THE EFFECT OF THREE-DAYS OF ANKLE IMMOBILIZATION ON JOINT MOTION AND NEURAL EXCITABILITY

A Thesis
by
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Abstract

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The effects of orthopedic injuries include changes to the nervous system, which lead to alterations in muscle function, biomechanics, and performance, possibly predisposing individuals to further injury. Ankle injuries are the most common injury among the physically active, with immobilization being a standard rehabilitation method. Boot immobilizers (BI) are commonly used following injuries, however, little is known about the effects of prolonged BI use on corticospinal and reflexive excitability, and on gait kinematics. A crossover design was implemented using twelve uninjured individuals (age: 20.8±1.4 yrs, H: 1.7±0.1 m, M: 75.2±9.9 kg). Participants were asked to wear one of two treatments – BI with a compression sock (CS) or just a CS – for three days, with at least a 7-day washout between conditions. Reflexive excitability was assessed using the Hoffmann reflex. Corticospinal excitability was measured using transcranial magnetic stimulation over the primary motor cortex. All neural measures were assessed for the tibialis anterior (TA), peroneus longus (PL), and soleus (SOL). Lower extremity 3-D joint angles were assessed using a Vicon motion capture system (Oxford UK), while participants walked on a split-belt treadmill. Differences between testing times were assessed using a repeated-measures analysis of variance. Changes in continuous joint angles were assessed using statistical parametric mapping (SPM). Reflexive excitability changes, as evidenced by the ratio of the maximum H-wave and maximum M-wave, were observed ($F_{3,33}$=4.026; p=0.015, η^2=0.268), with significant decreases pre- to post-BI (p=0.003). For

corticospinal excitability, similar changes were observed in resting motor threshold ($F_{6,36}$=4.351; p=0.002, η^2=0.42), also with decreases pre- to post-BI (p=0.046). No changes occurred in motor evoked potential size ($F_{6,36}$=0.702; p=0.65) or cortical silent period ($F_{6,42}$=0.631; p=0.704). Significant changes were observed at the knee and hip in the frontal and transverse planes, pre- to post-BI ($p<0.05$). SPM analysis revealed significant main effects of time in knee frontal and transverse angles during stance ($p<0.05$), and knee sagittal and transverse angles ($p<0.05$), as well as changes in hip transverse angles ($p<0.05$) during swing. Our results indicate that the BI increased corticospinal excitability and decreased reflexive excitability. In the context of injury, the former could be beneficial and the later, potentially problematic. Our results also indicated altered proximal joint (hip and knee) mechanics, however, the long term effects of these changes remains unclear. We postulate these data demonstrate the effects of a BI on neural excitability and lower extremity kinematics among healthy individuals, but future studies are needed to determine effects in injured populations.

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Dedication

I want to dedicate this to my parents. Thank you for all of your love and unwavering support, even if you still don’t fully understand what it is that I study. I love you both, and I will continue to do all that I can to make you proud.

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Chapter 1: Introduction

Orthopedic injuries generate alterations within the central and peripheral nervous systems (neuroplasticity) that effectively alter motor output, placing these individuals at increased risk of injury (Needle et al., 2017a). Previous studies have observed changes in motor output following upper (Bachasson et al., 2015) and lower extremity injuries (Kim et al., 2019; Needle et al., 2017a; Pietrosimone et al. 2012; Sefton et al., 2008), likely due to alterations in sensory feedback. Damage to capsuloligamentous mechanoreceptors, and its subsequent effect on muscle spindle & fusimotor function, as well as an increase in nociception contributes to sensory influx that negatively affects proprioception and subsequently can alter muscle function (Courtney et al., 2010). These adaptations are seen at the segmental level as evidenced by changes in reflexive excitability and within the brain and corticospinal tracts (Lepley et al., 2014; Lepley et al., 2015; McLeod et al., 2015; Sefton et al., 2008). Given the multiple levels at which adaptations can occur (e.g. joints, spinal cord, motor units, etc.), interventions capable of preventing or changing this response should be investigated.

Ankle injuries are the most commonly reported injury in athletic settings, making it imperative that research is dedicated to its treatment and prevention. Within these populations, 15% of all injuries reported were ankle injuries (Hootman et al., 2007), with 73% of athletes being subject to recurrent ankle sprains (Herzog et al., 2019). Chronic ankle instability (CAI), or the recurrent giving way of the ankle joint, can develop after acute ankle sprains in as many as 70% of those affected (Gribble et al., 2016). These negative outcomes appear to be related to neural adaptation and can occur independently of changes to the laxity or stiffness of the ankle (Needle et al., 2017b). Along with these physical changes, CAI has been shown to affect quality of life. Studies have shown individuals to have an increased fear of reinjury (Houston et al.,

2014) as well as higher body mass indexes, related to overall less physical activity (Hiller et al., 2011). Within the general population, 25% of all musculoskeletal injuries presented to primary care providers are ankle sprains (Czajka et al., 2014) and can come with considerable financial implications (Shah et al., 2016). Unfortunately, current interventions have generally been ineffective in curbing such consequences (Donovan & Hertel, 2012). In settings where ankle sprains are treated conservatively with immobilization (i.e. emergency room), patients are observed to have better long-term outcomes then those that report to rehabilitation medicine settings (Mailuhu et al., 2020).

Immobilization of the ankle joint is a common method of treatment following injury, as it limits range of motion while allowing the injury to heal and symptoms (e.g. pain & swelling) to subside. Traditionally, casting has been the primary means of immobilization performed in clinical settings, providing significantly more resistance to motion than other bracing options (Lin et al., 2012; Raikin et al., 2001). Currently, recommendations are to limit immobilization after ankle sprains. The 2013 National Athletic Trainers' Association position statement suggested minimizing immobilization in less severe injuries (Kaminski et al., 2013). Because of this, and increased patient satisfaction, dynamic immobilization techniques have become more common due to potentially better motor outcomes, such as less atrophy of the musculature surrounding the ankle joint (Kerkoffs et al., 2002). Analysis of biomechanical measures following prolonged cast immobilization has shown significant decreases in connective tissue remodeling (Reynolds et al., 1996), leading clinicians to err towards functional treatment models (Kaminski et al, 2013) and short-term immobilization (Kerkhoffs et al., 2001). Despite this, more contemporary research has shown potentially better long-term outcomes after casting when compared to alternate options (Lamb et al., 2009). Nonetheless, the advantage of dynamic

immobilization devices, such as walking boot immobilizers, is the allowance of attenuated weight bearing, allowing for some joint loading while still protecting the injured site. However, the treatment itself has not been studied extensively, particularly its effects on the nervous system and on biomechanics, and how those effects manifest themselves functionally. It is understood that ankle immobilization leads to positive rehabilitative outcomes, however, some disagreement still exists about which method is best.

Past studies on the neural effects of immobilization outside of injury models, have explored changes in the corticospinal tract's ability to activate muscle through, or corticospinal excitability, and through its ability to respond via the spinal cord's regulation of motor responses, or its reflexive excitability (Leukel et al., 2015; Lundbye-Jensen & Nielson 2008a; Lundbye-Jensen & Nielson 2008b; Roberts et al., 2007). Synapses retain a level of plasticity in an effort to best accommodate new information, or for these purposes, an immobilization device (Huber et al., 2006). Immobilization has been shown to affect sensory input, leading to an increase in corticospinal excitability due to gamma-aminobutyric acid (GABA)-mediated disinhibition (Leukel et al., 2015; Roberts et al. 2007). However, changes in reflexive excitability after immobilization have yielded inconclusive results, showing increases (Lundbye-Jensen & Nielson 2008a; Lundbye-Jensen & Nielson 2008b), no changes (Leukel et al., 2015), or those measures weren't considered (Roberts et al., 2007). The unloading effect of immobilization is associated with alterations in muscle spindle sensitivity, due to structural and histochemical changes (Kameda 1993, Józsa et al. 1990), as well as a decrease in presynaptic inhibition (Lundbye-Jensen & Nielsen, 2008a), leading to observed increases in Hoffmann reflex (H-reflex) amplitudes. These studies have primarily observed changes with the use of traditional casting and in the upper extremities. Very few studies have explored how the nervous system adapts

following a period of using a dynamic immobilization device, however, one study, involving a boot immobilizer (BI) (Stirling et al., 2018) found no change in central or peripheral excitability following 30-minutes of ambulation in dynamic immobilizers. Understanding the neural effects of acute immobilization is necessary, given its increased usage in treatment (Kerkhoffs et al., 2001). The nervous system has been shown to interact with the changing environment, thus changing biomechanical parameters (Chiel et al., 2009). However, few studies have focused on what those changes entail, particularly kinematic changes.

In addition to neural adaptation, immobilization devices place new biomechanical constraints on their wearers. As individuals face movement constraints imposed by immobilization devices (Jayaram et al., 2011), disinhibition within the primary motor cortex will modify inter-joint coordination (Cirstea et al., 2003) and negatively affect movement during daily tasks. These changes cause rewiring of the nervous system while wearers were using the device that may persist following removal of the modality. Lapses in coordination could present itself with changes in range of motion (ROM) that could ultimately affect performance. Wearing a BI creates a leg-length discrepancy (LLD), in which individuals must accommodate for a "longer limb." Studies observing kinematic differences have found conflicting results: no changes (Pollo et al., 1999), changes in the ankle, knee, and hip (Zhang et al., 2006), and changes in the knees and hips (Gulgin et al., 2018). The more contemporary studies, though, have both shown changes in the proximal joints' (knee and hip) ROM, hypothesized to be due to an increased effort to clear the "longer limb" during swing and fully allow the limb to accept an individual's load during stance. Additionally, due to the mechanical constraints of a BI, a reduction in ankle ROM would be expected, as was the case in Zhang et al. (2006). All of these

studies were done while wearing a BI, so analyzing such lower extremity kinematics after BI use could be beneficial in understanding what movement strategies can persist.

Acute ankle immobilization may serve as a way to better understand neuroplastic changes and its functional outcomes such that injury rehabilitation may be improved. No studies have concurrently measured neural & biomechanical adaptations following boot immobilization. Those looking at kinematics have only done so after immediate application. The proposed findings could aid in determining what joint mechanisms may predispose individuals to other musculoskeletal injuries, throughout the gait cycle. Using a healthy population is necessary to ensure that observed results were not due to nervous system changes associated with ankle injuries. In addition to the use of a BI, a sham immobilizer, or a compression sock (CS), would insure that observed changes were due to the use of a BI and not solely a change in cutaneous input. To minimize long-term disruption, an acute immobilization of 72 hours may be sufficient to generate transient changes in neural activity, given the altered sensory input provided by the BI, among these healthy individuals (McKay et al., 2002).

Specific Aim 1:

To determine the effects of 72 hours of immobilization on reflexive and corticospinal excitability to the lower leg muscles.

- Hypothesis 1.1: Reflexive excitability will increase after 72 hours of ankle immobilization. There will be an increase in reflexive excitability, seen after removal of the BI, when compared to the CS. This will be evidenced by an increase in the maximal H-wave (H_{Max}) and maximal M-wave (M_{Max}) ratio taken from Hoffmann reflex measurements.

- Hypothesis 1.2: Corticospinal excitability will increase after 72 hours of ankle immobilization. There will be an increase in corticospinal excitability, seen after removal of the BI, when compared to the CS represented by a decrease in resting motor threshold (RMT) and an increase in motor evoked potential (MEP) size. The cortical silent period (CSP) will decrease after the boot immobilizer intervention when compared to not wearing a brace.

Specific Aim 2:

To determine the effects of 72 hours of immobilization on unilateral hip, knee, and ankle joint kinematics.

- Hypothesis 2.1: ROM will be altered throughout one gait cycle (heel strike to heel strike of the same leg). The greatest change in ROM will be seen after removal of the BI, when compared the sham, CS, as measured by motion analysis using Vicon-Vantage Motion Capture (Oxford, UK). There will be a decrease in ankle ROM, and increases in knee and hip ROM.

Chapter 2: Review of Literature

Introduction

The central nervous system has the ability to adapt to changes in afferent input or efferent demand through a variety of means, including myelination morphology, cell proliferation, and synaptic behavioral modifications, also known as neuroplasticity (Ives, 2019). In the context of orthopedic injury, neuroplastic changes contribute to altered movement strategies, depression of activation of the musculature surrounding the affected joint, and sensory organ sensitization differences.

Ankle injuries are among the most common musculoskeletal injuries in athletic and general populations, justifying the study of its treatment. Dynamic immobilization is an increasingly common means of treatment, however, the majority of immobilization studies conducted focus on casting models. More so, these studies yield inconclusive results about its effects on the central nervous system, as well as the functional and biomechanical outcomes associated with these neural changes.

