Corticospinal excitability and reflex modulation in a non-stretched muscle following unilateral stretching

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ABSTRACT

Non-local effects of physical activity of a limb have been investigated in the literature focusing on cross-over or non-local muscles. The terms “cross-over” or “non-local muscle fatigue (NLMF)” have been subsequently introduced. However, the results of the studies are conflicting. While the NLMF research studies applied fatiguing interventions, other manipulations like muscle stretching have been shown to have non-local effects. However, the research outcomes regarding the non-local muscle stretching (NLMS) effects are conflicting as well. The accurate mechanisms underlying these possible effects throughout the neural pathway have not yet been elucidated. Hence, the objective of the present study was to examine the effects of soleus muscle stretching in the dominant leg on the crossover muscle.

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NLMF: Non-local muscle fatigue

MVC: Maximal voluntary contraction

MVIC: Maximal voluntary isometric contraction

EMG: Electromyography

MEPs: Motor evoked potential

TMEPs: Thoracic motor evoked potentials

SS: Static stretching

ROM: Range of motion

PF: Plantar flexor muscles

PIC: Persistent inward currents

TMS: Transcranial Magnetic Stimulation

POD: Point of discomfort

DNIC: Diffuse noxious inhibitory control

CNS: Central nervous system

H-reflex: Hoffman reflex

PAR-Q: Physical activity participation questionnaire

ICEHR: Interdisciplinary Committee on Ethics in Human Research

AMT: Active motor threshold

ICC: Intraclass correlation coefficient

ANOVA: Analyses of variances

DL: Dominant leg

NDL: Non-dominant leg

tSCS: Transcutaneous spinal cord stimulation

Chapter 1: Review of Literature

1-1 Introduction

Exercise of a limb may lead to fatigue in the exercised muscles. Fatiguing of a muscle group may additionally affect the non-exercised contralateral or remote muscles that were not exercised, and it has been labeled as crossover (homologous, contralateral, non-exercised muscles) (Martin& Rattey, 2007) or non-local muscle fatigue (NLMF: homologous or heterologous, contralateral, or ipsilateral, non-exercised muscles) (Halperin et al. 2015, Behm et al. 2021). Although several studies have been conducted, the NLMF effects are conflicting (Halperin et al., 2015, Behm et al., 2021). Some studies have reported NLMF with single discrete maximal contractions. For example, Martin and Rattey (Martin & Rattey, 2007) tested contralateral knee extensors after 100-s sustained MVCs performed with the dominant leg reporting force decrements with single discrete maximal contractions. Ben Othman (Ben Othman et al. 2017) fatigued the knee extensors with isokinetic and isometric contractions reporting NLMF effects with the contralateral knee extensors MVIC. Halperin et al. (Halperin et al. 2014b) exhausted the dominant knee extensors and elbow flexors with a 2x100-s fatiguing protocol and showed NLMF with non-dominant knee extensors. NLMF has also been reported with single plantar flexors MVC (Kennedy, 2013). However, Behm's recent meta-analysis (Behm et al. 2021) showed negligible NLMF effects with single discrete contractions but moderate magnitude effects when testing with fatiguing or endurance tasks. For example, Amann (Amann et al. 2013) reported NLMF with endurance performance after fatiguing quadriceps unilaterally with a constant load. Sambaher et al. (Sambaher et al. 2016) fatigued knee extensors bilaterally and reported NLMF with 12 repetitive 5-s MVCs of the dominant elbow flexors. However, it must be noted that there are still fewer studies with endurance testing results. Thus, further research seems necessary.

