitudes from the biceps brachii increased significantly (p < .001, η_p^2 = .680) and similarly (p = .365, η_p^2 = .052) for both men and women. For men there was a significant (p < .05, r = .591) positive relationship between muscle size and MEP amplitude, whereas for women there was a significant (p < .05, r = -0.525) negative relationship between MEP amplitude and skinfold thickness. Collectively, the data suggest that the amount of muscle size and subcutaneous fat thickness affects CSE, and these findings are sex- dependent.

Key words: corticospinal excitability, ultrasound, transcranial magnetic stimulation, motor evoked potential

4.2 Introduction

Ultrasound has become a unique technique for measuring human muscle and fat thickness. Apart from using ultrasound, other methods such as dual x-ray absorptiometry and magnetic resonance imaging (MRI) can also be used to estimate both human muscle and subcutaneous fat thickness (SFT) but ultrasound may be more advantageous due to its portability, lower cost and no radiation or magnetic field exposure (Wegener et al., 2011). In addition, ultrasound may be an alternative method for measuring localized SFT leading to an estimation of the overall body composition (Thiebaud et al., 2019). Hence there has been a growing interest in assessing muscle properties and body composition using ultrasound particularly in measuring thickness of muscle and subcutaneous fat in humans.

Ultrasound measurement of human muscle and SFT characteristics may also be useful for understanding changes in neurophysiological responses to stimulation. Using ultrasound, human subcutaneous fat is found separated from the muscle by a fascia which is seen as a continuous white layer on the scan image (Ng et al., 2009). Thus, ultrasound could be used to determine if muscle and subcutaneous fat thickness influences muscular responses to different types and intensities of stimulation. A common measure of nervous system excitability during a voluntary contraction is corticospinal excitability. Corticospinal fibers are unique connections originating in the motor cortex and terminate indirectly via interneurons of the spinal cord or directly onto spinal motoneurones. The contributions or these fibers to voluntary muscle contraction can be evaluated by applying transcranial magnetic stimulation (TMS) to the cortex. TMS elicits a motor evoked potential (MEP) in each muscle of interest, and it is recorded via electromyography (EMG). Typically, putting into consideration the peripheral excitability, the larger the response at a given stimulation intensity represents greater CSE to that given muscle.

It has been shown that fat and muscle have impedances of 0.138 $g \cdot cm^{-1} \cdot s^{-1}$ and 0.170 $g \cdot cm^{-1} \cdot s^{-1}$, respectively (Wagner, 2013), however, the influence of this on corticospinal excitability remains largely unknown. Using electrical stimulation with ultrasound imaging to measure multifidus muscle activation has been demonstrated to identify the existence of muscle activation deficit with changes in muscle thickness (Wattananon et al., 2020). Also, Doheny et al. (2010) developed a study to optimise the electrode configuration based on subcutaneous fat thickness during neuromuscular electrical stimulation and found that stimulus current threshold for excitation becomes less sensitive to electrode size as subcutaneous fat thickness increases. However, none of these studies considered sex-related differences. Based on the aforementioned and the fact that male typically have larger muscle size (Abe et al., 2014; Ichinose et al., 1998), and lower subcutaneous fat thickness (Müller et al., 2013; Thiebaud et al., 2019) than female there could be sex-related differences when considering muscle and SFT and their effect on CSE of the biceps brachii.

Therefore, the primary purpose of this study was to; 1) investigate if corticospinal excitability (CSE) of the biceps brachii is muscle and fat thickness dependent, 2) determine the effect of sex on the CSE. We hypothesized that 1) CSE of biceps brachii will become larger as the muscle size of the biceps brachii increases and reduces as subcutaneous fat thickness increases and 2) that the pattern of increment in CSE would be different in males compared to females.

4.3 Methodology

4.3.1 Participants

Eighteen healthy, recreationally active (≤ 5 hours of physical activity per week) comprising of ten male (28.2 ± 7.6 years of age, height 178.6 ± 4.8 cm. weight 85.3 ± 13.3 kg) and eight female (25.9 ± 5.2 years of age, height 160.6 ± 7.2 cm. weight 68.8 ± 15.6 kg) volunteers with no known

neurological impairments participated in the study. Participants filled out a magnetic safety checklist (Rossi et al., 2009) and an International Physical Activity Questionnaire (IPAQ) to determine level of physical activity (Craig et al., 2003). Participants with any known contraindications to magnetic stimulation, such as subjects with metal implants, or a history of epilepsy was excluded from the study. Participants were also screened for any contraindications to exercise by completing the Physical Activity Readiness Questionnaire (PAR-Q) form (Warburton et al., 2011). Hand dominance (16 right-hand dominance, 2 left-hand dominance) was also determined using the Edinburg handedness inventory (Veale, 2014), to ensure that the stimulation conditions were measured from the dominant arm. In addition, participants were also instructed to refrain from heavy exercise 24 hours before testing and to follow the Canadian Society for Exercise Physiology preliminary instructions (i.e., no eating for 2 hours, drinking caffeine for 2 hours, smoking for 2 hours, or drinking alcohol for 6 hours) prior to the commencement of testing (Canadian Society for Exercise Physiology, 2013). Prior to data collection, all participants received verbal explanation of the experimental protocol. If any participant had questions or concerns, these were answered before written informed consent was obtained. This study was conducted in accordance with the Helsinki declaration and all protocols were approved by the Interdisciplinary committee on Ethics in Human Research at Memorial University of Newfoundland (ICEHR No. 20220630 -HK). Additionally, the Tri-council guidelines in Canada were adhered to with the potential risks being fully disclosed to all participants.

4.3.2 Experimental Set-Up

A two-group subject experimental design was used. The study was started with a familiarization session of about 30 minutes at an earlier date after consent was taken before an actual testing session was carried out for data collection. The testing session took about 1.5 hours. During the initial visit, participants were familiarized with the stimulation techniques and the experimental setup. Participants received, TMS, brachial plexus stimulation and ultrasound to ensure they were comfortable receiving stimulations during the experimental protocol. This study was conducted in a single session and consisted of two parts: *part 1* assessed the muscle size and subcutaneous fat thickness while *part 2* assessed corticospinal excitability. Data measurements were taken from the dominant arm for part 1 and 2 sequentially.

4.3.3 Elbow Flexor Force

Participants were seated in a custom-built chair (Technical Services, Memorial University of Newfoundland, St. John's, NL, Canada) in an upright position, with the chest and head strapped in place to minimize movement, and the hips and knees flexed at 90º. The shoulder was placed at 0º, and the elbow was flexed at 90º. At the 0º position, both arms were slightly abducted and rested on a padded support. The forearm was held horizontal, positioned midway between neutral and supinated positions, and placed in a custom-made orthosis that was connected to a load cell (Omegadyne Inc., Sunbury, OH, USA). The load cell detected force output, which was amplified × 1000 (CED 1902, Cambridge Electronic Design Ltd., Cambridge, UK) and displayed on a computer screen. Data were sampled at 2000 Hz. (Signal 5.11 software, Cambridge Electronic Design Ltd., Cambridge, UK).

During the experiment, participants were instructed to give a maximal voluntary isometric contraction (MVC) for 3 secs and produce force as quickly as possible. Verbal encouragements

were given to the participant during the MVC to provide motivation and the computer screen was positioned so that the participants could see real-time feedback of their effort. Two MVCs with at 1 minute rest in between was performed to ensure maximal force are achieved and a third MVC was performed when the previous 2 were not within 5% of one another. Participants were instructed to maintain an upright position with their head in a neutral position during contractions of the elbow flexors. Once MVC force was determined, the force at 10% MVC was calculated for each participant which was held by participants in the protocol for assessing corticospinal excitability (figure 1a).