Injury and the Central Nervous System

The central nervous system (CNS) has an intrinsic capacity throughout one's lifespan to alter its functioning caused by signaling changes, in an effort to maintain some level of homeostasis (Ives, 2019). Orthopedic injuries in both the upper (Bachasson et al., 2015) and lower extremities typically present with microtrauma to sensory organs leading to altered proprioceptive feedback (Needle et al., 2017a; Pietrosimone et al., 2012). More specifically, tearing of the musculotendinous unit is associated with structural and functional alterations, as well as deformation to the muscle, skin, and surrounding structures that all relay information to

the somatosensory cortex. This is turn, hinders one's ability to recognize the affected area in space (Bachasson et al., 2015). Additionally, injuries resulting from ligamentous damage cause peripheral deafferentation, ultimately impacting joint proprioception, and decreasing the ability to stabilize the affected joint (Needle et al., 2017a).

Volitional movement primarily occurs through two neural mechanisms: reflexive and corticospinal pathways. Altered sensory organ input, as well as the influence of noxious stimuli (due to pain, inflammation, etc.) sensitizes afferent structures in an effort to protect the injured area (Courtney et al., 2010). These changes partially explain altered motor function following injury, and studying reflexive pathways are necessary to bettering our understanding. These pathways are typically measured using stretch reflex and Hoffmann reflex (H-reflex) testing, to elicit responses indicative of their neural circuitry (H-reflex, M-wave, F-wave, H_{Max}:M_{Max} ratio, etc.) (Needle et al., 2017a).

Studies imploring these methods following injury have found changes in reflexive excitability in the quadriceps following anterior cruciate ligament reconstruction (ACLR) (Lepley et al., 2014). In another study, subjects with chronic ankle instability (CAI) were assessed via paired reflex depression (PRD), which is associated with presynaptic inhibition. When compared to controls, the subjects were not able to successfully transition from a double-legged stance to a single-legged stance, indicating presynaptic pathways were affected by their CAI. Subjects with CAI also presented with more recurrent inhibition, or increased motor neuron inhibition via Renshaw cells, which is an estimate of postsynaptic inhibition (Sefton et al., 2008). However, when H_{Max}:M_{Max} ratio was assessed, there was no significant differences between those with CAI and the control group. Conversely, a metanalysis composed of 17 studies was

recently conducted, demonstrating that H_{Max}:M_{Max} ratio is smaller in those with CAI compared to uninjured individuals (Kim et al., 2019).

In cases where the musculature surrounding the joint is uninjured, decreased reflexive activation of said musculature is known as arthrogenic muscle inhibition (AMI). This weakened activation is due to a decrease in the neurons' ability to respond to stimuli, or its neural excitability, that innervates the musculature. AMI is thought to be a protective mechanism to attenuate load placed on the injured joint, but has been seen to have a negative impact on re-injury and muscle function (Hopkins & Ingersoll, 2000). Individuals are subject to recurrent injuries in the same joint because of these signaling and excitability changes, as well as increased laxity in the joint structure itself (Pietrosimone et al., 2012).

In addition to reflexive excitability, measuring corticospinal tracts is necessary to have a complete picture about how the nervous system responds to injury. Changes to corticospinal tracts can be measured using Transcranial Magnetic Stimulation (TMS). A magnetic stimulus is applied over the motor cortex, stimulating the muscles of interest and effectively obtaining motor evoked potentials (MEP's). MEP's are reflective of the ability of the motor cortex to send impulses to descending motor tracts, or corticospinal excitability (Hallett, 2007).

A full understanding of how corticospinal excitability can be affected after injury, however, is still being determined. Recent studies have found a decrease in excitability following musculoskeletal injury. Patients who underwent ACLR surgery did not present with changes to MEP size when compared to controls prior to their surgery. However, central activation (another measure of CNS involvement) of the quadriceps muscle decreased for the ACLR group, denoting a decrease in corticospinal drive (Lepley et al., 2015). In individuals with CAI, MEP amplitudes were found to be lesser than those who consisted of the control group (McLeod et al., 2015).

Resting motor threshold (RMT), a measure of the minimum stimulus required to illicit a motor response, was found to be increased bilaterally in CAI individuals, also suggesting decreased corticospinal excitability (Pietrosimone & Gribble, 2012).

All of the presented studies are evidence that the nervous system changes in response to injury, however, to what extent and what means still remains to be seen. The CNS in particular \ has been shown to change in relation to the complexity of the task (Duclay et al., 2011), possibly indicating that there is no true general mechanism of change, because the magnitude of sensory input may change from task to task.

Ankle Injuries

The most common orthopedic injury in athletic populations involve the ankle joint. In the National Collegiate Athletic Association alone, 15% of the injuries reported over a 16 year span and across 15 sports were ankle injuries (Hootman et al., 2007). Additionally it has been found that 73% of athletes of varying experience levels were subject to recurrent ankle sprains following an initial episode (Yeung et al., 1994). Frequent injuries combined with structural changes and ongoing symptoms such as pain, swelling, and crepitus lead to CAI (Hiller et al., 2011). CAI can develop after acute ankle sprains in as many as 70% of those affected (Gribble et al., 2016), and 72% of those with CAI have also reported feelings of disablement because they could not perform sport at their desired level (Konradsen et al., 2002). In the general population, ankle sprains are one of the most common musculoskeletal injuries presented to primary care providers at 25% (Czajka et. al., 2014). Their prevalence also places a considerable strain on health care costs in the range of $914-$1008 per injury (Shah et al., 2016).

Because of the prevalence of ankle injuries, it is important to also understand the biomechanical mechanisms by which they occur. It has been shown, that ankle sprains can occur after a sudden increase in inversion and internal rotation, 130-180 ms after initial contact after a side stepping task (Kristianslund et al., 2011). Additionally, after a similar cutting motion, maximal displacement of the ankle was seen 150 ms after heel strike, with rapid plantar flexion (Gehring et al., 2013).

Treatment for ankle sprains usually involve either cast immobilization, operative measures, functional treatment, or some combination of those methods. Upon conduction of a meta-analysis comparing the different methods, it was found that functional treatment yielded better results than immobilization via casting, and operative treatment provided better results that functional treatment after six weeks (Pijnenburg et al., 2000). In an analysis of 3 randomized trials, Mailuhu et al. (2020) found that individuals who obtain treatments in emergency department settings prior to neuromuscular training experience less recurrent ankle sprains than those whose initial care comes from therapy settings. This study suggested that one of the reasons for these improved outcomes could be the treatments used in emergency settings, i.e. immobilization and bracing techniques, pointing to their effectiveness. Given this data, clinical preference for different methods is a bit controversial, though, because it is impossible to accurately say in every case that one method is better than another.

Immobilization

Upon injury, immobilization of the ankle joint is a common method of treatment. It limits range of motion while alleviating symptoms such as pain and swelling, and allowing the injury to heal. There are a multitude of immobilization methods with major differences existing in range

of motion, differences in accessibility of the injured joint, hygiene, and ease of application of the device. Such devices include Plaster of Paris casts, pneumatic walkers, and stirrup braces (Raiken et al., 2001).

Studies have found there to be statistically significant differences between functional treatment and immobilization, pointing to a more favorable outcome with short term immobilization coupled with functional treatment (Kerkoffs et al., 2001). A systematic review comparing rehabilitation methods found that removable immobilization devices compared to cast immobilization alone yielded increased ankle dorsiflexion ROM as well as an increased ability to perform activities of daily living (Lin et al., 2012). Similarly, clinical preference has recently been given to dynamic immobilization techniques due to additional factors such as more favorable patient satisfaction and better motor performance (Kerkoffs et al., 2002). Based on this evidence, the 2013 National Athletic Trainers' Association Position Statement on treatment of ankle injuries states that functional treatment is preferable to immobilization (Kaminski et al., 2013)

Despite such preferences, there is evidence suggesting otherwise. A meta-analysis of 19 studies demonstrated that the majority of ankle sprains, regardless of the grade (I, II, or III), do not require serious intervention (e.g. surgery). However, a period of 10 days of immobilization has been shown to be sufficient in treatment of more serious ankle sprains (grade III) (Petersen et al., 2013). Likewise, upon assessment of the effectiveness of several braces when used after severe ankle sprains, a below-knee dynamic immobilizer resulted in faster recovery than in subjects only given tubular compression bandage, suggesting the efficacy of more stringent immobilization based on the type of sprain (Lamb et al., 2009).

CAI and even osteoarthritis can develop if adequate treatment is not provided. A prospective cohort study found that a full recovery of mechanical laxity following an ankle sprain takes longer than 8 weeks (Hubbard & Cordova, 2009). Despite a shift to dynamic immobilization and functional treatment, it may not always allow the ankle to fully heal and regain structural stability. According to a review, laxity still persisted after treatments, in the form of feelings of instability (7%-42%) and positive anterior drawer tests (3%-31%) (Hubbard & Wikstrom, 2010).

Although boot immobilizers (BI), are replacing casting because of the aforementioned reasons, the choice of an such over a traditional cast has been seen as being anecdotal rather than based on evidence. In clinical settings, the choice of immobilization device should be assigned on an individualized basis (Raikin et al., 2001). Due to discrepancies of the research presented, gaining more of an understanding of how boot immobilizers can affect wearers is necessary to ensure its that its use in rehabilitation programs is warranted.

Neural Effects of Immobilization

Gait pattern adjustments before and after wearing immobilization devices can be seen as learning an unfamiliar task. It places new sensory and motor constraints on the body, thus affecting the central nervous system. Synapses can remodel themselves according to how new information has to be sent from neuron to neuron, based on adapting to the immobilized device (Huber et al., 2006). However, studies implementing such methodologies have yielded inconclusive results. It is important to note that the differences in effects of immobilization on the nervous system could be a result of differences in immobilization, timing, upper versus lower extremities, and neural measures used.

Researchers have used upper and lower extremity models to demonstrate neural excitability changes. In upper extremity models, changes in reflexive and corticospinal excitability have been observed after a week in an arm cast (Clark et al., 2008; Lundbye-Jensen & Nielson, 2008a). Lundbye-Jensen and Nielson (2008a) determined that H-reflex amplitudes increased, indicating an increase in reflexive excitability, however there were no changes in corticospinal excitability (as measured by MEP's). It was suggested that due to these findings, as well as a reduction in corticomuscular coherence (CC), a measure of the synchrony between the cortex and the muscles of interest, immobilization of the arm results in less communication between reflexive and cortical drives. Also, the restrictions of information to Ia afferents, were possibly caused by presynaptic inhibition or post activation depression. Unloading effects had before been shown to increase H-reflex gains, as well as inhibition of the corresponding stretch reflex (Anderson et al., 1999). Conversely, Clark et al. (2008) found that MEP values increased after only a week of forearm immobilization, and remained elevated through the 3 week protocol implemented in that study, as well as 1 week following cast removal. When an immobilization method that allowed for some afferent input (a splint) was used RMT decreased but only in one muscle, suggesting an increase corticospinal excitability (Zannette et al., 2004). Another major difference between this study and the previous two, is that this protocol called for participants to be immobilization for 30-45 days (Table 1).

Measuring neural function in the lower extremities have similarly yielded ambiguous results. In a separate study done by Lundbye-Jensen and Nielsen (2008b), reflexive excitability was found to increase. The researchers posited that the disuse caused plastic changes to the spinal interneurons, thus resulting in an increased H-reflex amplitude. These alterations are possibly due to muscle spindle sensitization, as well as increased stiffness of intrafusal fibers that

can affect factors such as myelin sheath degeneration of the corresponding neurons, structural changes, and collagen network morphology (Józsa et al. 1990; Kameda, 1993).

Other studies have shown there to be increases in corticospinal excitability after 10 days to 8 weeks of joint immobilization, (Leukel et al., 2015; Roberts et al. 2007), with these measures returning to pretest values upon reevaluation. In one of the first studies exploring neuroplastic changes following immobilization in a healthy population, MEP amplitudes increased 24 hours after the removal of a full leg cast (Roberts et al., 2007). After 48 hours, though, MEP amplitudes returned to normal. This could possibly aid in determining the timing of injuries after removal of a cast, as similar decreases in MEP's (McLeod et al., 2015), have been observed. Interestingly, Leukel et al. (2015) used a conditioned H-reflex method to determine pathway specific changes in corticospinal excitability, as traditional MEP measures are indicative of a summation of neural activity to the muscle of interest. The study showed that after 8 weeks of ankle immobilization, slower, less direct corticospinal tracts were altered. This suggests that while excitability changes are present, they may be pathway specific. However, in these two studies there were no reported changes in reflexive excitability, as measured by the stretch and H-reflex (Leukel et al., 2015), or no measures taken (Roberts et al., 2007) (Table 2).