1-2 Non-Local Muscle Fatigue Mechanisms

Research has proposed several mechanisms contributing to the NLMF effects, including central and peripheral neural pathways. However, other global factors, such as metabolite dispersion and psychological factors, may contribute to the phenomenon (Behm et al., 2021). The key pathways responsible for NLMF effects have been discussed by Halperin (Halperin et al. 2015) as follows:

(i) Biomechanical - This mechanism refers to the muscles not directly involved in a fatiguing task but may be necessary for task performance by stabilizing the body. Research shows that the activities of the muscles, such as the abdominal stabilizers, upper lumbar erector spinae, and lumbosacral erector spinae, are more highly activated during an unstable versus stable squat movement (Anderson & Behm, 2005). The increased EMG activity of these muscles has been attributed to their postural and stabilization role (Anderson & Behm, 2005). The trunk and pelvis muscles form the core musculature, which impacts spinal and pelvic stability and are responsible for generating and transferring energy from large to small body parts during sports activities (Baechle et al. 2000). For example, the trunk muscles can exhibit high electromyographic (EMG) activity during upper and lower body activities to stabilize the core or trunk (Behm et al. 2021). Most of the critical mover muscles for the distal segments, such as lassitimus dorsi, pectoralis major, and hamstrings, attach to the core of the pelvis and spine (Kibler et al. 2006). Most of the major stabilizing muscles for the extremities also attach to the core (Kibler et al. 2006). These muscles span several spinal segments and function as prime mover muscles to integrate multiple joints and produce force (Kibler et al. 2006). The hip/trunk area contributes around 50% of the kinetic energy and force to the entire throwing motion (Kibler, 1996). Thus, fatigue in these muscles can impact subsequent tasks, and these muscles have the potential to influence NLMF effects (Behm et al., 2021).

(ii) The accumulation of metabolic by-products and their systemic distribution via cardiovascular and lymphatic systems has been introduced as another mechanism for NLMF (Halperin et al. 2015). Potassium (K^+), hydrogen ions (H^+), and blood lactate were reported to increase in the non-exercised muscles following contralateral exercise (Johnson et al. 2014), leading to force reduction and fatigue (Fitts, 2008). Perturbations to cerebral oxygenation and metabolism have also been shown to inhibit neural drive to the non-exercised muscles. Oxygenation reduction has been shown during maximal exercise (Nybo & Rasmussen, 2007). After a 20-min hypoxic and high-intensity cycling to exhaustion, MVC and muscle activation were decreased in the rested elbow flexors. The authors attributed this reduction to lower cerebral oxygen delivery that hinders central drive to the rested muscles (Nybo & Rasmussen, 2007).

(iii) NLMF may result from the psychological effects of the fatiguing exercise. Keeping the pace of performance to the desired level is uncomfortable and sometimes painful. Therefore, it will need extended focus and concentration (Behm et al. 2021). Even cognitively fatiguing tasks by themselves may affect subsequent physical activity as they cause the performer to find the following task to be more challenging (Marcora et al. 2009). Besides the research showing the effect of neuromuscular alterations on non-local muscles, some studies reported no significant alterations in the neuromuscular function of non-local muscles. Greenhouse-Tucknott (Greenhouse-Tucknott et al. 2020) investigated the effect of prior handgrip exercise on both behavioral-perceptual and neuromuscular alterations during submaximal contraction of the knee extensors. They reported no significant alteration in knee extensor transcranial magnetic stimulation response. However, previous handgrip exercises increased the perception of fatigue and behavioral responses to the sustained knee extensor contraction. However, physiological manipulations can reveal perception changes. Gandevia et al. (Gandevia et al. 2005) paralyzed one hand unilaterally with ischaemic blockade and showed greater perception-mismatch in both hands' flexion-extension than in non-paralyzed condition. They observed a more significant mismatch with the intensity level of the motor command. They suggested a sensory tolerance limit whereby activity ceases or is reduced based on the sum of all neural feedback and feedforward signals. No physiological mechanism seems to explain non-local effects thoroughly. Therefore, the global sensory tolerance limit suggests a cumulative impact. This global negative feedback loop is strengthened by sensory afferents from muscles directly or indirectly involved in the exercise. The result of this is qualitative changes in the phenomenological experience that are posited to impact performance. Thus, this sensory threshold might affect the ability to tolerate the subsequent exercise (Behm et al. 2021).