4.3.4 Ultrasound Evaluation

Ultrasound was used to measure the participants' muscle size and subcutaneous fat thickness. All ultrasound scans were made with the subject in a relaxed seated position in a chair with feet distributed evenly on the floor. The elbow was at 90^0 and forearm on an armrest adjusted so that when the ultrasound probe was placed on the table it was held perpendicularly to acquire a transverse image of the arm. This position was chosen to avoid compressing the subcutaneous fat layer and apply just enough pressure on the transducer to allow contact between its surface and the skin (figure 1b). All assessments were made by the same examiner using a real-time ultrasound device (Chison Sonobook 9, USA) equipped with an 8 MHz linear transducer (L12M-T). Ultrasound gel (EcoGel 200) was applied to the skin to act as a coupling medium between the skin and the transducer, and measurements were taken in the sagittal plane for the biceps brachii with the transducer aligned to match the level of skinfold measurement.

Image acquisition and thickness measurement was done from the reference point used for the skinfold. The linear probe was placed perpendicular to the skin and was moved along this line till a suitable image was obtained and frozen. All scans were repeated until image resolution was

sufficiently clear for the subcutaneous fat/skeletal muscle interface and the skeletal muscle/bone interface to be simultaneously identified. A vertical line was placed on the circumferential line which was corresponding to the center of the probe providing a suitable image. The measurement of thickness of muscles of the flexor compartment was recorded between the superficial fat-muscle interface and the periosteum of the humerus (figure 2). The force applied to achieve contact with skin was just sufficient to get a suitable image to avoid the compression of muscle due to unnecessary excessive force. Three measurements of muscle thickness were recorded by the observer while arm was kept in fixed position.

4.3.5 Skinfold Measurement

Using the description of the International Society for the Advancement of Kinanthrometry (ISAK) recommended technique, measurement of subcutaneous fat was taken using a skinfold (SF) caliper (Lange Skinfold Caliper Model C-130, Cambridge Scientific Industries). Participants were asked to flex their elbow at 90° with their palm facing up. The distance between the acromion and olecranon was measured from the posterior aspect of the arm and registered as the arm length. The arm circumference was then measured at the mid-level of arm length. The same level (mid arm length), in the longitudinal midline of the anterior aspect of the arm, the SF measurement point and point for subsequent ultrasound evaluation (figure 3) was marked with a water-resistant pen. Then approaching the skin surface at 90° with the finger and thumb; aligning these on the SF landmark; and raising the SF with parallel sides. The calliper was applied 1 cm laterally from the raised fold and midway along the fingernail of the index finger. The grasped skinfold was shaken with both fingers to avoid including the muscle layer in the measurement. Measurements are taken by releasing the calliper spring and reading the dial to 0.2 mm after two seconds (Müller et al.,

2013). Furthermore, to ensure consistency in maintenance of the pressure by fingers on the skinfold, all measurements was made by the same investigator.

4.3.6 Electromyography (EMG)

EMG activity of the biceps brachii muscle was recorded using 10 mm diameter MediTrace Pellet Ag/AgCl electrodes (disc shape, Covidien, Mansfield, USA). EMG was recorded using a bipolar configuration (centre to centre) with an inter-electrode distance of 20mm over the mid-muscle belly of the participant's biceps brachii. The ground electrode was placed on the lateral epicondyle of the arm. Prior to the electrode placement, the participant's skin was prepared for all electrodes to reduce impedance and to obtain the best signal-to-nose ratio of EMG. The electrode site was shaved, abraded (using abrasive gel) to remove dead epithelial cells, and sanitized with isopropyl alcohol. EMG was collected on-line at 5 kHz using a CED 1402 interface and the associated Signal (version 5.11) software (Cambridge Electronic Design (CED) Ltd., Cambridge, UK). EMG signals were amplified (gain = 300) and filtered with a 3-pole Butterworth filter with cut-off frequencies of 10 –1000 Hz with the CED 1902 amplifier

4.3.7 Stimulation Conditions

4.3.7.1 Brachial plexus electrical stimulation (Erb's Point Stimulation)

Stimulation of the brachial plexus was used to measure participants' maximal compound muscle action potential (Mmax). Erb's point was electrically stimulated via a cathode and anode (Meditrace Ag-AgCl pellet electrode, disc-shaped 10 mm diameter, Covidien, Mansfield, USA) positioned on the skin overlying the supraclavicular fossa and over the acromion process, respectively. Current pulses were delivered as a singlet using a constant-current electrical stimulator (square wave pulse, 200-μs duration at 100-300 mA: model DS7AH, Digitimer Ltd, Welwyn Garden City, UK). The electrical stimulation was then gradually increased until the M-

wave of the biceps brachii no longer increased (Stefanelli et al., 2019). The stimulator setting used to evoke Mmax at 10% MVC of flexion was recorded and then increased by 10% to ensure maximal M-wave throughout all trials (Aboodarda et al., 2015; Crone et al., 1988). This stimulator intensity was used for the remainder of the experimental protocol. Two Mmax trials were performed pre- and post- TMS trials, with the average amplitude of the Mmax recorded.

4.3.7.2 Transcranial magnetic stimulation (TMS)

TMS stimulations were delivered using a Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK). A circular coil (13.5 cm outside diameter) was positioned over the vertex of each participant's skull, with the direction of current flow in the coil preferentially activating the left or right motor cortex, depending on hand dominance. The stimulating coil was positioned directly over the vertex of participants' head to induce MEPs in the active (10% MVC) biceps brachii muscle. The vertex would be located by measuring the mid-point between the participant's nasion and inion and the participant's tragi (Power & Copithorne, 2013). The intersection of these two points was defined as the vertex and this point was clearly marked with a felt-tipped dry-erase marker. Electrical currents flowed in an anticlockwise direction through the circular coil and the induced current formed in the cortex flowed from anterior to posterior or vice versa to activate the right or left motor cortex (A side up for right side, B side up for left) and subsequently activate the dominant biceps brachii as the coil is held tangentially and firmly over the participant's skull. Stimulation intensity started at approximately 30% maximal stimulator output (MSO) at 10% MVC and was increased gradually until active motor threshold (AMT) was determined for participants. AMT was defined as a clearly discernable MEP in the biceps brachii with an amplitude ≥50 μV in five out of ten stimulation trials. Once AMT was determined, eight

experimental intensities (90, 100, 110, 120, 130, 140, 150, and 160% of MEP AMT) were calculated to create a stimulus response curve (SRC) for the trials.

4.3.8 Experimental Protocol

Participants completed a single experimental session (~1.5 hrs.). The procedure involved performing an elbow flexion contraction at 10% of MVC. The participants were first evaluated for anthropometric measures with which an ultrasound measurement for biceps brachii muscle size and skinfold and ultrasound measurement of subcutaneous fat thickness over the biceps brachii were performed. Participants were then prepared for EMG and the stimulation conditions. Participants performed submaximal isometric contractions for 5 s to get accustomed to producing force output. Participants then completed two elbow flexors MVCs for 3 secs with 1 minute rest between each MVC, which were required to have force measurements (N) within 5% of one another to ensure maximal force output. A third MVC was then performed if the two MVCs exceed 5% of each other. The MVCs were then proceeded by a 10-min rest period. After 10 min of rest, the intensities for each stimulation type were set. CSE (MEP) and peripheral excitability (Mmax) measurements were then taken at the biceps brachii during 10% MVC. The Mmax was recorded before and after the TMS trials. Participants received 7 MEPs concurrently for each of the 8 stimulation intensities with 1 minute rest between successful trials. The order of the stimulation intensity was randomized.