The aforementioned studies explore effects after extended periods of unloading, and primarily in casts. with few inquiries having explored short term immobilization. However, in one study, comparisons were made using a boot immobilizer and a pneumatic leg splint, and compared them to a control treatment of no device. In immobilizing the ankles of individuals for 30 minutes, there were no significant changes in the nervous system's ability to call upon its resources. Neural excitability has been shown to change as peripheral stimuli changes after about 45 minutes (McKay et al., 2002). Stirling et al. (2018) came to the conclusion that nervous

system changes can be attributed to long term immobilization. Because of its prevalence in treatment (Kerkhoffs et al., 2001), continued short term immobilization research (exceeding 45 minutes) is necessary to better understand its effects.

Table 1

Upper extremity immobilization studies observing neural excitability

Authors	Joint(s) Immobilized	Length of Time	Condition	Neural Measures	Findings
Clark et al., 2008	Forearm & wrist	3 weeks	Cast	H-reflex measures, MEP, CSP, Central activation	Changes in nervous system can be seen after 1 week of immobilization
Lundbye-Jensen & Nielsen, 2008a	Forearm & wrist	1 week	Cast	H-reflex measures, MEP, CC	Reduction of Ia afferents caused by presynaptic inhibition or postactivation depression; altered afferent input
Zannette et al., 2004	Wrist	30-45 days	Splint	RMT, MEP	Decreased RMT in single muscle

Table 2
Lower extremity immobilization studies observing neural excitability

Authors	Joint(s) Immobilized	Length of Time	Condition	Neural Measures	Findings
Leukel et al., 2015	Ankle	8 weeks	Cast	H-reflex measures, MEP	Slower, indirect corticospinal tracts affected increased; no change in reflexive measures
Roberts et al., 2007	Knee & ankle	10 days	Cast	MEP	Excitability peaks 24 hours after cast removal
Lundbye-Jensen & Nielsen, 2008b	Foot & ankle	2 weeks	Cast	H-reflex	Disuse causes plastic changes in spinal interneurons
Stirling et al., 2018	Ankle	30 minutes	Boot immobilizer, pneumatic leg splint	H-reflex, MEP, CSP	Excitability changes in dynamic immobilization devices caused by long term wearing

Neural Effects on Biomechanics

As it relates to brain activity, when individuals were tasked to learn a new locomotor pattern, an increase in excitability in the motor cortex (M1) was observed (Jayaram et al., 2011). For the purposes of this experiment, learning to walk without a dynamic immobilizer can be seen as learning a new locomotor pattern. The changes in balance that may occur could be due to

changes in proprioceptive feedback from ankle receptors and muscle spindles in the area (Horak & Nashner, 1986). Conversely, adaptations to a decrease in neural firing can affect interjoint coordination. Participants were assessed after a cerebrovascular accident, and asked to do a series of reaching tasks. Significant differences were observed in the middle of reaching, as well as at the end of the reach in the shoulder and elbow compared to healthy controls (Cirstea et al., 2003). This could possibly be an effect of a decrease in firing in Purkinje cells, which normally secrete GABA, an inhibitory neurotransmitter.

Significant relationships have also been determined between limb movements during a gait cycle, suggesting that the coordination of one limb effects that of another, particularly in the lower extremities (Svoboda et al., 2016). A concept known as the kinematic chain, this study found that inferior segments were correlated to the movement of superior segments and to adjacent segments (knee-hip, hip-pelvis) (Svoboda et al., 2016). Little is known about the biomechanical effects that the ipsilateral limb would have on the contralateral limb during and after use of a boot immobilizer.

When used, boot immobilizers create differences in the limb length of the instrumented leg, known as a leg length discrepancy (LLD). LLD's can alter whole body kinematics and kinematics during gait, regardless of the discrepancy (Azizan et al., 2018). It is important to note, though, that other factors such as pain and arthritis can also contribute to changes in gait in those with natural LLD (Azizan et al., 2018). There have only been three studies specifically observing LLD's via a BI during gait. The first known study used 10 subjects and three-dimensional gait analysis to determine that there were no differences between a Bledsoe boot use, or walking with shoes on (Pollo et al., 1999).

In another study, Zhang et al. (2006) compared ground reaction forces (GRF) and three-dimensional biomechanical characteristics between BI's and sneakers. They found that there were no differences in vertical GRF between the two conditions, however, there was a decreased muscle activity at the knee and hip abductors via EMG in the walking boot conditions. Range of motion (ROM) only increased at the knee in the frontal plane, and ankle and hip ROM decreased in the sagittal plane (Zhang et al., 2006). In the only study observing bilateral difference, Gulgin et al. (2018) found there to be kinematic as well as kinetic changes in comparing three conditions: a BI and barefoot, a BI with sneakers, and bilateral tennis shoes. They confirmed that a boot immobilizer changes gait in the same manner that a LLD would; having to alter ROM in order to clear the elongated limb during swing, and having to extend farther in order to accept one's load during stance. This resulted in significant differences in the hip and knee in the sagittal, frontal, and transverse planes. This would mean that walking boots usage results in proximal kinematic changes.

All of these studies observed changes with a BI on, leaving room to understand what happens upon removal of a BI. Despite the lack of research in the biomechanical changes that occur with the use of a boot immobilizer, previous findings could aid in understanding how seemingly uninvolved changes to other segments could create changes to the entirety of the lower body, possibly predisposing individuals to injury, up or down the kinematic chain.

Summary

The prevalence of ankle injuries warrants the need to better understand current and evolving treatment methods. Although functional treatment (short term immobilization and physical therapy) has been seen as the preferred method of clinicians, its efficacy, particularly

after the use of dynamic immobilizers is still being researched. It still remains to be seen how the nervous system responds to dynamic immobilization devices, as the majority of studies that have been done have used casting treatments. Additionally, little is known about the neural effects of a short bout of dynamic immobilization, as it's typically prescribed.

Those neural changes could affect biomechanical measures during gait cycles while using a dynamic immobilization device, because the wearer is essentially learning a new motor pattern. They have to create new affordances to essentially an extension of their bodies, possibly including changes in joint angles and ground reaction forces. This could create new injuries, or, upon removal of the device, could predispose the individual to other injuries due to the body's unfamiliarity with this new motor plan. Research is necessary to understand to what extent these neural and biomechanical changes could lead to possible injuries after the use of a dynamic immobilizer.

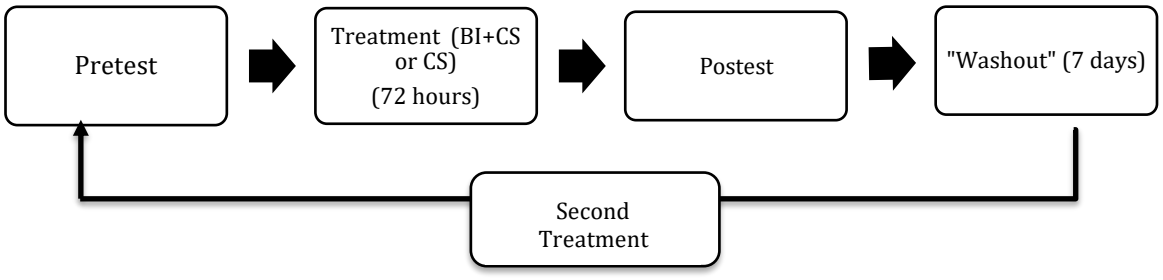
Chapter 3: Materials and Methods

Experimental Design

This study used a double crossover design with pretest and posttest measures. The independent variable of this study was the time of each treatment (Pre-BI, Post-BI, Pre-CS, Post-CS), with the kinematic measures having one additional time point (Post-BI15). Dependent variables included reflexive excitability (H_{Max}:M_{Max}), cortical excitability (RMT, MEP_{Max}, MEP size), cortical inhibition (CSP), and lower extremity kinematics, including peak joint angles at the ankle, knee, and hip during stance and swing, as well as continuous analyses. All participants received both BI and CS treatments. After posttest measurements for the first condition, subjects underwent a "washout" period of no less than 7 days to ensure minimize the potential of carryover effects from the previous condition.

Measurements were taken directly before (pretest) and after (posttest) treatments (Figure 1). After the posttest measurements, subjects underwent a "washout" period of 7 days to insure no contamination of results from previous treatments. Following the washout period, each subject was introduced to the other treatment, so that all participants experienced both treatments.

Figure 1
Study design



Note: *All subjects followed the above timeline, following placement into either the BI+CS or CS treatment. They completed the pretest, the treatment for 72 hours, a posttest and finally the washout period for 7 days.*

Participants

Twelve uninjured men and women (6M, 6W, aged 18-30) were recruited from Appalachian State University and the surrounding community in Boone, NC through class presentations and flyers. We did not anticipate any neural differences due to sex (Pitcher et al., 2003). While we acknowledge walking biomechanical differences between sexes, we did not expect those differences to affect the response to immobilization. Inclusion criteria included uninjured men and women aged 18-30. Exclusion criteria included a history of lower limb long-term (>1 week) immobilization, a history of repeated rolling or giving-way at the ankle joint, and a history of lower extremity surgery. Further exclusion criteria for safe practice of TMS included presence of epilepsy or history of seizure, immediate family members with epilepsy, recurrent syncope or fainting episodes, recent concussion (within 6 months), hearing problems or cochlear implants, pregnancy, implanted metal (including splinters, fragments, clips, etc.), pacemaker, neurostimulator, or other medical device, suffers from recurrent migraine headaches (recurrent= 4 or more in a year), history of skull fracture or abnormalities, history of surgery to brain or heart, taking medications associated with risk of seizure, or history of previous problems during TMS or magnetic resonance imaging testing. The left leg was used in the instrumentation of CS and BI, and for neural excitability testing. Anthropometric measures including height and mass were recorded.

Reflexive Excitability

For all neural testing procedures (reflexive and corticospinal excitability) electromyography (EMG) electrodes were placed on the left tibialis anterior (TA), peroneus longus (PL), and the soleus (SOL). In order to prep the skin, the area was shaved (if necessary), cleaned with alcohol wipes, and abraded (Perotto & Delagi, 2005). Electrodes were connected to

an EMG analyzer (B&L Engineering, Santa Ana, CA), and connected to an analog-to-digital convertor (National Instruments, Austin, TX) to be sampled in custom LabVIEW software (National Instruments, Austin, TX) at 2000 Hz.

To assess reflexive excitability, participants were asked to lie prone on a table. The H-reflex was elicited by electrical stimulation to the popliteal fossa of the left leg using a bar electrode connected to a constant current stimulator (DS7R, Digitimer LTD, Hertfordshire, UK). The location of the sciatic nerve prior to its bifurcation into the common peroneal nerve and the sciatic nerve, was identified as the point in which contraction was observed in all 3 muscles at the lowest possible intensity (Hoffman et al., 2003). Once the location was identified, the maximum reflexive (H) wave and direct (M) responses were determined by applying 1 ms square pulses every 10 seconds. Starting at 0 mA the pulses increased by 2 mA until a maximal response (M_{Max}) was seen in all three muscles (Hoffman et al., 2003). Peak-to-peak amplitudes of the H-wave (50-100ms after stimulus) and M-waves (10-40ms after stimulus) were plotted against stimulus intensities. Stimuli were triggered and synchronized with EMG data in custom LabVIEW software. The maximum H-wave (H_{Max}) and M_{Max} were extracted offline in separate LabVIEW software to create an H_{Max}:M_{Max} ratio that served as a dependent variable of interest. Reflexive measurements were taken first upon reporting for pre- and post-testing. During post-BI testing, upon completion of gait analysis procedures after 15-minutes of free ambulation (listed below), participants completed a second round of reflexive excitability testing to help assess duration of any potential changes.