(iv) As the ability of spinal and supraspinal processes in terms of the net excitation-inhibition determines the full activation of a muscle, fatigue in these structures may reduce voluntary activation or neural drive to the muscles (Gandevia, 2001). Fatigue-induced changes in the environment of the exercising muscle activate group III/IV muscle afferents (Amann, 2011). These afferents may inhibit the central drive from fully activating non-local or global muscle function (Sidhu et al. 2014). However, the role of group III/IV afferents is conflicting as the knee extensors occlusion (more activation of group III/IV afferents) did not result in NLMF effects in the contralateral limb (Kennedy et al. 2015). Thus, more research has been suggested to clarify the role of group III/IV afferents in NLMF effects.

Corticospinal excitation or inhibition has also been reported with NLMF. Some research illustrated increased motor evoked potentials (MEP) of the non-local muscles with fatiguing of the target muscles (Stedman et al., 1998; Matsuura and Ogata, 2015). However, MEP reductions in non-fatigued limbs have also been reported (Bonato et al. 1996; Takahashi et al. 2011). Thoracic motor evoked potentials (TMEPs) have also been investigated. Aboodarda (Aboodarda et al. 2015) fatigued elbow flexors unilaterally and bilaterally and reported a significant increase in TMEP.M_{max}^{-1} ratio following bilateral fatiguing protocol. They ascribed the changes to the supraspinal motor output reduction because of EMG decrements (Aboodarda et al. 2015). Increased tonic level of interhemispheric or transcallosal inhibition has been suggested as a contributing factor (Takahashi et al. 2011). Indirect connections from pre-motor areas or upstream of the motor cortex have also been proposed to involve in NLMF effects (Halperin et al. 2015). The studies regarding the cross-extensor reflex (Sherrington, 1910) and cross-education (Carroll et al. 2006) suggest a shared neural network between contralateral limbs that may affect the NLMF. The reduction of the intracortical facilitation in the motor cortex related to the non-exercised contralateral muscle has been shown with unilateral fatigue (Baumer et al. 2002).

Therefore, NLMF seems to be attributable to neurophysiologic, biochemical, psychological, and biomechanical mechanisms. It seems plausible that the combined overall effects can contribute to differing extents on NLMF. However, an accurate estimation of each factor's contribution and the determination of the possible mechanisms involved in NLMF need further investigation.

1-3 Stretching

There are different interventions available to study NLMF effects (Chaouachi et al. 2017). Most NLMF research studies applied fatiguing interventions, while there are other manipulations possible to investigate NLMF effects. There is evidence that other interventions may also have global or crossover effects (Chaouachi et al. 2017). For example, the increase in the strength of the contralateral untrained limb has been reported following a unilateral strength training program, and this effect has been attributed to cross-education (Zhou, 2000). Another intervention with global and/or crossover effects includes stretching (Behm et al. 2021b, 2021d, Chaouachi et al. 2017). Static stretching (SS) has been known as a principal warm-up activity. It is used during the task-specific warm-ups to increase range of motion (ROM) (Behm and Chaouachi 2011, Behm et al. 2016, 2021), decrease injury incidence (Behm et al. 2021b), and pain (Behm et al. 2021b), and improve athletic performance (Young et al. 2007).

Although short durations of stretch are more common (≤15-s), research agrees that isolated static stretching (>60-s) may be needed to yield more substantial benefits in joint ROM (Pulverenti 2020). However, research studies show that prolonged stretching can impair subsequent athletic performance when not incorporated into a complete warm-up (Behm and Chaouchi, 2011; Kay and Blazevich, 2012; Behm et al., 2016; Lima et al., 2019). However, the intensity, duration, stretching mode, and the individual's background are the other influencing factors affecting performance.