4.3.9 Data analysis

Data for the muscle size and subcutaneous fat thickness were recorded in millimeters (mm) from the image produced using the B-mode ultrasound. Measures of CSE (i.e., MEP, Mmax amplitudes) was analyzed off-line using Signal 5.11 software (CED, UK) after the experimental

protocol was completed. Peak-to-peak amplitudes of evoked potentials (MEP, and M-wave) were recorded from the initial deflection of the voltage trace to the return of the trace back to baseline EMG. MVC force output was measured as the peak-to-peak amplitude from no force to maximum force.

To create the SRCs for each intensity, raw MEP amplitudes were plotted across stimulation intensities (90-160% MEP AMT) and were used to determine the raw area under the curve (AUC) measurements. The AUC represents the summation of total corticomotor output over the range of TMS intensities used in the experiment (Iyer & Madhavan, 2019). The larger the value of the AUC, the greater the corticomotor output. The AUC for each SRC was obtained by trapezoidal integration of the curve's function using Prism 9 for MacOS (version 9.2.0; GraphPad Software LLC, CA, USA).

4.3.10 Statistical Analysis

Statistical analyses were computed using SPSS (SPSS 28.0 IBM Corporation, Armonk, New York, USA). Assumptions of normality (Shapiro-Wilk test) and sphericity (Mauchley test) were tested for all dependent variables. If the assumption of sphericity was violated, the corrected value for non-sphericity with Greenhouse-Geisser epsilon was reported. A one-way ANOVA with repeated measures was also performed on within each trial for MEP to ensure consistency of the data. To determine the relationship between muscle size and subcutaneous fat thickness during the various CSE measures at 10% MVC, Pearson correlations was used.

A paired-sample t-test was used to determine if a statistically significant mean difference was present between pre- and post- Mmax amplitudes. Additionally, an independent t-test was also done to assess if a statistically significant mean difference existed in the group statistics between

sexes. All statistics were performed on group data and the statistical significance was set at $p < .05$.

4.4 Results

All data is reported in text as mean ± standard deviation (SD) and illustrated in figures as mean ± standard error (SE). Group data on anthropometrics, ultrasound, force and stimulation values for men and women is reported in Table 1. Overall, males had a 35.1% larger ($t_{(16)}$ = 3.12, p = .003, d = 7.27) biceps brachii, 40% lower ($t_{(16)}$ = 3.22, p = .003, d = 2.20) subcutaneous fat thickness, and 106% greater ($t_{(16)}$ = 3.31, p = .002, d = 1.57) elbow flexor force.

4.4.1 Active Motor Threshold of the biceps brachii

There was a significant difference ($t_{(16)}$ = 2.32, p = .017) between MEP intensity required to obtain threshold at 10% MVC between males and females. Males reached threshold earlier than females (43.3 ± 7.92 *versus* 52.9 ± 9.61 MSO, respectively).

4.4.2 MEP Amplitude

For the participants, group data demonstrated no significant effect for the interaction between sex and stimulation intensity ($F_{(2.575, 41.205)}$ = 1.22, p = .312). In addition, no significant main effect of sex was present ($F_{(1, 16)}$ = .87, p = .365), indicating the MEP amplitudes were similar between sexes. Figure 4 shows raw MEP amplitudes at stimulation intensities ranging from 100-160 %AMT for one participant. A significant main effect was found for stimulation intensity ($F_{(2.575, 41.205)}$ = 33.98, p = < .001) signifying that mean MEP amplitudes increased with increased stimulation intensity (figure 5A) and the %MSO used at each stimulation %AMT also increased throughout but was higher in females for each stimulation intensity (Figure 5B). Pairwise comparisons indicated that mean MEP amplitudes were significantly different for each stimulation intensity greater than 90% (p < .05 for all comparisons), except for no significant mean differences between 130% and 140% stimulus intensities (p = .12), 140% and 150% stimulus intensities (p =

1.00), between 140% and 160% stimulus intensities (p = 1.00) and between 150% and 160% stimulus intensities (p = 1.00).

The AUC for the biceps brachii, which was derived from the stimulus response curves, was not significantly different between sexes ($t_{(16)}$ = 0.89, p = .194). There was also no change ($t_{(16)}$ = 3.12, p = .161). between the pre- and post- Mmax values following the TMS trials and no difference between sexes.

4.4.3 Correlation between Variables

Table 2 shows correlation data between dependent variables. For the whole group there were some significant ($p < 0.05$) correlations between dependent variables. Significant positive correlations existed between the %MSO at AMT and fat thickness, and AUC and muscle size. For men, significant ($p < 0.05$) positive correlations existed between MEP amplitude at AMT and muscle size, %MSO at AMT and MVC, and AUC and muscle size. For women, significant ($p < 0.05$) positive correlations existed between %MSO at AMT and fat thickness (Figure 6A) and %MSO at AMT and muscle size.

Table 3 shows correlation data between dependent variables and the MEP amplitude at each stimulation intensity. For males, significant ($p < 0.05$) positive correlations existed between MEP amplitude and muscle size (Figure 6B) at almost every stimulation intensity based on %AMT. For females, significant ($p < 0.05$) negative correlations existed between MEP amplitude and skinfold thickness (Figure 6C) at 110, 120, and 130 %AMT.

There were no correlations found between Mmax and any other dependent variables (data not shown in tables).

4.5 Discussion

This is the first study to directly examine the effects of muscle size and subcutaneous fat thickness of biceps brachii on CSE and to determine if any of these effects were sex- dependent. The main observations from this study were males had a greater biceps brachii thickness with less subcutaneous fat overlaying the biceps brachii compared to females. While there was a difference between %MSO at AMT of the biceps brachii, there were no differences in MEP amplitude size or AUC between men and women. Females required higher stimulation intensity to reach threshold compared to males and have to receive a higher TMS output in order to achieve similar MEP amplitudes. For males there was a positive relationship between muscle size and MEP amplitude, whereas for females there was a negative relationship between MEP amplitude and skinfold thickness. Thus, for females, higher skinfold thickness leads to lower MEP amplitudes, whereas in males, greater muscle mass leads to higher MEP amplitudes. Our findings illustrate that the measurement of corticospinal excitability projecting to the biceps brachii is impacted by muscle size and subcutaneous fat thickness and this impact differs between men and women.

4.5.1 Sex Differences in Muscle Size and Subcutaneous Fat Thickness

In the current study males had a significantly greater muscle size and arm circumference and significantly less subcutaneous fat thickness (both ultrasound and skinfold caliper measures) compared to females. Thus males tended to have more muscle tissue and less adipose tissue than females which has also been previously shown in a study that used ultrasound to measure the biceps brachii muscle size and SCF thickness overlaying the biceps brachii among healthy and unhealthy group (Li et al., 2020). Males also had a higher elbow flexor MVC than females, which has also been shown elsewhere (Hunter et al., 2004; Keller et al., 2011). This sex difference in MVC could have arisen from differences in muscle size or muscle contractile mechanism

associated with the prevalent muscle type in men (type I) and women (type II) as reported in several sex-based muscle fatigue studies (Hunter, 2016; Hunter et al., 2006; Wüst et al., 2008).