Corticospinal Excitability

Subjects were seated in an arm-chair with a lycra elastic cap placed on their heads. The vertex of the skull was identified by the subject and marked on the cap to serve as a reference point for TMS procedures. Cortical excitability was assessed using a single-pulse magnetic stimulator with a 2T maximal output connected to a double-cone coil (Magstim 200-2, Magstim LTD, Whitland, UK). Subjects were first familiarized to TMS procedures by explaining the procedures and anticipated sensations, and the coil was placed at the vertex and stimuli were applied at gradually increasing intensities, starting at 15 percent maximal stimulator output and increasing 5 percent, until a visible ankle twitch is observed. The coil was then moved around the apex of the head, in a 5 cm radius beginning 1 cm anterior and lateral (right) to the vertex, with a suprathreshold stimulus every 5 seconds in order to determine a "hotspot" indicated by a motor response in the TA. Once the hotspot was determined, 40-50 magnetic pulses were delivered in a randomized order, separated by 4-7 seconds, to generate a stimulus-response curve. The motor responses to these pulses were recorded and the resting motor threshold (RMT) was derived from plotting the peak to peak amplitudes of the MEP's against the varying intensities (Devanne et al., 1997; Needle et al., 2013). Lastly, participants contracted their TA muscles at 10% of their maximal isometric contraction as thirty pulses (10 each) at 90, 110, and 130% of the subject's RMT were randomly provided. Upon completion of gait analysis procedures listed below, participants completed a second round of this facilitated testing. This was to help determine any long term changes. Stimulus intensities were set, triggered, and EMG responses were synchronized and recorded in custom LabVIEW software.

Custom LabVIEW software was used to extract variables of corticospinal excitability and cortical inhibition offline. Peak to peak MEP amplitudes were extracted and normalized by

dividing by M_{Max} (from H-reflex testing). Stimulus-response curves were fitted to a modified Boltzmann equation using a Levenberg-Marquardt algorithm to extract the maximum MEP size (MEP_{Max}), and to derive the RMT (Needle et al., 2013). For facilitated (TA contracted) trials, the peak-to-peak average of normalized MEP's were extracted and averaged across each intensity to determine the MEP size at 90%, 110%, and 130% of subjects' RMT (Bruce et al., 2020). Facilitated trials were also used to assess for CSP. T-tests were used to compare logarithmically-transformed EMG activity normalized to pre-stimulus levels to a 4 ms window, to help determine when EMG levels had returned to resting values (Nilsson et al., 1997). A trained investigator confirmed these durations on a visible plot, extracting the time from the onset of the MEP, to the resumption of pre-stimulus activity, as the CSP (Kimberley et al., 2009).

Gait Analysis

To capture kinematic data, an eight camera Vicon-Vantage System (Oxford, UK) was used as subjects ambulated on a split-belt instrumented treadmill (Bertec, Columbus, OH). Infrared cameras sampling at a frequency of 100 Hz contributed to detecting a custom reflective marker template placed on subjects during all testing sessions, using the recommendations of Visual 3D (C-Motion Inc., Germantown, MD) documentation. For system calibration purposes, individual markers were placed on both anterior superior iliac spines, greater trochanters, medial and lateral femoral condyles, medial and lateral malleoli, the second and fifth metatarsal joints, and the calcanei. Reflective marker clusters were placed on the sacrum, lateral thighs, and on the lateral shanks. During dynamic trials markers for the greater trochanters, medial and lateral femoral condyles, medial and lateral malleoli were removed. All markers were secured by Velcro wraps and tape.

With a preselected speed of 1 m/s on each belt, participants walked on the treadmill with each foot on one belt, for two minutes as the cameras collected data in Nexus software (Vicon, Oxford, UK) (Figure 2). Data were reconstructed, automatically labeled, filtered, and cut, only using the middle 60 seconds of the treadmill walking. For Post-BI trials, participants were allowed to freely walk around the laboratory for 15 minutes without the BI, and then tested in the same manner as previously described on the treadmill to create an additional time point (Post-BI15). This was to aid in determining any long-term changes to gait kinematics.

Gait cycles were partitioned based on heel-strike to heel-strike of the left leg using Visual 3D (C-Motion Inc., Germantown, MD). ROM in the sagittal (X), frontal (Y), and transverse (Z) planes (Table 3) were then time normalized for the stance and swing phases separately, so that each phase was some percentage of 100 frames. To assess continuous changes in lower extremity joint angles, statistical parametric mapping (SPM) was used. Peak joint angles were then averaged for each intervention using a custom LabVIEW program (National Instruments, Austin, TX).

Figure 2
Marker placement and split-belt treadmill

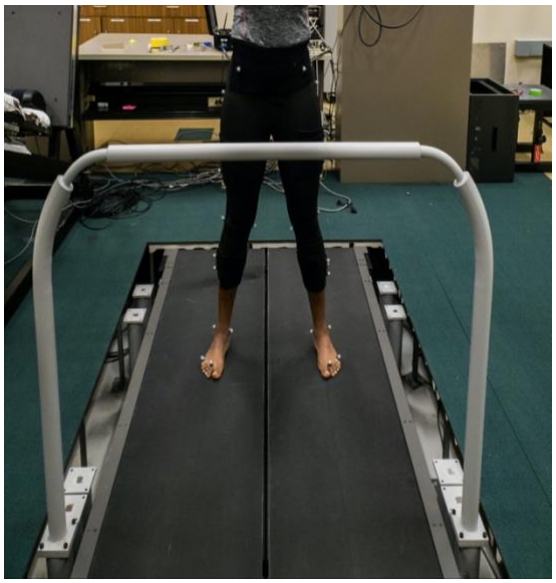


Table 3
Reference for positive and negative angles in V3D

		Positive	Negative
Ankle	X	Dorsiflexion	Plantarflexion
	Y	Eversion	Inversion
	Z	Ext. Rotation	Int. Rotation
Knee	X	Extension	Flexion
	Y	Adduction	Abduction
	Z	Int. Rotation	Ext. Rotation
Hip	X	Flexion	Extension
	Y	Abduction	Adduction
	Z	Ext. Rotation	Int. Rotation

Intervention

Because of the burden on activities of daily living caused by the BI treatment, we allowed each participant to choose which treatment they wanted to undergo first, with the decision made upon recruitment. All subjects were sized for the appropriate BI (Aircast FP Walker) (Figure 3), according to their left shoe size, after the pretests and prior to instrumentation. Prior to instrumentation of the BI, subjects were also provided a CS (Rolyan Stockinette) (Figure 4) to wear under the BI, to control for cutaneous afferent information and allow the CS to serve as a true control condition. The second condition only involved wearing the CS. Participants were instructed to go about their normal daily routines. They were instructed with proper application and removal procedures. It was asked that the BI only be removed during sleeping and bathing times. During the initial pretest, subjects were asked to wear a Fitbit Zip (Fitbit Inc, San

Francisco, CA) on their waistbands to determine changes in activity levels. The number of steps at the end of every day a treatment was implemented was recorded by the subjects and obtained via the Research Electronic Database Capture (REDCap). A follow-up questionnaire was sent to all participants a week after each treatment to insure the maintenance of their well-being, including questions of lingering pain and discomfort following BI use.

Figure 3

Boot immobilizer

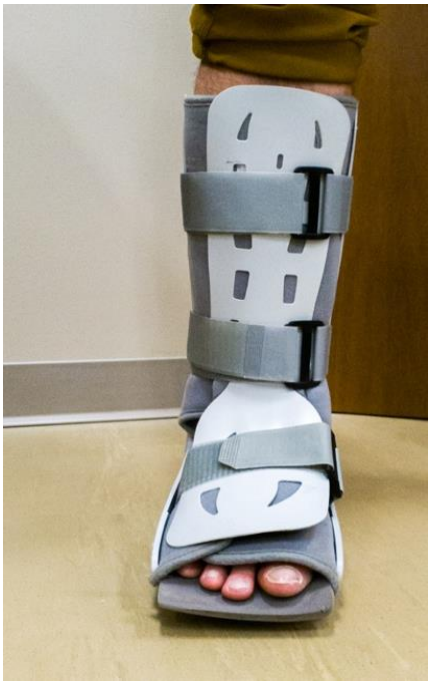
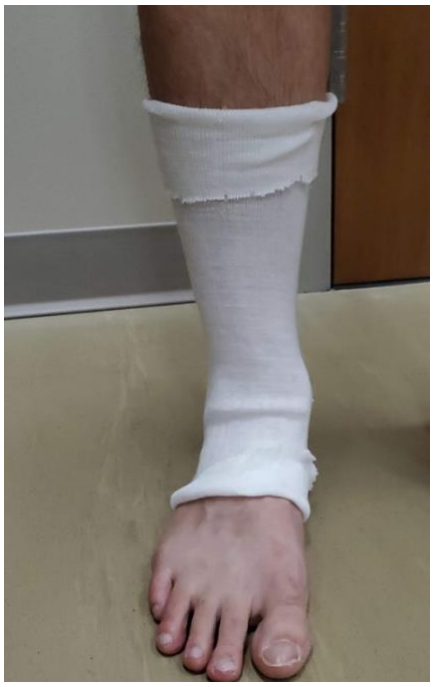


Figure 4

Compression sock



Statistical Analysis

A factorial ANOVA with two within-subjects factors (Muscle, 3 levels; Time, 4 levels) was used to analyze H_{Max}:M_{Max} ratio, RMT, and MEP_{Max}. A repeated measures factorial ANOVA (Intensity, 3 levels; Time, 4 levels) was used to compare MEP size and CSP durations.

SPM was used to conduct a repeated-measures ANOVA with one within-subjects factor (Time, 5 levels) to compare ensemble ankle, knee, and hip angles, in the frontal, transverse, and

sagittal planes during the stance and swing phases, separately. MATLAB code (MathWorks, Inc., Natick, USA) was used to conduct SPM analyses, using functions from the spm1d open-source package (Pataky, Vanrenterghem, Robinson, 2016). In the case of significant main-effects, post-hoc tests were conducted using SPM paired t-test modules to compare Pre-BI to Post-B, Pre-BI to Post-BI15, and Pre-CS to Post-CS. For peak joint angles, a repeated measures ANOVA within one within-subjects factor (Time, 5 levels) was used to compare positive and negative joint angles at each joint for the stance and swing phases.

In the case of significant interaction effects for both neural and kinematic measures, Fisher's least significant difference pairwise comparisons was used post hoc to determine differences, with the difference within conditions (Pre-BI to Post-BI; Pre-CS to Post-CS) being the tests on interest. Effect sizes were determined through partial eta-squared, where 0.01 was a small effect, 0.06 was a medium sized effect, and 0.14 was a large effect size. An a priori level of significance was set at 0.05 (Cohen, Miles, Shevlin, 2001)

Chapter 4: Results

Twelve participants were recruited for this study (6M/6F, age: 20.8±1.4 yrs, height: 1.7±0.1 m, mass: 75.2±9.9 kg). Nine participants chose the BI condition first. Eleven subjects completed all testing procedures (one subject did not tolerate TMS procedures). The average time between the posttest of the first condition and the pretest of the second condition was 12.4±6.9 days.

Reflexive Excitability

There were no time-by-muscle main effects $F_{6,66}$=1.946; p=0.086, η^2=0.15) for H:M ratio. There were, however, significant time ($F_{3,33}$=4.026; p=0.015, η^2=0.268) and muscle ($F_{2,22}$=1.946; p<0.001, η^2=0.793) main effects observed. Pairwise comparisons revealed a decrease in H_{Max}:M_{Max} ratio, Pre-BI to Post-BI (p=0.003, Figure 5), with no differences between other time points. Pairwise comparisons also revealed a greater H:M ratio in the SOL than the TA (p<0.001) and the PL (p<0.001, Table 4). Individual differences were plotted Pre-BI to Post-BI, with three subjects' data being unusable (Figure 6).