Many studies reported performance decrements due to stretching. For example, Fowles (Fowles et al. 2000) applied 33 min of maximum passive stretch on 12 recreationally active subjects. Although the ROM was increased from 31.3 to 37.8°, they showed a 28% reduction in maximal voluntary isometric contraction (MVIC) force after the stretch that was recovered by 80% after 5-min. However, the final MVC was still 9% below pre-values after 60 min (Fowles et al. 2000). Behm and colleagues (Behm et al. 2001) stretched the quadriceps statically for 20 min and reported 12% MVIC force impairments, 20% reductions in EMG activity, and 12% decrease in evoked twitch force. In another study, Power et al. (Power et al. 2004) stretched quadriceps, plantar flexors (PF), and hamstrings for 270s and showed a significant 9% reduction in the quadriceps MVC and a 5.4% increase in the quadriceps voluntary inactivation rate. The quadriceps EMG activity reduced after the stretch in their study, and it was still lower than the pre-test in both quadriceps and PF after 120 min (Power et al., 2004). Since the early 2000s, a substantial number of static stretching studies have demonstrated subsequent performance impairments (see reviews by Behm and Chaouachi 2011, Kay and Blazevich 2012, Behm et al. 2016 & 2021, Chaabene et al. 2019). Although there are many practical and procedural problems with these studies, such as unrealistic stretching durations (>60-s per muscle group), lack of a full warm-up in conjunction with the static stretching, and ecologically invalid testing times, among other problems (Behm and Chaouachi 2011, Kay and Blazevich 2012, Behm et al. 2016, 2021, Chaabene et al. 2019), the prior stretching research does indicate that prolonged, isolated (no warm-up), static stretching can have negative impacts upon subsequent performance.

1-3-1 Acute Local Effects of Static Stretching- Impairment Mechanisms

The SS-induced impairment mechanisms have been attributed to both peripheral (muscle & tendons) and central (neural) components (Behm et al. 2019). Peripheral components include the alterations in viscoelastic properties of the muscle-tendon unit (Ce et al. 2019), decreases in muscle stiffness and muscle- tendon stiffness due to SS (Konrad et al. 2017). These can adversely affect the force-length relationships (Behm et al. 2019), force transmission efficiency (Pulverenti et al. 2020), and electromechanical coupling (Behm et al. 2019). However, the effect of the force-length relations as a mechanism needs to be mentioned cautiously. Kay & Blazevich (Kay & Blazevich, 2009) demonstrated impairments with no change in the gastrocnemius length. Peripheral changes also include the reduction in myofibrillar Ca^{2+} sensitivity (due to the accumulation of metabolites) (Sugi et al. 2013) and likely changes in titin properties (Herzog et al. 2014). SS-induced contractile fatigue or damage has been proposed as another mechanism (Behm et al. 2019). The damage to the excitation-contraction coupling process or contractile elements has been shown to decrease evoked twitch forces (Behm et al. 2001, Power et al. 2004). Moreover, the SS-associated tension can hinder blood flow and oxygen supply to the tissue. This results in the accumulation of metabolites, reactive oxygen, and nitrogen species (Behm et al. 2016b). These mechanical factors further inhibit the full-force transmission to the tendon insertion point (Ce et al. 2019).

In addition to the SS-induced peripheral alterations, there is increasing evidence that the passive stretch-induced performance impairments can also be attributed to a reduction in neural activation of muscle (Pulverenti et al. 2020). These neural factors may have originated from central or peripheral components. The type Ia/II (muscle spindles) afferents, type III (mechanoreceptors), and type IV (Metabo/nociceptors) fibers have been suggested to be involved in the force-capacity reduction of the stretched muscle (Ce et al. 2019). It has been proposed that small myelinated and unmyelinated muscle afferents (like those belonging to groups III and IV) run an inhibitory metabolically induced signal, and they are responsible for the decline in reflex output and force reduction (Avela et al. 1999). These polymodal afferents are sensitive to several parameters associated with either metabolic fatigue or muscle damage (Avela et al. 1999). These receptors are also known to make a robust input to inhibitory interneurons. The metabolic stimulation of these muscle afferents due to fatigue may inhibit presynaptic Ia terminals or inhibit interneurons in the oligosynaptic pathways (Avela et al. 1999). The disfacilitation of the α-motoneuron pool due to a progressive withdrawal of spindle-mediated fusimotor support has also been suggested to reduce the activation. Fatigue in intrafusal fibers has been reported following maximal voluntary contractions (Asmussen and Mazin, 1978). Intrafusal fatigue reduces the voluntary drive to the α-motoneurons via the γ-loop (Macefield et al. 1991). Avela (Avela et al. 1999), using repeated stretching of the calf muscles, showed reductions in the sensitivity of short-latency reflexes due to a reduction in the activity of the large-diameter afferents. They mentioned that the sensitivity of the muscle spindles was reduced because of stretching (Avela et al. 1999).