4.5.2 Sex Differences in CSE

4.5.2.1 Stimulator Intensity at AMT

In the present study females required a higher stimulation intensity to reach AMT compared to males. A significant correlation between %MSO at AMT to fat thickness was also found for females but not males. This implies that the size of subcutaneous fat thickness may be proportional to the intensity of TMS output required to excite the cortical neurons. Furthermore, females had higher fat thickness overlaying the biceps brachii than males. Thus, the difference in fat thickness may be responsible for the difference in the cortical excitation seen at AMT in both sexes. This finding is in agreement with our hypothesis, that corticospinal excitability will decrease as subcutaneous fat thickness increase. Interestingly, there was no relationship between fat thickness and %MSO at AMT for males. It is possible that since males had much lower fat thickness than females and the range of fat thickness was much closer in males that we did not find any correlation. Future studies could incorporate participants ranging from normal weight to obese to further study the impact of muscle and SCF thickness on CSE.

One possible influence as to why intensities were different at threshold may be attributed to the high impedance (resistivity) offered by the subcutaneous fat since a positive correlation existed between stimulation intensity at AMT with subcutaneous fat thickness. Previous work has employed neuromuscular electrical stimulation to examine the interrelationships between subcutaneous fat thickness and current required for electrical stimulation of the human muscle (Doheny et al., 2010; Petrofsky et al., 2008). They found that higher currents were required for stimulation to reach threshold and elicit muscle contraction of biceps brachii in people with thicker

SCF. Similarly, Baars and colleagues (2006), in an attempt to identify the attenuating influence of skin and subcutaneous fat tissue on surface electromyography demonstrated that there is an inverse relationship between electrode muscle distance and sEMG amplitude, which is explained by tissue thickness. Thus, when researchers are stimulating the participant to determine neurophysiological responses from EMG they need to be aware that the SCF thickness overlying a given muscle has an impact on the signal and that this impact may be different between sexes.

4.5.2.2 MEP Amplitudes at Various Intensities

In this study, males produced higher MVCs, had larger bicep brachii muscle thickness and had lower SCF thickness than females. Males' muscle size had a significant positive relationship with the elicited MEPs peak-to-peak amplitude at almost all stimulation intensities whereas a negative relationship was seen for females. While there was no significant difference in the MEP amplitude for both male and female, females required a higher MSO to achieve a similar MEP amplitude from 100-160% MSO at an absolute force that was lower than males. Thus, we believe that both muscle size and SCF thickness impacts the CSE response recorded from a muscle. However, there are no other studies that reports the relationship between CSE and muscle size. Previous work done in our lab to determine the differences in supraspinal and spinal excitability during various force outputs of the biceps brachii among chronic and non- resistance trained individuals showed that elbow flexor force output was greater in the chronic resistant trained group than the non-resistant group and that the CSE responses were different between these groups (Pearcey et al., 2014; Philpott et al., 2015). In these studies, it was acknowledged that differences in force outputs between groups were due to both changes in nervous system excitability and muscle size. However, relationships between muscle size and SCF thickness was not reported.

Based on the findings in the current study, muscle size itself may have had a partial impact on the differences in CSE of the biceps brachii in those training studies.

There was a positive correlation between AUC (from 90% - 160% of AMT), a measure of CSE and muscle size for males but not for females. It is possible that electrophysiology properties of the muscle necessary for adequate muscle activation are enhanced with increased muscle size which results in an increase for descending motor drive to the spinal cord and a corresponding increase in CSE. In stroke patients muscle thickness, as measured by ultrasound, was significantly smaller on the paretic side compared to the non-paretic (Berenpas et al., 2017). Berenpas et al. (2019) then demonstrated that functional electrical stimulation has the capacity to counteract reduced motor nerve excitation and muscle atrophy and increase muscle mass. Furthermore, they found that, MEP AUC and amplitudes were generally lower on the paretic side compared to the non-paretic side. Thus, a larger muscle mass in the non-paretic produced a greater excitation in that muscle.

In CSE studies, MEP amplitude it usually normalized to Mmax in order to account for the overall effect of a change in peripheral excitability would have on CSE. In the current study we found no sex differences in Mmax, nor did we find any correlation between Mmax and any dependent variables. Our findings are supported by other studies. (Nozoe et al., 2020) investigated changes in lower-leg motor nerve conduction, muscle strength, and muscle wasting in patients with acute stroke. They measured their motor conduction velocity (MCV) and compound motor action potential (CMAP) amplitude and quadriceps muscle thickness (QMT) on both sides of the common peroneal nerve. Their results showed limb muscle wasting was detected in QMT with a significant difference between paretic and non-paretic side, but MCV and CMAP amplitude were not significantly different between limbs (Nozoe et al., 2020). Berenpas et al. (2017), also showed

no correlation between the muscle thickness and compound motor action potentials (CMAPs) AUC.

Our CSE results could be interpreted a few different ways; 1) males and females have similar CSE of the biceps brachii but females require a higher MSO to achieve a similar MEP amplitude because of differences in SCF thickness and muscle size, 2) males and females have different CSE of the biceps brachii because females have a lower MEP response at the same absolute force then males and females require a greater MSO to activate a smaller muscle mass, 3) SCF thickness and muscle size correlate differently with different types of stimulation. TMS is given at the brain and the MEP response was impacted by SCF thickness and muscle size whereas electrical stimulation was given at the brachial plexus and the Mmax response was not impacted. Thus, perhaps the distance from site of stimulation to the recording of the response may be impacted more or less by SCF thickness and muscle size. Furthermore, how the stimulation is produced and what it travels through on its way to the muscle may also be affected by the amount of SCF thickness and muscle size.

4.5.3 Comparison Between Ultrasound and Skinfold Caliper Measurements of Subcutaneous Fat (SCF) Thickness

A secondary objective to this study was to compare our ultrasound and skinfold measures of SCF thickness overlying the biceps brachii. Our data indicate a significant positive correlation between the ultrasound and skinfold measurements of SCF thickness overlaying the biceps brachii and this result was not sex- dependent. Our findings were similar to the results obtained in previous studies which compared the measurement of subcutaneous fat at different sites (chest, abdomen, thighs, triceps and back) using skinfold calipers and ultrasound (Nösslinger et al., 2022; Orphanidou et al., 1994). However, a paired t-test also indicated a significant difference between

the two measures, whereby SCF thickness measures from ultrasound were lower than skinfold caliper measures. Ultrasound provides a direct measurement for subcutaneous fat thickness, thus the difference in measurements may imply presence of tissue approximation seen in the indirect method of subcutaneous fat thickness with the use of skinfold caliper. Thus, the lower SCF thickness measure from ultrasound compared to skinfold calipers was not unexpected.