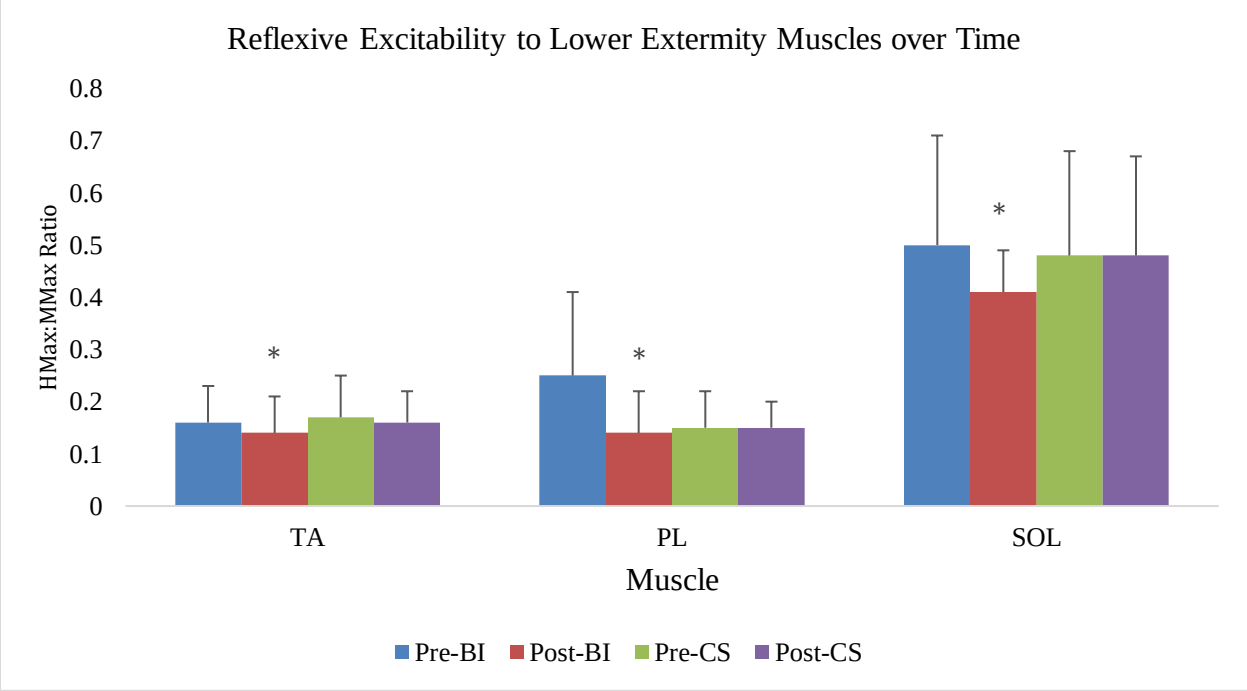
Table 4

H_{Max}:M_{Max} Ratio

	Pre-BI	Post-BI	Pre-CS	Pre-CS
TA	0.16±0.07	0.14±0.07[a]	0.17±0.08	0.16±0.06
PL	0.25±0.16	0.14±0.08[a]	0.15±0.07	0.15±0.05
SOL[bc]	0.50±0.21	0.41±0.08[a]	0.48±0.20	0.48±0.19

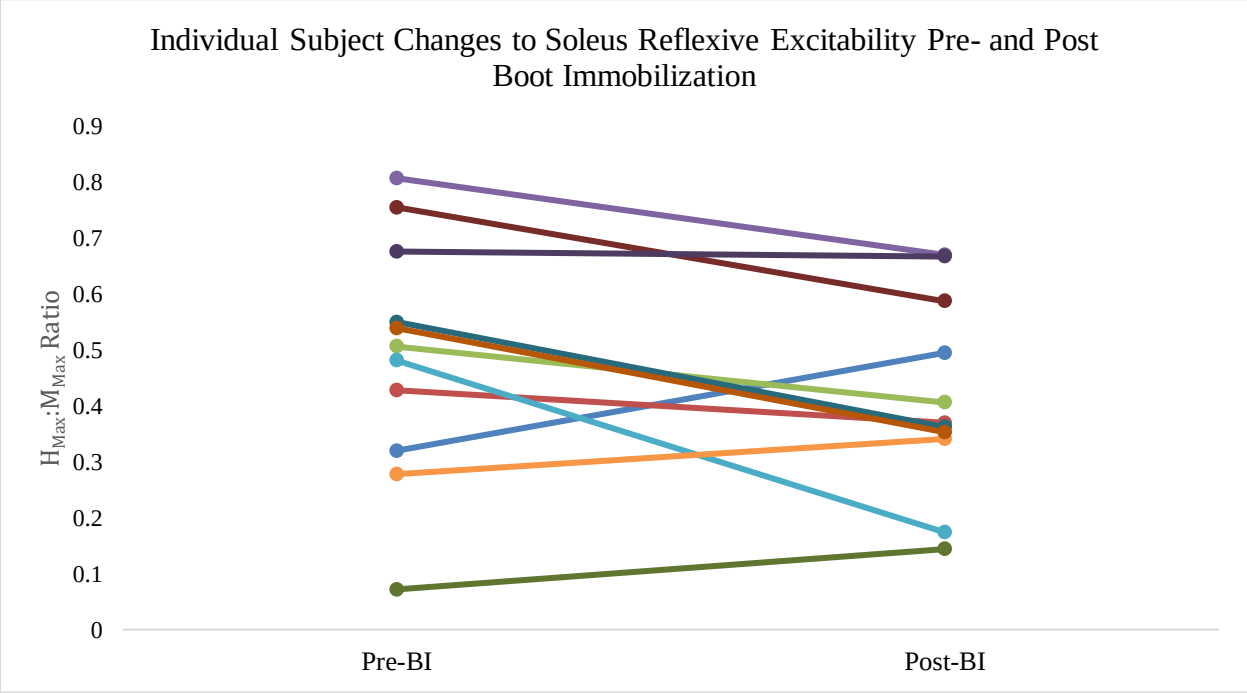
Note: Pairwise comparisons of decrease in H:M ratio Pre-BI to Post-BI. [a] Significantly different from Pre-BI. [b] Significantly different from TA.[c] Significantly different from PL

Figure 5



Note: Pairwise comparisons of decreases in TA, PL, and SOL H:M ratio Pre-BI to Post-BI
*Significantly different from Pre-BI

Figure 6



Corticospinal Excitability

For RMT, there was a significant muscle-by-time interaction effect ($F_{6,36}$=4.351; p=0.002, η^2=0.42) (Table 5). Pairwise comparisons revealed that RMT significantly decreased Pre-BI to Post-BI in the TA (p=0.046 Figure 7) and in the SOL (p=0.005). Additionally, the TA (p=0.035) and PL (p=0.033) had significantly greater RMT than the SOL, Post-BI. Individual differences were plotted Pre-BI to Post-BI, with three subjects' data being unusable (Figure 8).

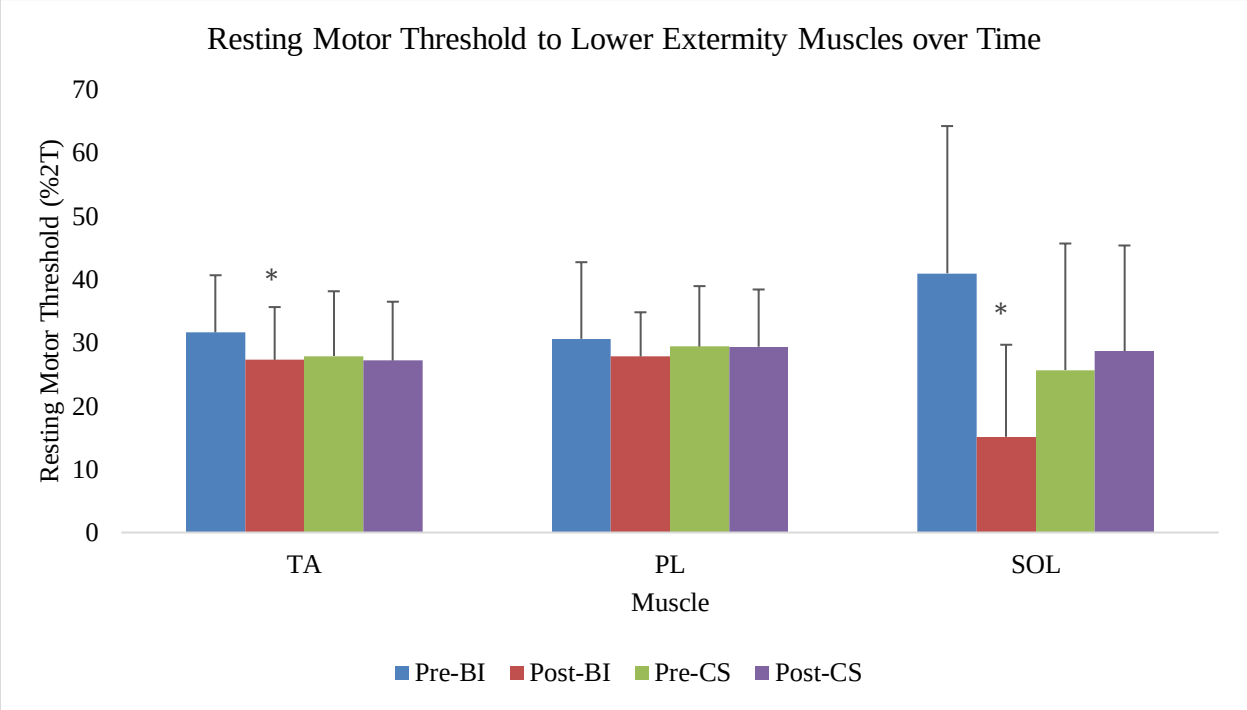
Table 5

RMT (%2T)

	Pre-BI	Post-BI	Pre-CS	Post-CS
TA	31.61±9.02	27.27±8.33[a]	27.87±10.23	27.14±9.31
PL[b]	30.59±12.10	27.80±6.98	29.37±9.54	29.34±9.04
SOL[b]	40.93±23.26	15.11±14.54[a]	25.66±19.98	28.66±16.67

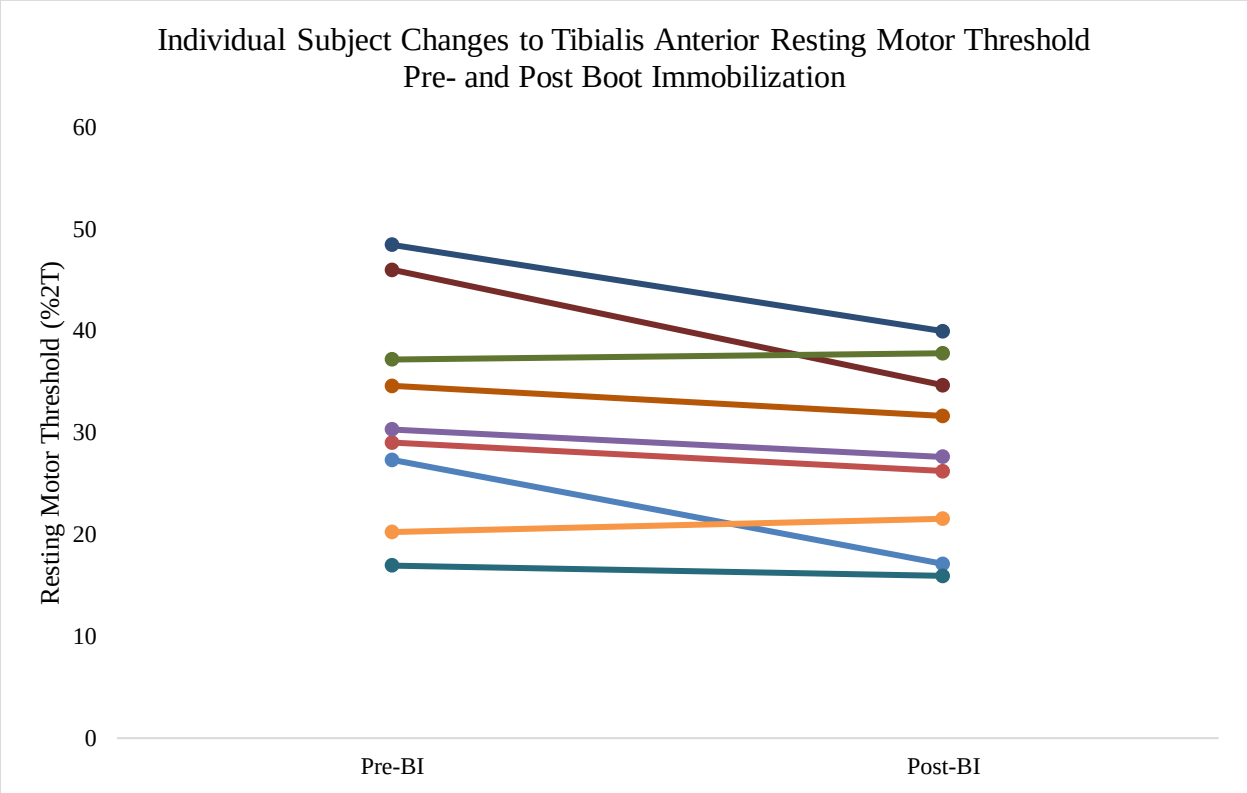
Note: Post hoc analysis showing RMT decreased Pre-BI to Post-BI. [a] Significantly different from Pre-BI. [b]Significantly different from TA

Figure 7



Note: Pairwise comparisons of decreases in TA, PL, and SOL H:M ratio Pre-BI to Post-BI
*Significantly different from Pre-BI

Figure 8



For MEP_{Max}, there was no significant muscle-by-time interaction effect ($F_{6,36}$=0.702; p=0.65, η^2=0.105), and no main effect of time ($F_{5,18}$=0.57; p=0.642, η^2=0.087). However there was a significant effect of muscle ($F_{2,12}$=4.232; p=0.041, η^2=0.414). Pairwise comparison revealed greater maximum MEP values in the TA than the PL, p=0.008 (Table 6).