The reduction in neural activation of muscle could be at any level in the nervous system (Pulverenti 2020). However, the neural activation is related to the decrease in the activity of the excitatory sympathetic system (due to the stimulation of the exteroceptive reflex) (Guissard et al. 2001) and the strength of motoneuron dendrites (persistent inward currents, (PIC) as the determinant of the amplification of central neural drive) (Trajano et al. 2017, 2020). The reduced neural drive is partly attributed to the stretch-induced reduction in spinal motoneuron facilitation, mediated by a withdrawal of excitatory muscle spindle feedback (Pulverenti et al. 2019). However, it is not known if other parallel mechanisms within the central nervous system contribute to reducing neural drive following the stretch (Pulverenti et al. 2019) because the previous studies have not tested specific locations within the nervous system (Budini et al. 2017, 2019; Pulverenti et al. 2019; Opplert et al. 2020). Pulverenti et al. (Pulverenti et al. 2019) aimed to target specific sites of the nervous system involved in SS-induced impairments. However, no effects of stretching were shown on corticospinal excitability (Pulverenti et al. 2019) and cortical silent period (Pulverenti et al. 2020). Corticospinal excitability (the ability of the corticospinal-motoneuronal pathway to receive, initiate, and transmit excitatory synaptic input) is sensitive to net excitation and inhibition from sensory/afferent feedback (Chen et al. 2004). It can be estimated by measuring the amplitude/area of motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) (Rothwell et al. 1999). It has been suggested that it might take more time for the corticospinal excitability to be detected following a period of the stretching usually applied in the studies. As specific neuronal sites involve the impaired activation of muscle after passive static stretching, Pulverenti (Pulverenti et al. 2020) suggests further investigation to understand better stretch-induced reductions in different levels of the neural system. Therefore, the stretch-induced impairments seem to depend mainly on the neural mechanisms at the spinal or supraspinal levels. The intrafusal, type III, or type IV muscle afferents seem crucial in stretch-induced impairments.

1-3-2 Non-local Stretching Effects

Research studies show the effects of stretch influencing the non-stretched limbs (Behm et al. 2015). Although an increase in the ROM in the non-stretched limbs has often been reported (Behm et al. 2021b, Ce et al. 2019), studies have also shown non-local impairments in strength, power, endurance, sprint, balance, and other performance measures following the stretch (Behm et al. 2021d, Caldwell et al. 2019).

1-3-3 Non-Local Range of Motion Improvement

Non-local improvements in ROM following a bout of unilateral stretching have been shown in the literature. The earliest evidence demonstrated unilateral hamstrings static stretches (6x6-s) resulted in a 14.4% improvement in the straight leg raise ROM (Clark et al. 1999). Chaouachi et al. (Chaouachi et al. 2017) reported 6-8% increases in the contralateral non-stretched hip flexors ROM after a unilateral static and dynamic hip flexion stretch. However, Lima et al. (Lima et al. 2014) implemented unilateral plantar flexors SS (6 sets of 45-s with 15-s rest) and investigated the EMG and center of pressure in both legs after stretch. They also reported a significant ROM increase, but alterations in postural sway and gastrocnemius lateralis muscle activity only with the stretched limb.

The effectiveness of stretching duration on ROM in the stretched muscle has been questioned, with the studies showing similar improvements in ROM with different protocols. For example, Marchetti et al. (Marchetti et al. 2019) investigated the effects of 120-s vs. 240-s stretch on hip flexion ROM in resistance-trained participants and showed ROM improvements with both durations. Thus, it is unknown if the duration of unilateral stretching impacts non-local ROM. However, Behm et al. recent meta-analysis (Behm et al. 2021) showed that the stretching duration did not result in statistically significant differences.