4.6 Methodological Considerations and Future Studies

There are several methodological considerations for the study. First, our data analysis, included a sample of n = 18 (male 10, female 8), rather than sample of n = 20 (male 10, female 10). Hence, there is a decrease in statistical power (Chaumon et al., 2021). Second, TMS induced raw MEP peak-to-peak amplitudes were used to represent the measures of corticospinal excitability of the biceps brachii (Ah Sen et al., 2017; Iyer & Madhavan, 2019). However, the MEP amplitude as a measure of CSE can be influenced by three components including supraspinal, spinal and peripheral and the types of stimulation and location are different between the three. Thus, the effect of muscle and SCF thickness on muscle responses to stimulation may be different due to location and stimulation type. For example, we showed correlations between MEP amplitude and muscle size or skinfold thickness but there were no correlations between Mmax and these same variables. Both the stimulation site and type of stimulation differ between the production of a MEP and Mmax. Thus, future studies comparing various stimulation types and how the subsequent CSE responses relate to muscle and SCF thickness are warranted. Third, our choice of flexor arm muscle to measure CSE could be limited at generalizing the results for arm muscles and to other skeletal muscles in the body. Muscle, size, architecture, and function and the SCF thickness overlaying each muscle is quite different. Thus, future studies could compare CSE of several muscles and how the subsequent CSE responses relate to muscle and SCF thickness. All

CSE responses were taken during a low intensity contraction. The relationship between MEP CSE and muscle thickness or SCF thickness may change when measured at higher intensity muscle contractions. Finally, we did not control for menstrual cycle in our study. However, menstrual cycle does not appear to have an effect on CSE in females (El-Sayes et al., 2019)

4.7 Conclusion

The present study demonstrated that muscle size and subcutaneous fat thickness are important factors that, in part, affect CSE projecting to the biceps brachii. It was also found that the CSE projecting to the biceps brachii in female and male are similar, but female require higher stimulation intensity compared to male to get a similar response. This is, in part, due to females having lower muscle thickness and higher SCF thickness compared to males. Overall, CSE responses are influenced by muscle size and SCF thickness and should be taken into consideration during experimental design used to measure CSE of the biceps brachii.

4.8 Figure Legends

Figure 1: (A) Schematic diagram of the TMS stimulations. **(B)** Protocol for ultrasound evaluation. Following the ultrasound evaluation participants were asked to maintain 10% MVC at the stimulation intensity was set. Participants received seven TMS trials to the motor cortex at each intensity (90% - 160% of AMT) to obtain the MEP data for CSE. Maximum voluntary contraction (MVC), transcranial magnetic stimulation (TMS), motor evoked potential (MEP), active motor threshold (AMS).

Figure 2: Method of providing real-time measurement of muscle size and subcutaneous fat thickness of biceps brachii.

Figure 3: Protocol for determining mid-arm and site for measuring muscle size and subcutaneous fat thickness of biceps brachii.

Figure 4: Corticospinal responses during 10% MVC recorded from biceps brachii. Average MEP traces from the dominant sides from 100% - 160% of threshold intensity.

Figure 5: (A) Group data for biceps brachii MEP amplitudes with 10% MVC. Data presented as mean ± *SE* (*n* = 18). **(B)** Group data for mean stimulator output at each intensity to elicit MEP of biceps brachii with 10% MVC. Data presented as mean ± *SE* (*n* = 18).

Figure 6: (A) Group data for correlation between %MSO at AMT and subcutaneous fat thickness. **(B)** Group data for correlation between MEP amplitudes at 120% of AMT and muscle size. **(C)** Group data for correlation between MEP amplitudes at 120% of AMT and skinfold.

4.9 List of Tables

Table 1:Anthropoemetrics, ultrasound, force and stimulation values for men and women. Data presented as Means ± Standard deviation. Maximum Voluntary Isometric Contraction (MVC), Active Motor Threshold (AMT), Maximal Muscle Compound Action Potential (Mmax).

	All Participants (n = 18)	**Male (n =10)**	**Female (n= 8)**	***P***
Anthropometrics				
- Age (years)	27.1 ± 6.54	28.2 ± 7.55	25.9 ± 5.19	.235
- Weight (Kg)	88.0 ± 16.29	85.3 ± 13.33	68.8 ± 15.63	**.014**
- Height (cm)	170.6 ± 10.86	178.6 ± 4.80	160.6 ± 7.21	**<.001**
- BMI (Kg/m^2)	26.7 ± 4.35	26.7 ± 3.45	26. 6 ± 5.54	.496
- Arm Circum. (mm)	333.7 ± 42.24	347.7 ± 39.87	316.3 ± 40.77	.060
- Skinfold (mm)	8.1 ± 3.70	5.8 ± 1.83	11.0 ± 3.47	**<.001**
Ultrasound Assessment				
- Fat thickness (mm)	6.6 ± 2.74	5.1 ± 1.91	8.5 ± 2.52	**.003**
- Muscle Size (mm)	25.9 ± 8.95	30.7 ± 9.22	19.9 ± 3.42	**.003**
Force				
- MVC	278.6 ±149.54	361.5 ± 153.5	175.0 ± 43.85	**.002**
Stimulation				
- AMT (%MSO)	47.6 ± 9.76	43.3 ± 7.92	52.9 ± 9.61	**.017**
- AUC (mV/ms)	74.0 ± 32.67	80.1 ± 32.18	66.3 ± 33.75	.194
- Mmax (mV)	9.7 ± 4.51	10.7. ± 4.08	8.5 ± 5.00	.161

Bold values = $p < .05$

Table 2: Correlation between variables. Percentage of maximum stimulation output at threshold ($AMT_{(\%MSO)}$), Peak-to-peak amplitude at threshold ($AMT_{(MEP)}$), Area under curve (AUC), Maximum voluntary isometric contraction (MVC).

Correlation	$AMT_{(\%MSO)}$	$AMT_{(MEP)}$	AUC	Fat thickness	Muscle Size	MVC	Skinfold
All Participants							
- $AMT_{(\%MSO)}$	1.000	-0.367	-0.331	**0.666**	-0.088	-0.361	**0.698**
- $AMT_{(MEP)}$		1.000	**0.726**	-0.167	0.186	0.136	-0.251
- AUC			1.000	-0.223	**0.426**	0.351	-0.324
- Fat thickness				1.000	-0.346	-0.369	**0.796**
- Muscle Size					1.000	**0.641**	**-0.309**
- MVC						1.000	**-0.432**
- Skinfold							1.000
Men							
- $AMT_{(\%MSO)}$	1.000	-0.009	0.057	0.279	0.196	**0.698**	0.384
- $AMT_{(MEP)}$		1.000	**0.561**	-0.136	**0.591**	0.268	0.030
- AUC			1.000	-0.284	**0.687**	0.441	0.086
- Fat thickness				1.000	-0.162	0.222	**0.660**
- Muscle Size					1.000	0.481	0.078
- MVC						1.000	0.305
- Skinfold							1.000
Women							
- $AMT_{(\%MSO)}$	1.000	**-0.727**	-0.587	**0.717**	**0.867**	-0.197	0.679
- $AMT_{(MEP)}$		1.000	**0.910**	-0.241	-0.583	-0.037	-0.525
- AUC			1.000	0.042	-0.470	-0.184	-0.479
- Fat thickness				1.000	**0.705**	-0.478	**0.642**
- Muscle Size					1.000	-0.439	**0.732**
- MVC						1.000	-0.529
- Skinfold							1.000

Bold values = $p < .05$

Table 3: Correlation between variables and MEP amplitude at different stimulation intensity. Motor evoked potential (MEP), Maximum voluntary contraction (MVC).