Table 6

MEP_{Max}

	Pre-BI	Post-BI	Pre-CS	Post-CS
TA	0.18±0.03	0.13±0.08	0.18±0.11	0.22±0.12
PL	0.07±0.06	0.07±0.05	0.10±0.09	0.09±0.08
SOL	0.84±1.44	0.74±0.97	0.97±2.11	0.04±0.04

Note: No significant time-by-intensity effects. Values are ratio of MEP_{Max} normalized to $M_{Max.}$ [a] Significantly different from PL

For MEP size at 90, 110, and 130 percent of RMT, there were no significant time-by-intensity interaction effects for the TA ($F_{6,54}$=0.823; p=0.557, η^2=0.084), PL ($F_{6,54}$=0.740; p=0.620, η^2=0.076), or SOL ($F_{6,48}$=0.798; p=0.577, η^2=0.091) . Similarly there were no significant effects of time observed for the TA ($F_{3,27}$=1.658; p=0.200, η^2=0.156), PL ($F_{3,27}$=0.403; p=0.752, η^2=0.043), and SOL ($F_{3,24}$=2.031; p=0.136, η^2=0.202). Significant main effects of intensity were observed for the TA ($F_{2,18}$=33.666; p<0.001, η^2=0.789), PL ($F_{2,18}$=23.335; p<0.001, η^2=0.721), and SOL ($F_{2,16}$=11.568; p=0.001, η^2=0.591). Pairwise comparison revealed that for all muscles MEP size was significantly greater at 130% than 110% RMT (TA: p=0.026, PL: p=0.018, SOL: p=0.017), greater at 110% than 90% RMT (TA: p<0.001, PL: p=0.001, SOL: p=0.012), and greater at 130% than 90% RMT (TA: p<0.001, PL: p=0.001, SOL: p=p=0.008) (Table 7).

Table 7

MEP Size

		Pre-BI	Post-BI	Pre-CS	Post-CS
90	**TA**	0.13±0.07	0.09±0.05	0.14±0.11	0.15±0.07
	PL	0.06±0.04	0.04±0.04	0.05±0.04	0.05±0.03
	SOL	0.01±0.01	0.03±0.06	0.02±0.01	0.01±0.01
110[a]	**TA**	0.25±0.10	0.21±0.11	0.31±0.15	0.29±0.09
	PL	0.10±0.06	0.10±0.08	0.12±0.06	0.11±0.07
	SOL	0.02±0.02	0.05±0.09	0.03±0.02	0.03±0.02
130[ab]	**TA**	0.42±0.24	0.29±0.11	0.36±0.18	0.36±0.09
	PL	0.16±0.10	0.13±0.11	0.15±0.06	0.13±0.07
	SOL	0.04±0.03	0.03±0.02	0.4±0.03	0.03±0.02

Note: Pairwise comparison showed MEP size greater at 130%, than 110%, and 90% at all muscles. Values are ratio of MEP size normalized to M_{Max}. [a] Significantly different from 90%. [b] Significantly different from 110%

For CSP, there was no significant time-by-intensity interaction (F=0.631; df 6,42; p=0.704, η^2=0.083), as well as no main effects of time (F=1.022; df 3,21; p=0.403, η^2=0.127) or intensity (F=3.412; df 2,14; p=0.062, η^2=0.328) (Table 8).

Table 8

CSP

	Pre-BI	Post-BI	Pre-CS	Post-CS
90	135.50±59.11	165.63±63.07	171.00±55.93	166.50±57.93
110	158.75±67.09	187.38±83.70	164.63±54.57	190.50±72.19
130	183.25±97.34	193.88±97.89	237.75±49.93	248.13±93.61

Note: No significant time-by-intensity interaction. Values in ms.

Ankle Kinematics

SPM analysis revealed no significant changes during stance or swing in ankle frontal, sagittal, or transverse angles ($p>0.05$). When comparing peak joint angles, there were no significant main effects of time in the stance or swing phase (Table 9).

Table 9

Peak ankle joint angles

		Pre-BI	Post-BI	Post-BII5	Pre-CS	Post-CS	F	p-value
Stance	Plantarflexion	67.23±5.24	67.04± 5.59	66.47 ±4.00	65.14±3.90	65.12±4.39	1.662	0.178
	Dorsiflexion	90.24±4.87	88.89±5.25	88.61± 4.87	87.62± 4.13	87.72± 5.58	1.547	0.207
	Inversion	-16.40±8.87	-14.03±5.26	-13.36±5.54	-13.27±8.47	-11.62±4.09	1.432	0.241
	Eversion	-2.45±9.13	0.43±5.74	1.21±4.22	1.19±8.23	3.06±5.81	1.817	0.144
	Int. Rotation	3.61±8.94	4.64±6.98	3.99±6.60	4.28±5.26	1.74±4.89	0.544	0.697
	Ext. Rotation	21.24±9.40	22.31±7.69	21.34±8.36	23.05±6.70	18.97±5.71	0.817	0.522
Swing	Plantarflexion	66.71±6.57	66.42±6.57	66.64±5.17	65.54±4.28	66.34±5.45	0.315	0.866
	Dorsiflexion	83.61± 5.13	83.15± 4.50	82.35± 3.70	83.31± 3.91	82.73± 3.57	0.433	0.784
	Inversion	-15.06±8.43	-13.44±5.07	-12.37±5.19	-13.09±8.59	-10.87±4.58	1.007	0.417
	Eversion	-6.22±8.05	-4.24±5.91	-3.14±5.02	-3.01±7.24	-2.00±5.95	1.168	0.341
	Int. Rotation	5.63±8.83	6.38±7.03	5.94±6.48	8.44±6.74	4.40± 3.83	0.731	0.577
	Ext. Rotation	12.78± 8.57	14.76±8.39	12.74±7.75	15.46±7.31	10.41±4.46	1.083	0.380

Note: Values in degrees.

Knee Kinematics

In the stance phase, SPM analysis revealed significant main effects of time for knee angles in the frontal (p=0.001) and transverse planes (p=0.049). Post hoc analyses, though,

revealed no significant differences between testing conditions (Figure 9). During the swing phase, there were significant differences in knee sagittal angles ($p=0.025$) and knee frontal angles ($p<0.001$). Post hoc analyses similarly revealed no significant differences between conditions (Figure 10).

Figure 9

Knee frontal and transverse angles during stance

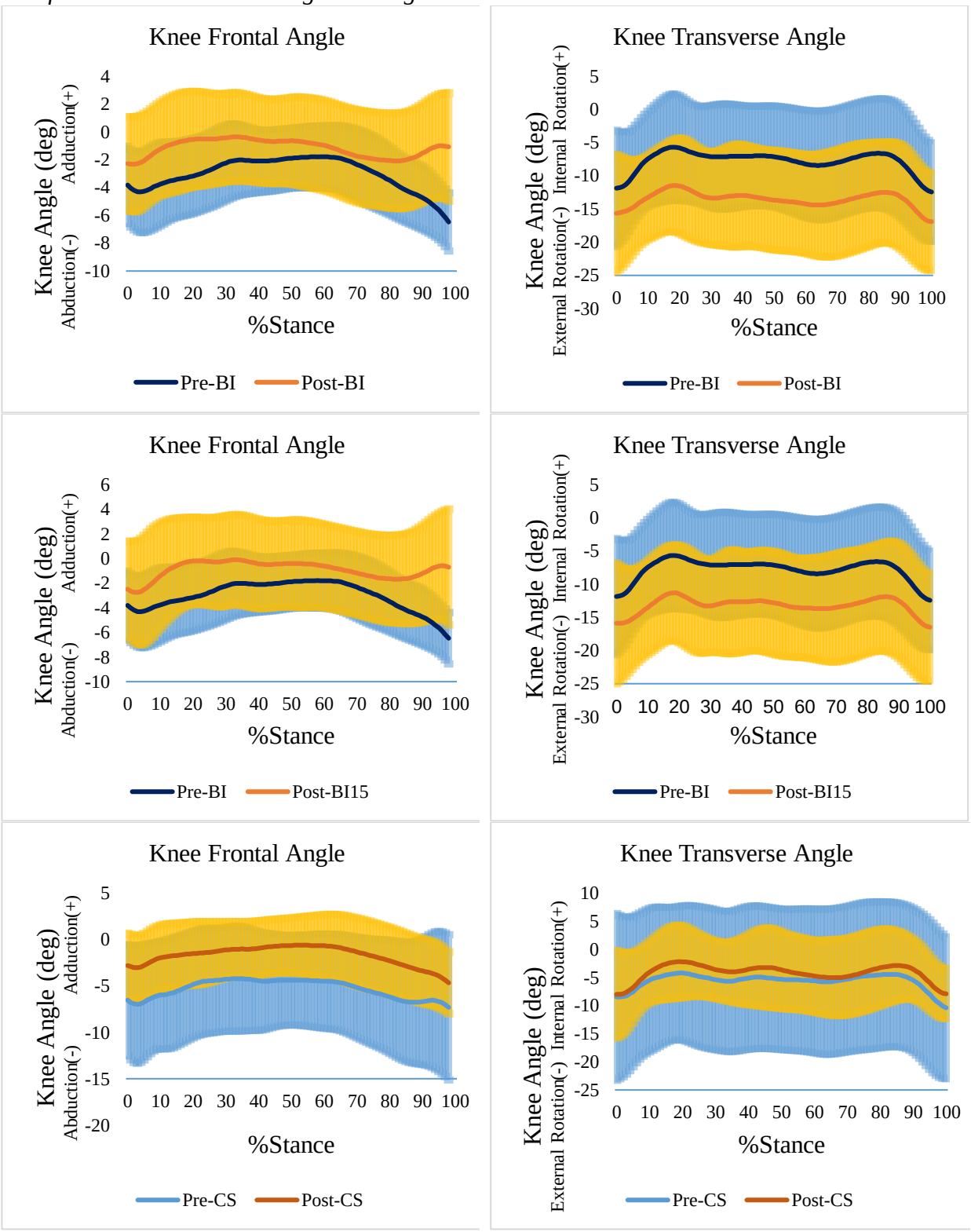
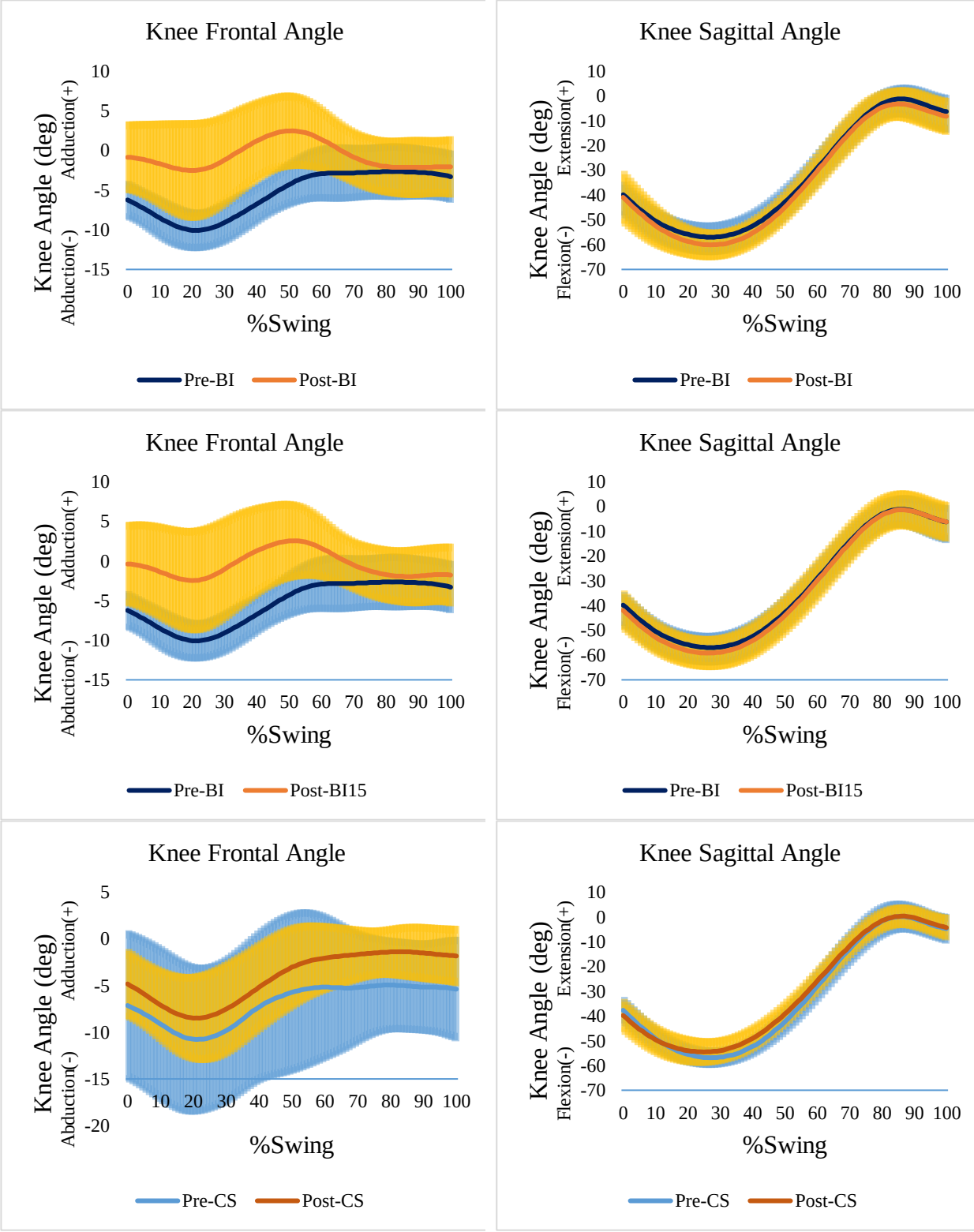


Figure 10

Knee sagittal and frontal angles during swing



In comparing peak knee angles in the stance phase, there was a significant main effect of time for negative knee frontal angles ($F_{4,40}$=5.720; p=0.001, η^2=0.364), with pairwise comparisons revealing decreased knee abduction Pre-BI to Post-BI (p=0.011), and Pre-BI to Post-BI15 (p=0.048) (Table 10). There were no other differences observed for knee sagittal or transverse angles. There were significant overall effects during stance for negative knee frontal angles and knee transverse angles, however, post-hoc testing revealed no significant differences before and after the BI or CS interventions.