The effects of the stretching intensity on the stretched limb's ROM have also resulted in contradictions. For example, Young et al. (Young et al. 2006) performed static stretching with submaximal and maximal intensities on the right calf muscle groups. They reported no significant difference in ROM following the different stretching intensities. There is also no information on non-local responses to the stretch intensity (Behm et al. 2021). Again, Behm and colleagues (2021) meta-analysis showed no statistically significant effect of passive static stretching intensity. The results of this meta-analysis supported previous findings showing similar increases in ROM using 50 and 85% of the point of discomfort (POD) (Marchetti et al. 2019), initial POD and maximum POD, and 120% of maximum POD (Takeuchi and Nakamura, 2020), initial and near maximal POD (Santos et al. 2020), or four weeks of low or high-intensity stretch training (Valença et al. 2020).

1-3-4 Acute Non-local Performance Effects

Besides the non-local stretch-induced ROM improvements, there is evidence that SS results in a force, jump height, and muscle activation deficits in a non-local unstretched limb (Behm et al. 2021c). For example, Marchetti et al. (2014) used extensive SS in the upper limb consisting of ten stretches of 30-s with 15-s rest and demonstrated force reduction and an increased peak propulsion duration of the maximal concentric jump. In another study, Marchetti et al. (2017) applied a prolonged SS on the pectoralis major muscle (6 x 45-s). They showed muscle activation reduction in both non-stretched pectoralis major (32.6%) and triceps brachii (12.6%). However, Chaoauchi et al. (2017) showed no significant impairments of isokinetic leg flexion torque following eight repetitions of 30-s each of unilateral hip flexion static stretching. Jelmini et al. (2018) used three static stretching repetitions of 45-s each in the finger flexors and reported no change in handgrip force or EMG activity. However, the rate of force generation decreased by 10%. Caldwell et al. (2019), however, found a near-significant, small-magnitude improvement (p=0.06, d=0.22, 12.1%) in contralateral unilateral drop jump height and no change in ground contact time following four repetitions of 30-s of unilateral hamstrings SS. In agreement with previous reviews (Behm.

2018, Behm & Chaouchi. 2011, Behm et al. 2016b), Behm and colleague's meta-analysis (Behm et al. 2021d) showed that the young participants experienced SS-induced force decrements in the stretched (small magnitude) muscle group. They reported strength impairments in the contralateral non-stretched limbs (with small magnitude) for the first time. Thus, with this conflict in the literature, it is not known whether there is sufficient evidence to substantiate that unilateral SS generally has global (contralateral or non-local) consequences (i.e., positive, negative, or no significant effect) on performance (Behm et al. 2021d).

1-3-5 Non-Local Stretching Mechanisms

The improvement of acute ROM in the non-stretched muscle cannot be attributed to peripheral or local mechanisms (Behm et al. 2021b). Moreover, as passive stretching does not induce substantial accumulation of metabolites in the stretched muscle or the need for extensive core stability (Behm 2018), the minor accumulation and dispersion of metabolites or fatigue in core muscles do not explain changes in the non-stretched muscles. Thus, the involved mechanisms responsible for the non-local improvements in ROM have been attributed to psychophysiological influences (i.e., increased stretch or pain tolerance) (Behm et al. 2016) and the reduction in the stimulation of sympathetic excitation (Behm et al. 2021d). The concept of increased stretch tolerance has been suggested to involve the tolerance of the musculotendinous unit to greater stress without a change in tension for a given length (Magnusson et al. 1997). Stretching has also been shown to elevate muscle sympathetic nervous activity (Cui et al. 2006). This activity results in the facilitation of stretch reflexes (Kamibayashi et al. 2009) that may influence both stretched and non-stretched muscles (Ray et al. 1995). It has been shown that joint ROM is highly dependent on muscle resistance caused by tonic reflexes (Guissard and Duchateau, 2006). There is evidence that the increase of the motor unit discharge frequency and the decrease of ½ relaxation time are influenced by sympathetic activity and demonstrate adrenergic stimulation of the muscle fiber contractility (Roatta et al. 2008). Hence, sympathetic-induced (fight or fight) reflexive and motor unit-induced effects on muscle tonicity could increase resistance to global muscle extensibility (Behm et al. 2021b).