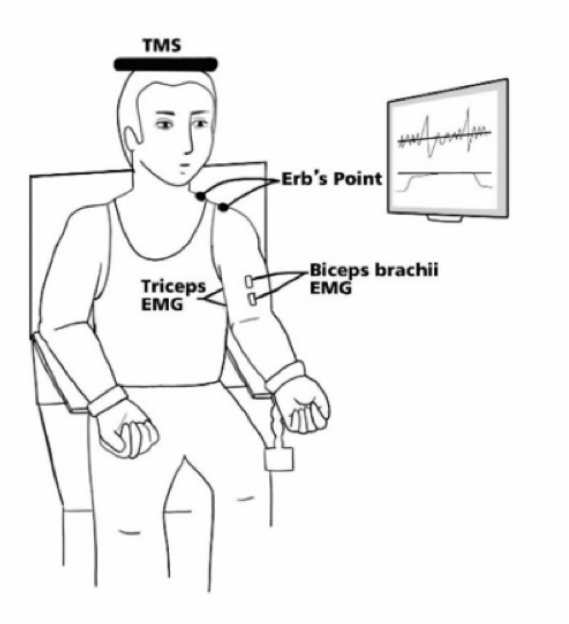
Correlation	**MEP Amplitude at**						
	100%	**110%**	**120%**	**130%**	**140%**	**150%**	**160%**
All Participants							
- **Fat thickness**	-0.167	-0.271	-0.331	-0.321	-0.121	-0.092	-0.139
- **Muscle size**	0.186	0.117	0.342	0.385	0.350	**0.502**	**0.525**
- **MVC**	0.136	0.102	0.294	0.316	0.446	0.288	0/328
- **Skinfold**	-0.251	-0.314	-0.396	-0.340	-0.264	-0.178	-0.299
Men							
- **Fat thickness**	-0.136	-0.137	-0.164	-0.364	-0.115	-0.317	-0.452
- **Muscle size**	**0.591**	**0.674**	**0.712**	**0.585**	0.375	**0.746**	**0.702**
- **MVC**	0.268	0.237	0.378	0.331	**0.553**	0.371	0.381
- **Skinfold**	0.030	0.130	0.118	0.155	0.165	-0.028	-0.106
Women							
Fat thickness	-0.241	-0.505	-0.473	-0.123	0.276	0.407	0.502
Muscle size	-0.583	**-0.750**	**-0.750**	-0.571	-0.175	-0.288	-0.065
MVC	-0.037	0.112	0.084	-0.102	-0.246	-0.361	-0.398
Skinfold	-0.525	**-0.715**	**-0.812**	**-0.634**	-0.232	-0.124	-0.160