In the swing phase, a significant main effect of time was observed for positive ($F_{4,36}$=6.051; p=0.001, η^2=0.402) and negative ($F_{4,36}$=5.382; p=0.002, η^2=0.374) peak knee frontal angles. Pairwise comparisons revealed decreased knee abduction Pre-BI to Post-BI (p=0.006), and Pre-BI to Post-BI15 (p=0.012). There was also an increase in knee adduction Pre-BI to Post-BI (p=0.034), and Pre-BI to Post-BI15 (p=0.031). A main effect of time was shown for peak knee transverse angle during the swing phase, with pairwise comparison revealing an increase in external rotation Pre-BI to Post-BI (p=0.044).

There were significant overall effects for negative knee sagittal angles ($F_{4,36}$=4.270; p=0.006, η^2=0.322) during swing, however, post-hoc testing revealed no significant differences before and after the BI or CS interventions.

Table 10

Peak knee joint angles

		Pre-BI	Post-BI	Post-BI15	Pre-CS	Post-CS	F	p-value
Stance	Flexion	-40.61±5.12	-40.75±9.16	-43.83±6.46	-38.62±6.15	-40.53±6.59	1.132	0.355
	Extension	-4.30±5.22	-5.68±7.10	-4.11±8.09	-1.50±4.84	-2.78±3.90	2.163	0.091
	Abduction	-7.09±1.89	-3.35±3.41[a]	-3.69±4.66[a]	-10.17± 7.47	-5.68± 3.02	5.720	0.001
	Adduction	-0.85±2.48	0.63± 3.44	1.29± 3.75	-2.99± 5.55	0.10± 3.06	2.938	0.032
	Ext. Rotation	-14.59±7.25	-19.44±7.67	-20.08±7.81	-12.77±12.90	-10.93±5.90	3.478	0.016
	Int. Rotation	-3.85± 8.80	-9.49± 7.29	-8.44± 7.54	-1.09±13.84	0.29± 5.80	3.318	0.019
Swing	Flexion	-57.15±4.73	-60.34±4.80	-59.41± 5.58	-57.06±2.74	-54.67±4.21	4.270	0.006
	Extension	-0.85±4.48	-2.26±5.87	-0.77±6.64	0.55±5.20	0.57±3.30	1.414	0.249
	Abduction	-10.60±2.06	-4.70± 4.33[a]	-4.55±5.13[a]	-11.67± 7.50	-9.17± 3.87	5.382	0.002
	Adduction	-1.25± 2.98	3.50± 4.27[a]	3.71± 4.45[a]	-2.57± 5.91	0.04± 2.81	6.051	0.001
	Ext. Rotation	-19.58±7.55	-25.58±7.12[a]	-24.82±7.54	-19.21±11.62	-15.40±6.05	3.786	0.011
	Int. Rotation	-6.97±8.25	-11.49±8.28	-10.62±8.43	-4.43±14.34	-3.57±6.14	2.253	0.083

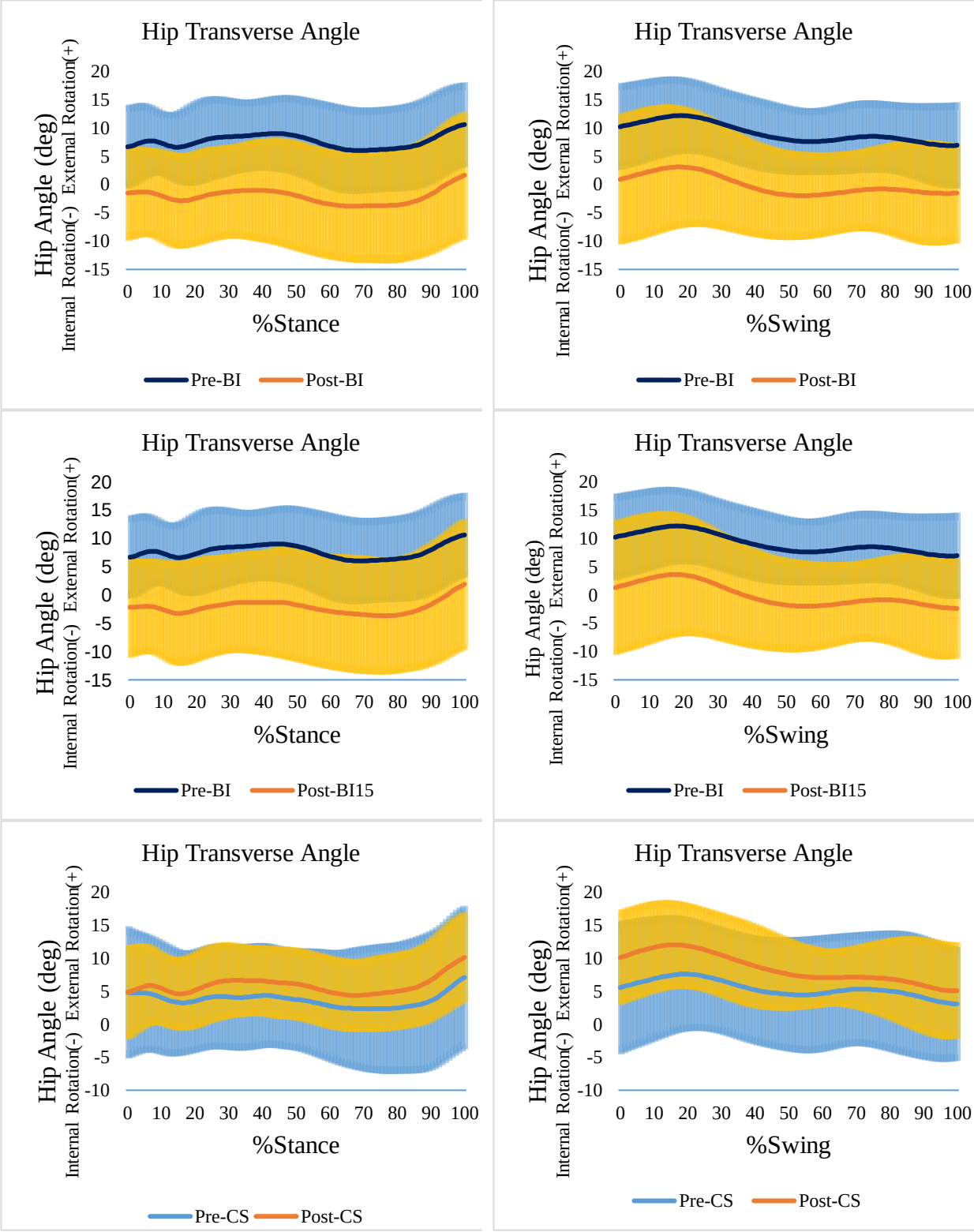
Note: Knee kinematics significantly different Pre-BI to Post BI. Values in degrees. [a] Significantly different from Pre-BI.

Hip Kinematics

SPM analysis revealed a significant difference in hip transverse joint angles during the stance phase. Post hoc analysis revealed more negative hip transverse angles between Pre-BI and Post-BI ($p<0.003$) and Pre-BI to Pre-BI15 ($p<0.001$) throughout the entire range of motion (Figure 9). During the swing phase, there was a significant difference in hip joint angles in the transverse plane. Post hoc analysis revealed more negative hip transverse angles between Pre-BI and Post-BI and Pre-BI to Post-BI15 ($p<0.001$) (Figure 11).

Figure 11

Hip transverse angles during stance and swing



During the stance phase, a significant main effect of time was noted for positive ($F_{4,36}$=5.252 p=0.002, η^2=0.327) and negative ($F_{4,36}$=5.120; p=0.002, η^2=0.309) peak hip transverse angles (Table 11). Pairwise comparisons showed a decrease in external rotation during stance, Pre-BI to Post-BI (p=0.009), and Pre-BI to Post-BI15 (p=0.003). Concurrently, an increase in peak internal rotation was also observed, Pre-BI to Post-BI (p=0.005), and Pre-BI to Post-BI15 (p=0.010). There were significant overall effects during stance for positive hip frontal angles however, there was no significant main effect Pre-BI to Post BI, Pre-BI to Post BI15, or Pre-CS to Post-CS.

During swing, peak hip transverse angles also demonstrated a main effect of time Pre-BI to Post-BI (p=0.013), and Pre-BI to Post-BI15 (p=0.010). An increase in peak internal rotation was also observed, Pre-BI to Post-BI (p=0.012), and Pre-BI to Post-BI15 (p=0.016).

Table 11

Peak hip angles

		Pre-BI	Post-BI	Post-BI15	Pre-CS	Post-CS	F	p-value
Stance	Extension	-5.91±6.92	-4.61±6.84	-4.89±6.96	-6.21±6.58	-8.73±5.88	1.488	0.224
	Flexion	28.58±6.57	28.45±7.91	29.43±4.47	28.39±6.81	25.01±5.25	1.309	0.283
	Abduction	-3.97±3.69	-3.11±6.26	-3.12±6.27	-6.26±6.93	-1.65±3.61	2.516	0.056
	Adduction	5.66± 4.62	5.70± 5.16	5.83± 4.95	3.85± 5.43	7.79± 3.85	2.627	0.049
	Int. Rotation	3.33± 7.19	-5.72± 8.79[a]	-6.57± 8.34[a]	-0.95± 8.10	0.96± 5.72	5.120	0.002
	Ext. Rotation	12.41± 6.25	3.35± 9.70[a]	3.71± 10.41[a]	8.96± 8.76	11.67± 4.74	5.252	0.002
Swing	Extension	1.29±6.19	3.60±6.54	3.85±7.38	1.67±6.17	0.59±4.11	1.287	0.293
	Flexion	30.74±6.97	33.16±6.33	32.47±6.69	31.62±7.56	28.11±3.83	1.796	0.151
	Abduction	-0.73±4.47	-0.03±6.74	-0.23±6.85	-0.73±4.47	1.32±3.95	1.474	0.230
	Adduction	6.56±3.72	7.30±5.19	7.15±4.94	4.78±5.07	8.95±3.46	2.498	0.060
	Int. Rotation	4.63± 7.00	-4.14±8.58[a]	-4.48± 8.62[a]	1.46± 9.20	3.83± 6.57	4.022	0.008
	Ext. Rotation	13.08± 5.63	4.22± 10.02[a]	4.77± 10.17[a]	8.65± 8.24	13.03± 6.05	4.379	0.005

Note: Hip kinematics significantly different Pre-BI to Post BI. Values in degrees. [a] Significantly different from Pre-BI.

Physical Activity

Comparing step count, one subject did not report their data. No significant differences were observed for total step count between conditions (t_9=1.031, p=0.329). There were also no

significant differences between average step count between conditions (t_9=1.327, p=0.217) (Table 12).