The pain perception system alerts any potential threats or damage to individuals in conditions like stretching a muscle (Moayedi et al. 2013). This neural biofeedback response may excite the sympathetic nervous system (Berne and Levy, 1983). Behm et al. (2021b) suggest that the sustained stretching near or at the point of discomfort (POD) would eventually be sensed by the individual as non-damaging and not harmful, and the fight or flight response would be downregulated over time. The downregulation of the sympathetic increased excitement allows the individual to relax and extend the muscle even farther (greater stretch tolerance). Due to a global effect of the sympathetic nervous system that may affect the entire body (Cui et al. 2006), including non-local muscles (Ray et al. 1995), this cortical perceived lack of threat or injury and the downregulation of the sympathetic nervous system result in an increased stretch tolerance and ROM in a non-local and non-stretched muscle.

Additionally, the global modulatory pain systems, such as diffuse noxious inhibitory control (DNIC), seem related to increased global stretch tolerance (Mense, 2000). DNIC is activated by nociceptive stimuli such as stretching to the POD (Mense, 2000). The nociceptive receptor transfers the pain signals to multi-modal, dynamic range convergent cortical neurons located in the subnucleus reticularis dorsalis, where it can suppress pain transmission monoaminergically (Mense, 2000), reducing pain perception not only at the painful location but also globally (Pud et al. 2009). DNIC inhibits pain sensation with the global distribution of monoamines such as endorphins, enkephalins, and other compounds to contribute to global analgesia (Pud et al. 2009), thus contributing to greater non-local stretch or pain tolerance. This effect (non-local pain tolerance) has been shown by applying a painful cold pressor test to the hand and wrist and finding an increased passive knee extension ROM (Stove et al. 2019).

Besides the effects of the stretch on non-local ROM, performance deficits have been shown globally following the stretch. Cramer et al. (2004) initially found isokinetic torque deficits in contralateral quadriceps after a unilateral quadriceps stretch and suggested a central nervous system (CNS) inhibiting effect. The reduction of the muscle spindles excitatory reflexive activity following prolonged SS (>30-s) has been suggested (Guissard et al. 2001). Type I and II muscle spindle afferents innervate spinal motoneurons (Prochazka and Ellaway, 2012). These afferents also project to the somatosensory and primary motor cortex (Phillips et al. 1971). Therefore, they may have global consequences. The global effect of the reduction in the stimulation of the sympathetic nervous system and inhibition of excitatory spindles may adversely affect the performance of non-stretched muscles (Behm et al. 2021b). Exteroceptive reflexes arise from cutaneous receptors, and they have polysynaptic innervations to motoneurons (Jenner and Stephens, 1982). They may inhibit sympathetic nervous system excitation (Wu et al. 1999), contributing to muscle tone reduction, heart rate, and blood pressure (Wu et al. 1999). However, it has been reported that exteroceptive reflex activity only persists for several seconds following the stretch (Guissard et al. 2001). A similar response duration has been observed with the attenuation of facilitatory reflex activity from proprioceptive Ia and Ib afferents. However, their activity seems too transient to impair subsequent performance persistently (Behm et al. 2021). It appears that monoamines such as noradrenaline from the sympathetic nervous system can affect motoneuron PIC, which might impact non-local responses (Behm et al. 2021). Stretching may affect PIC-dependent amplification of the central drive to the stretched muscle, which would diminish force production (Tarajano et al. 2020). However, it is unknown whether the PICs' diminution would also affect contralateral motoneurons (Behm et al. 2021). Monoamines like serotonin and noradrenaline control PIC activity. These neurohormones are released mainly from sympathetic nerve fibers (Lee and Heckman, 2000). The reduction in the sympathetic activity due to static stretching (Inami et al. 2014) would decrease noradrenaline globally and may affect non-local motoneuron PIC activity (Behm et al. 2021).