Bold values = $p < .05$

4.10 List of Figures

Figure 1

A



B

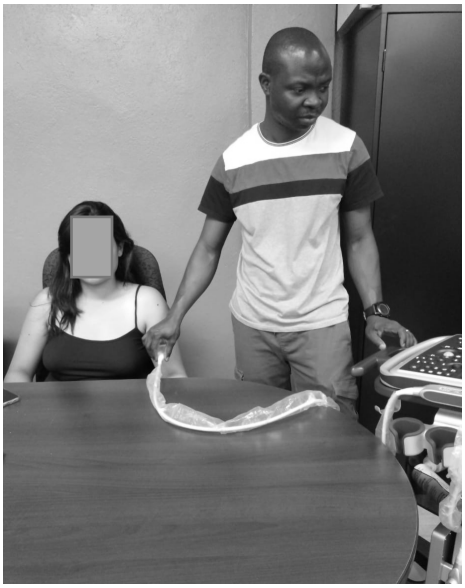


Figure 2

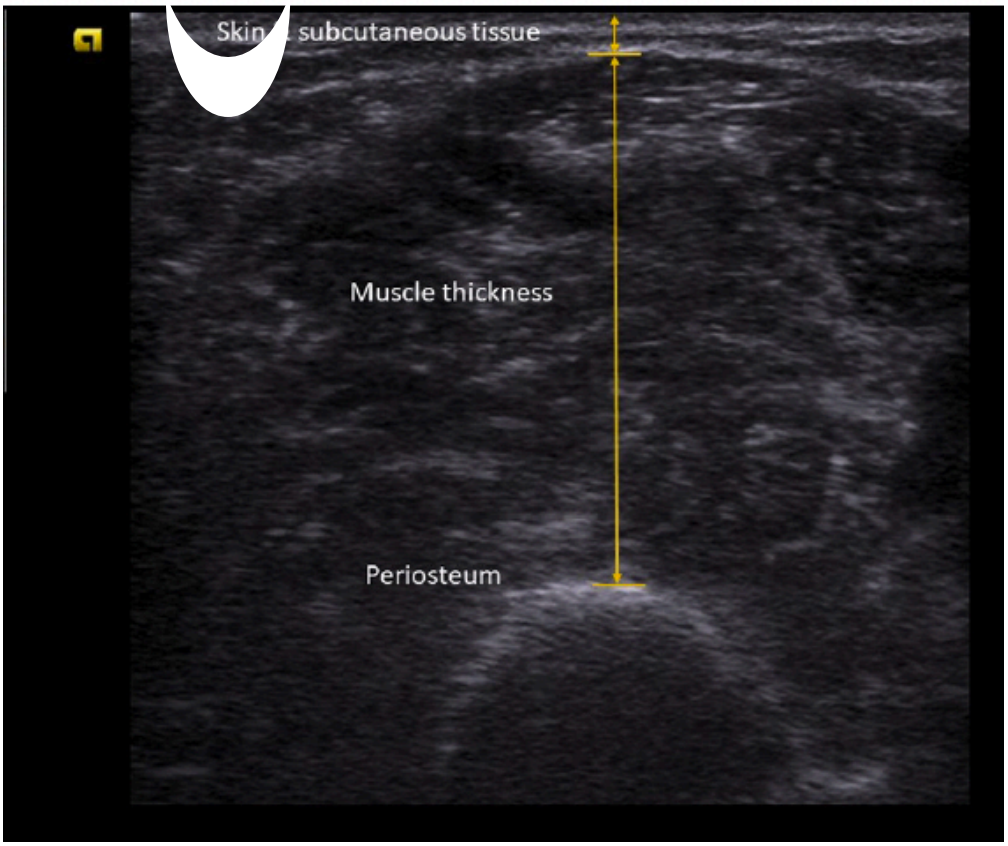


Figure 3

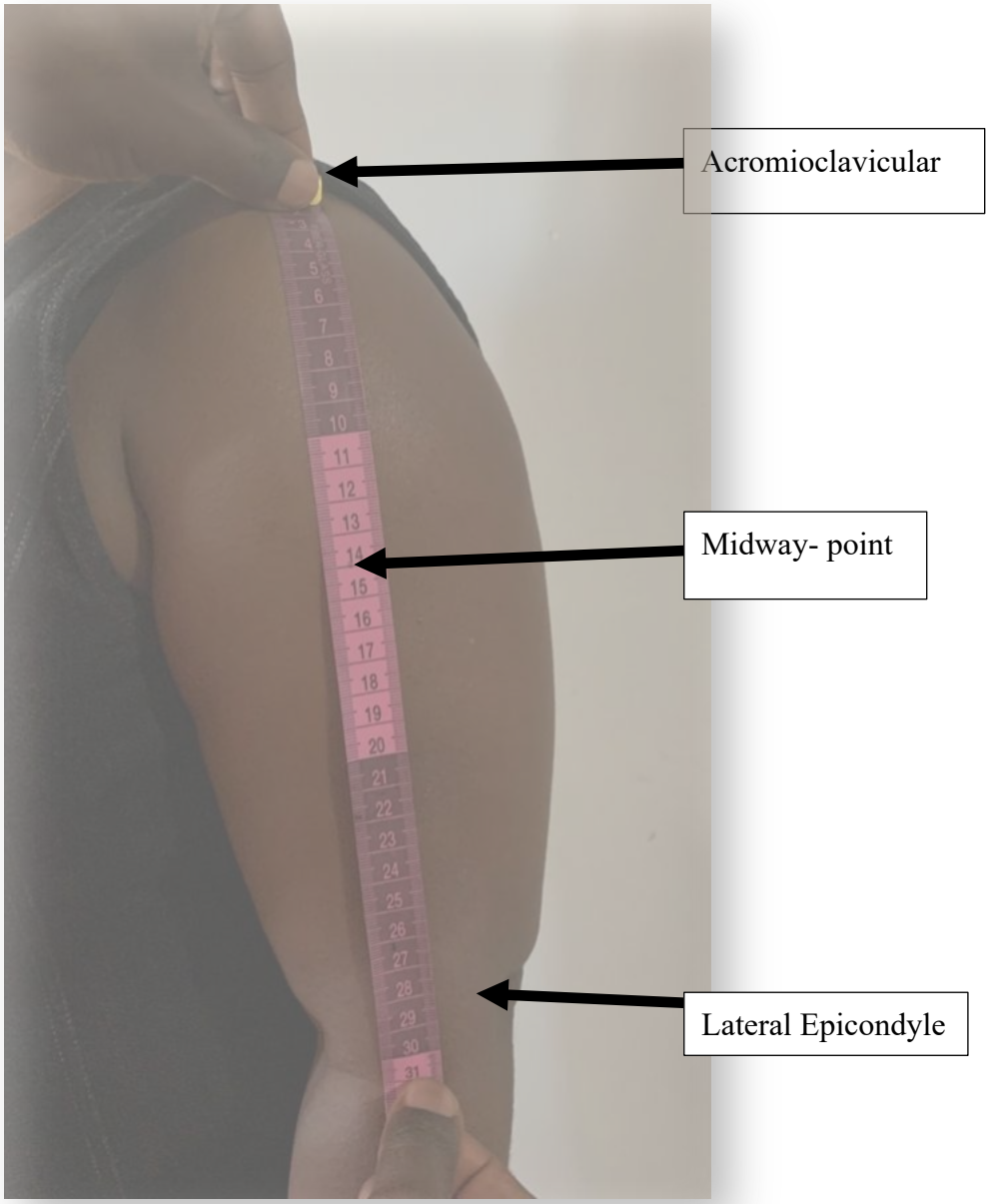


Figure 4

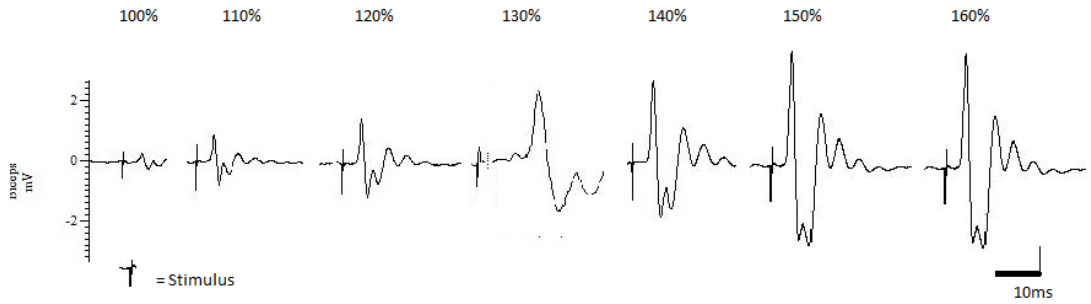
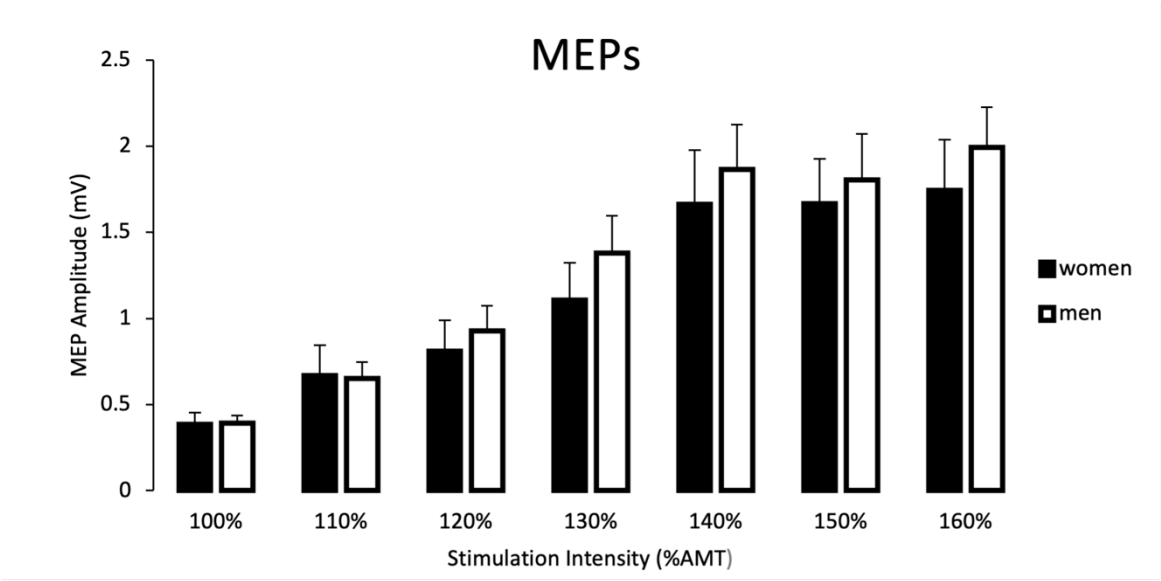


Figure 5

A


MEPs
MEP Amplitude (mV)
2.5
2
1.5
1
0.5
0
100%
110%
120%
130%
140%
150%
160%
Stimulation Intensity (%AMT)
women
men