Table 12

Step counts with BI and CS

	Sum	**Average**
BI	11004±7105	3950±2234
CS	14008±7366	5339±2949

Chapter 5: Discussion

The purpose of this study was to determine the effects of 72 hours of ankle immobilization on reflexive and corticospinal excitability to lower leg muscles, as well as determine the effects of on unilateral hip, knee, and ankle joint kinematics. We hypothesized that reflexive and corticospinal excitability would increase, as evidenced by an increase in H_{Max}:M_{Max} ratio, a decrease in RMT and CSP, and an increase in MEP size. Our findings partially supported these hypotheses, as RMT deceased; however, H_{Max}:M_{Max} ratios decreased for all muscles Pre-BI to Post-BI, while MEP size and CSP did not change Pre-BI to Post-BI. Lastly, we posited that the BI would alter ROM throughout the gait cycle. Our results partially support this, in that significant differences in knee adduction angles and increased hip internal rotation were seen, with no changes at the ankle.

Reflexive Excitability

Wearing a BI for 72 hours compared to a CS resulted in decreased in H_{Max}:M_{Max} ratios, not specific to any lower leg muscle. We hypothesize that 72 hours of decreasing ankle joint load and minimizing changes in joint position reduced mechanical stimulation of afferents, which in turn reduced sensory drive to spinal neural pools (interneurons, motor neurons, synapses on other sensory axons, etc.), and eventually caused functional and perhaps structural changes to the pool that resulted in reduced ability to respond to sensory inputs. More simply, the 72 hours of decreased muscle spindle discharge, due to BI use, led to decreased motor neuron pool excitability resulting in a decreased H_{Max}:M_{Max} ratio. Further, it is possible that other subcortical descending pathways (e.g. reticulospinal pathways) could also modify reflex sensitivity, although this may be less likely as changes were across all muscles and not isolated to the flexors

or extensors (Chakravarty & Mukherjee, 2010). Although some variability was observed across subjects, differences over time persisted across all muscles. While unclear the duration of time that these changes persisted, some individuals had neural excitability measured 15-minutes after free ambulation. A paired t-test on these data revealed that reflexive excitability did not significantly change between post-testing and post-walking for the TA ($p=0.058$), PL ($p=0.387$), or SOL ($p=0.428$). These indicate that depressed excitability is maintained for at least 15 minutes following immobilization.

Our results conflict with previous research done using different immobilization paradigms. The majority of studies use casting models to observe changes in reflexive excitability primarily and have done so in the upper extremities (Clark et al., 2008; Lundbye-Jensen & Nielson, 2008a). These upper extremity models typically reveal increases to reflexive excitability, however, multiple differences exist between upper and lower extremity models, notably the decreased cortical input to muscles in the lower extremities. Lower extremity models that have implemented casting have found both increases (Lundbye-Jensen & Nielson 2008b) and no changes (Leukel et al., 2015) in reflexive excitability after 2 weeks and 8 weeks, respectively, possibly indicating the time immobilized impacts responses to reflexive excitability. These previous investigations hypothesized increased excitability represented neural changes that occur because of near complete sensory deprivation leading to *increased* spindle sensitivity. However, as mentioned, these investigations utilize casting, whereas the use of a BI (such as in our study) is more commonly used, and likely still allows for some small amounts of joint loading compared to a full immobilization.

Only one study has looked at reflexive excitability after wearing a BI and found no differences after a 30-minute intervention (Stirling et al., 2018). This indicates that, similar to

casting models, reflexive excitability changes could be time-dependent in a BI. These data collectively suggest that immobilization may lead to sub-acute decreases in reflexive excitability in ambulatory devices but would increase when completely unloaded.

Ankle sprains and CAI are both associated with consistent decreases in reflexive excitability secondary to arthrogenic muscle inhibition (Kim et al., 2019). Pain and swelling from injury, and/or deafferentation contribute to decreased reflexive excitability (Needle et al., 2017a). Subsequently, the weakened reflexes seen in injured individuals is believed to pose a disadvantage, impairing the ability of stabilizing muscles to provide protection to the ankle joint. Our results suggest that immobilization may amplify such changes, due to the decrease in reflexive excitability; however, studies utilizing immobilization in injury models are needed. It remains unclear how decreased loading would interact with injury symptoms (e.g. pain, swelling) to modify neural excitability.

Corticospinal Excitability

Corticospinal excitability increased after 72 hours of BI use, as evidenced by increased RMT; however, no changes were observed for MEP size, MEP_{Max}, or CSP. These findings suggest a decreased activation threshold of cortical neurons, without modifying motor neuron recruitment and/or intracortical GABA-ergic activity (Devanne et al., 1997). This is in line with our a priori hypotheses, which stated that modified spindle sensitivity from unloading would increase muscle spindle sensitivity and thus increase cortical excitability to the immobilized muscles. In our case, we observed changes within the muscles that contribute to sagittal plane ankle motion (i.e. TA & SOL). These results are divergent from what was observed in reflexive excitability; however, it's possible that cortical changes occurred in such a way to counteract

depressed reflexive excitability to maintain appropriate muscle activation. Our findings are similar to previous studies that found there to be a significant correlation between motor threshold and muscle spindle afferent activity (Needle et al., 2018). Intersubject neural variability persisted with changes Pre-BI to Post-BI, however, the reported significant differences reflect all participants. Neural excitability data was not available after 15 minutes of free ambulation, to compare long term effects.

Previous lower limb immobilization studies have produced varying results. Casting models have shown increases in corticospinal excitability as measured by MEP size (Leukel et al., 2015; Roberts et al., 2007) with no changes previously observed for RMT (Roberts et al., 2007). These studies immobilized participants from 10 days to 8 weeks. The longer wear coupled with limb unloading may have caused changes in how neural resources were called upon, resulting in increased MEP size. As previously mentioned, the only study that measured corticospinal excitability after wearing a BI did so for only 30 minutes, potentially not leaving enough time for changes to occur, also citing no CSP changes (Stirling et al., 2018).

Ankle sprains and CAI are associated with decreases in cortical excitability from similar models of arthrogenic inhibition (Needle et al., 2017a). The BI appears to increase RMT which is often decreased among those with CAI, posing a potential benefit. However, questions remain as to if lowering cortical membrane thresholds is an advantage, due to the array of signal processing within the nervous system (Faisal et al., 2008). Nevertheless, the consequences of a decreased RMT remain to be seen, as future research should explore if this effect persists in an injured population. Additionally, measurements were only taken in the immobilized ankle (left leg), leaving room for continued understanding surround proximal and contralateral neural excitability.

Lower Extremity Kinematics

After 72 hours of BI, we observed no changes in ankle ROM. This is surprising as we hypothesized immobilization would contribute to a stiffer ankle joint, resulting in decreased motion. Additionally, changes in excitability have been shown to be associated with changes in joint stiffness (Needle et al., 2013). While no changes in ROM were detected in this investigation, we cannot conclude that stiffness did not change as joint moments were not calculated in this investigation. While contrary to our hypothesis, the maintenance of ankle ROM may be a worthwhile effect when considering injury models. Ankle sprains and CAI are associated with decreases in ankle dorsiflexion and increases in frontal and transverse motion. Our lack of differences may be beneficial in preventing these deficits, but further investigation among injured populations would be needed.

Proximal changes at the hip and knee were observed using both SPM analyses that look at continuous gait cycle data as well as discrete variables. Notably, decreased hip internal rotation during stance and swing phases were observed using SPM, while multiple main effects were detected at the knee joint that did not reveal any changes post hoc. It is possible that the lack of changes observed post hoc would not be the case with a larger sample size. We additionally explored peak joint angles during stance and swing for the knee and hip joints. During stance peak knee abduction and peak hip external rotation decreased, while hip internal rotation increased. During swing peak knee internal rotation, knee adduction, and hip internal rotation increased. Peak knee abduction also decreased during swing. These changes imply gait cycle disruptions in which there is more internal hip rotation and knee adduction.

Many of the changes observed can be explained by the induced leg length discrepancy (LLD) from the BI. During the stance phase, the induced LLD would result in an increased

demand on knee adduction, and hip and knee internal rotation throughout the loading response, as ankle pronation would be unavailable. This effect seemed to carry on following BI removal (Post-BI), as well as 15 minutes after removal (Post-BI15). During the swing phase, the increased LLD would create an increased demand on proximal joints to clear the limb, which could be achieved by increasing knee and hip flexion. The increased hip internal rotation that was found during swing would not aid this foot clearing, but could represent the inability to return to a resting position following the loading response. These results indicated that subjects were more likely to maximize frontotransverse hip motion during the swing phase to clear the foot, even after BI removal.

Only three studies have examined kinematics while wearing a BI similar to the one used in this study (Gulgin et al., 2018; Pollo et al., 1999; Zhang et al., 2006). Pollo et al. (1999) found that wearing a BI had no significant effects on ROM during gait, while Zhang et al. (2006) found that a BI can induce changes at the ankle, knee, and hip. Unlike our study they found that ankle eversion and hip adduction were reduced and knee flexion increased. More contemporary research by Gulgin et al. (2018) reported increased hip internal rotation, but unlike this study they also found changes in hip adduction, hip flexion, knee abduction, knee flexion, and knee internal rotation. This study did observe such trends, but it is possible that those trends could have been more present with more participants, like that of the 40 used in the aforementioned study. The 12 participants used in this study were similar to the 10 to 12 participants used in other immobilization studies (Clark et al., 2008; Lundbye-Jensen & Nielsen, 2008a; Lundbye-Jensen & Nielsen, 2008b). Another major reason for discrepancies in findings could be that all of the previous studies measured kinematics with the BI still on, thus creating an acute LLD. No studies have observed kinematic changes upon removal of a BI, and it is possible that

compensatory mechanisms change when there is no LLD. What does appear similar to our study, with at least the Zhang et al. (2006) and Gulgin et al. (2018) studies, is that there were proximal kinematic changes, as opposed to their being changes at the ankle, however, that in part could be due to obtaining kinematic data with a BI on.

Previous literature has shown that walking speed can affect gait kinematics (Fukuchi et al., 2019), which is why a preselected speed was used for this study, as opposed to allowing participants to self-select their speeds. Furthermore, 1 m/s is close to average walking speed for this demographic (Bohannon, 1997), and analysis was simplified, as we did not have to account for any differences due to gait speed. How a BI affects other variables such as joint stiffness, joint moments (and other kinetic measures), the contralateral limb, and in an injured population after using a BI still remains to be seen. Further studies taking these variables into account would help to create a more complete understanding of gait after BI usage.

Physical Activity

One potential covariate for changes in our dependent variables could be the amount of activity performed under the BI and CS conditions. Subjects were encouraged to continue activities of daily living with the BI; however, the restrictions of the boot could be likely to limit physical activity. To account for this we tracked this with a FitBit Zip (Fitbit Inc, San Francisco, CA). Although decreased physical activity was observed, there were no statistically significant differences in the amount of physical activity between conditions, indicating inactivity was not likely to affect our dependent variables.

Chapter 6: Conclusion

Seventy-two hours of BI use led to increases in cortical excitability, but decreased reflexive excitability. The former result is potentially desirable when considering injury models, but the latter is potentially problematic. Therefore, treatments to improve reflexive excitability after immobilization (e.g. focal joint cooling, joint mobilization) could be warranted. Further, the BI did not modify ankle kinematics, which is potentially beneficial, but it did modify proximal joint mechanics. The long-term effects of these proximal changes, and the way it may contribute to injury recovery, remain unclear.

No previous studies have measured neural excitability concurrently with reflexive excitability before and after the use of a boot immobilizer. Understanding the changes in healthy ankle are a prerequisite to investigating these changes among those with ankle injury, thus future studies are necessary to help determine the effects of BI's on injured populations. This study, unlike others, only observed unilateral changes, and understanding bilateral gait changes, as well as kinematic changes would create a full picture of the effects of BI's on lower extremity biomechanics. Lastly, neural measurements were focused at the ankle joint. Because of the proximal changes seen, it would be worthwhile to measure neural excitability at the knee and hip.

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Vita

Jasmine Cash is originally from Charlotte, NC, and is the daughter of Arlene and Walter Cash. She obtained her Bachelor of Art from the University of North Carolina-Chapel Hill in 2017, with a major in Exercise and Sport Science, and a minor in Neuroscience. Jasmine earned her Master of Science from Appalachian State University in Exercise Science in 2020. During her time at Appalachian State University she worked as a teaching assistant instructing introductory biomechanics labs. She was also a research assistant primarily focused on work regarding the neurophysiology of orthopedic injuries (primarily at the ankle) and rehabilitation in the Biomechanics and Electrophysiology Laboratories. Jasmine hopes to continue on in this field, with a greater emphasis on those with neurodegenerative diseases.