B

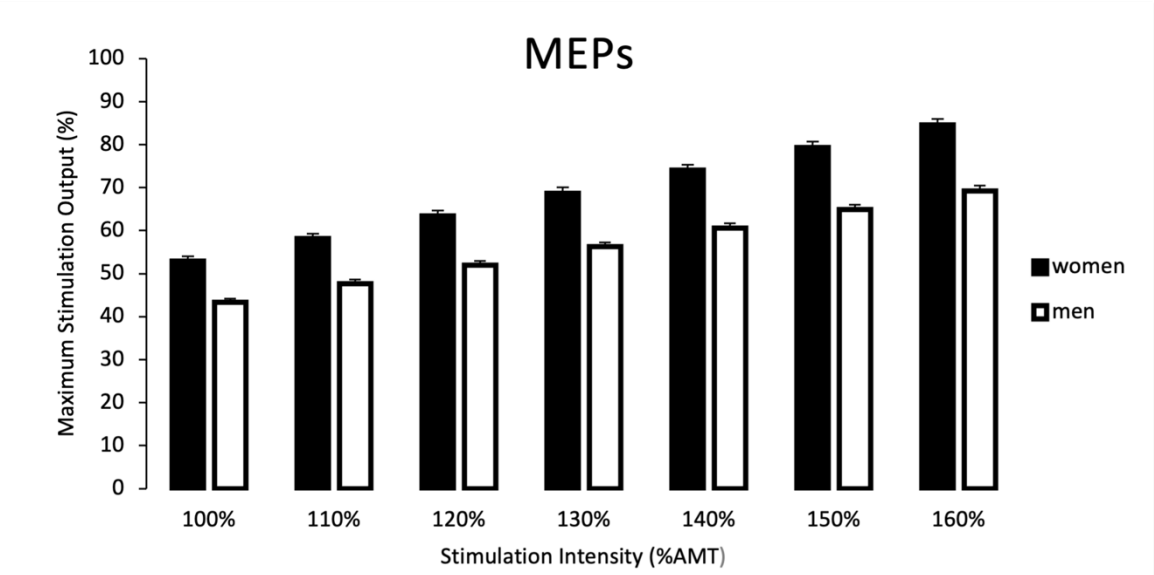
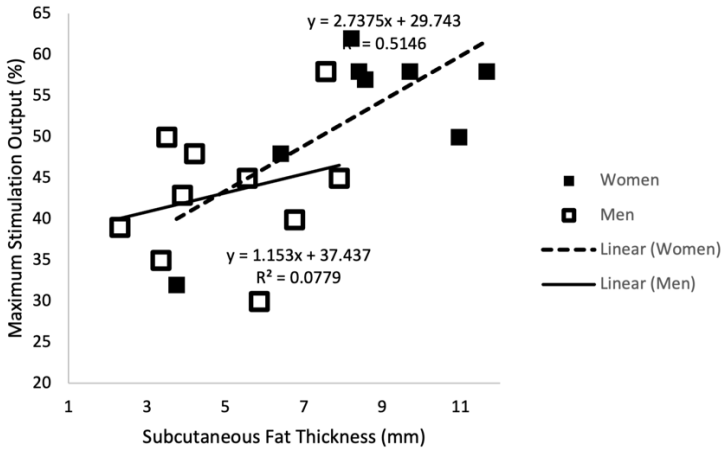
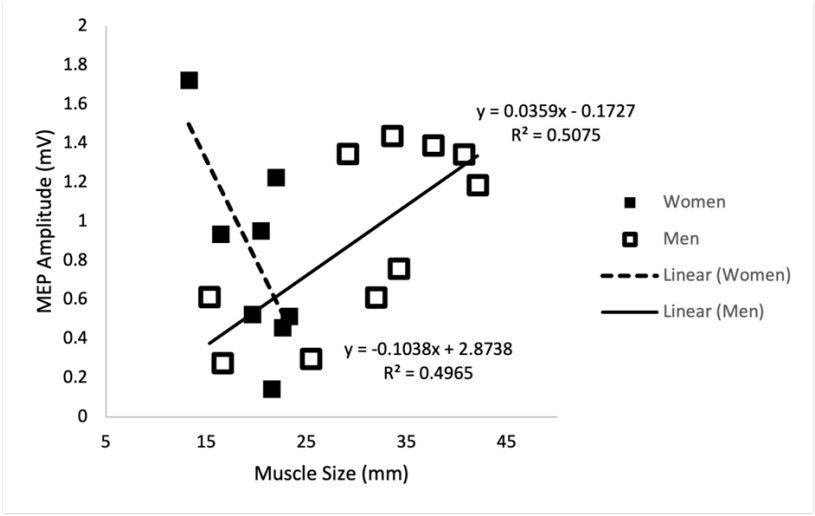

MEPs
Maximum Stimulation Output (%)
100
90
80
70
60
50
40
30
20
10
0
100%
110%
120%
130%
140%
150%
160%
Stimulation Intensity (%AMT)
women
men

Figure 6

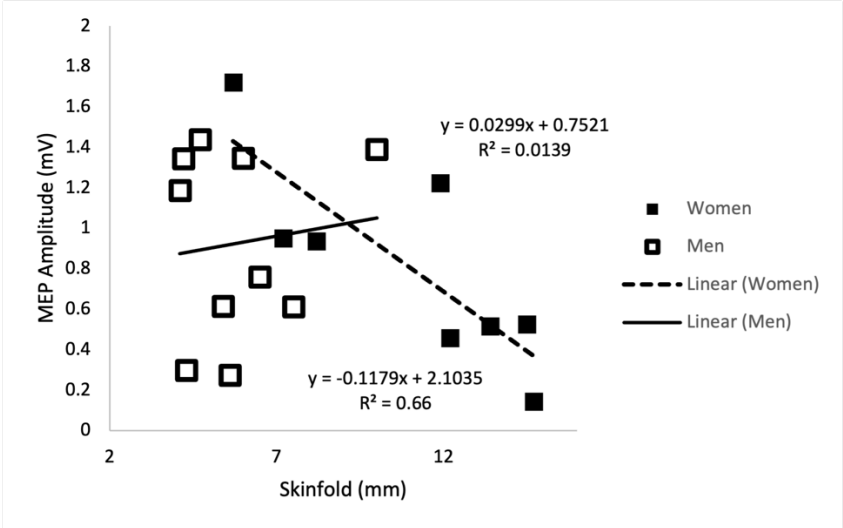
A


y = 2.7375x + 29.743
R² = 0.5146
y = 1.153x + 37.437
R² = 0.0779
Maximum Stimulation Output (%)
Subcutaneous Fat Thickness (mm)
Women
Men
Linear (Women)
Linear (Men)

B


y = 0.0359x - 0.1727
R² = 0.5075
y = -0.1038x + 2.8738
R² = 0.4965
MEP Amplitude (mV)
Muscle Size (mm)
Women
Men
Linear (Women)
Linear (Men)

C


y = 0.0299x + 0.7521
R² = 0.0139
y = -0.1179x + 2.1035
R² = 0.66
MEP Amplitude (mV)
Skinfold (mm)
Women
Men
Linear (Women)
Linear (Men)

4.11 References

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Appendix 1: Ethical Approval

MEMORIAL UNIVERSITY

Interdisciplinary Committee on Ethics in Human Research (ICEHR)

St. John's, NL Canada A1C 5S7
Tel: 709 864-2561 icehr@mun.ca
www.mun.ca/research/ethics/humans/icehr

ICEHR Number:	**20220630-HK**
Approval Period:	October 14, 2021 – October 31, 2022
Funding Source:	
Responsible Faculty:	Dr. Duane Button School of Human Kinetics and Recreation
Title of Project:	*The effect of muscle size and subcutaneous fat thickness on corticospinal excitability of the biceps brachii*

October 14, 2021

Mr. Olalekan Olarogba
School of Human Kinetics and Recreation
Memorial University of Newfoundland

Dear Mr. Olarogba:

Thank you for your correspondence addressing the issues raised by the Interdisciplinary Committee on Ethics in Human Research (ICEHR) for the above-named research project. ICEHR has re-examined the proposal with the clarifications and revisions submitted, and is satisfied that the concerns raised by the Committee have been adequately addressed. In accordance with the *Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans (TCPS2)*, the project has been granted *full ethics clearance* for **one year**. ICEHR approval applies to the ethical acceptability of the research, as per Article 6.3 of the *TCPS2*. Researchers are responsible for adherence to any other relevant University policies and/or funded or non-funded agreements that may be associated with the project. If funding is obtained subsequent to ethics approval, you must submit a Funding and/or Partner Change Request to ICEHR so that this ethics clearance can be linked to your award.

The *TCPS2* **requires** that you **strictly adhere to the protocol and documents as last reviewed** by ICEHR. If you need to make additions and/or modifications, you must submit an Amendment Request with a description of these changes, for the Committee's review of potential ethical concerns, before they may be implemented. Submit a Personnel Change Form to add or remove project team members and/or research staff. Also, to inform ICEHR of any unanticipated occurrences, an Adverse Event Report must be submitted with an indication of how the unexpected event may affect the continuation of the project.

The *TCPS2* **requires** that you submit an Annual Update to ICEHR before **October 31, 2022**. If you plan to continue the project, you need to request renewal of your ethics clearance and include a brief summary on the progress of your research. When the project no longer involves contact with human participants, is completed and/or terminated, you are required to provide an annual update with a brief final summary and your file will be closed. All post-approval ICEHR event forms noted above must be submitted by selecting the ***Applications: Post-Review*** link on your Researcher Portal homepage. We wish you success with your research.

Yours sincerely,

James Drover, Ph.D.
Vice-Chair, ICEHR

JD/bc

cc: Supervisor – Dr. Duane Button, School of Human Kinetics and Recreation