Maintenance of a joint position during prolonged static stretch involves a level of cognitive demand to sustain focus or concentration (Behm et al., 2020). Cognitive activity for a prolonged period can induce mental fatigue (impaired cognitive functioning), in which the subsequent performance would be negatively affected (Marcora et al. 2009). Mental fatigue leads the individual to perceive the activity more demanding, and therefore, they may stop earlier or exert less effort (Marcora et al. 2009). Halperin et al. (Halperin et al. 2015) showed that mental fatigue could be a major contributor to non-local muscle fatigue, as non-local muscle fatigue is more observable with prolonged fatiguing tests that would require sustained concentration. Behm et al. (Behm et al. 2021d) suggested that the attention necessary to maintain a prolonged SS to or near the POD could induce some mental fatigue. That would impair the ability of the individual to subsequently fully activate and co-ordinate the contraction of multiple muscle groups (activate agonists, synergists, and core stabilizers, and inhibit antagonist muscles) to achieve maximal force output (Behm et al. 2021d).

Transcranial magnetic stimulation (TMS) is a measure over the cortex that evokes motor-evoked potentials (MEPs). It has been used to investigate the changes in corticospinal excitability during NLMF. The effects of stretching on corticospinal excitability have also been investigated in the literature. Budini et al. (2017) applied two bouts of 30-s stretching on the plantar flexors to the maximal individual ankle dorsiflexion. They showed that corticospinal excitability in the relaxed muscle remained unchanged following stretching. Another study was conducted to investigate corticospinal excitability following the plantar flexors stretch (5 x 60-s) to the maximal dorsiflexion position. Again, no stretching effect was found on MEP changes (Budini et al. 2019). Pulverenti et al. (2019) stretched the plantar flexor muscles with 5x60-s of intense, passive static stretching and could not find differences in MEP amplitudes.

The Hoffman (H-) reflex measures presynaptic inhibition at the Ia- motoneuron synapse and the excitability of the neural reflex arc (Misiaszek, 2003). It assesses motor units activated by the afferent pathway (Duclay et al. 2005). There is insufficient information about the H-reflex changes with non-local stretching effects, with no studies examining crossover H-reflex responses.

1-4 SUMMARY

Based on the inconsistencies regarding the NLMF and non-local muscle stretching (NLMS) effects (Behm et al. 2021c), it seems necessary to investigate possible non-local mechanisms in neural pathways and different levels of the nervous system. Although it was reported that stretch increases ROM (Clark et al., 1999; Chaouachi et al., 2017; Lima et al., 2014) and changes other functional factors in both the stretched and non-stretched limbs (Behm et al. 2021c), it seems the stretching effects on subsequent physical activity in the stretched and non-stretched limbs are not consistent (Behm et al. 2021d). This is especially critical when it comes to sports performance and rehabilitation. The NLMS effects can be important during a warm-up session as it may increase the range of motion in both the dominant and non-dominant limbs. On the other side, static stretching of a limb may impair performance in the stretched and non-stretched limbs. In terms of rehabilitation, stretching an uninjured limb may have positive effects on the injured limb.

While stretch-induced performance impairments seem to depend on the stretched limb's peripheral and neural components, peripheral mechanisms should not play a role in non-local responses with a non-stretched muscle. Thus, it is likely that neural responses could affect the non-stretched limb. These neural mechanisms have not been investigated comprehensively in the non-stretched limb.

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Chapter 2: Research Study

Abstract:

Purpose: Muscle stretching's effects on the range of motion (ROM) and force deficit in non-stretched, non-local muscle, and the underlying mechanisms, is an ongoing issue in research. This study aimed to investigate crossover stretching effects on the plantar flexor muscles.

Methods: Fourteen recreationally active females (n=5) and males (n=9) performed a single 5-s pre and post-test maximal voluntary isometric contraction (MVIC) with each plantar flexor muscle and were tested for the ROM in both legs. They were also tested pre and post-test for the H-reflex and transcranial magnetic stimulation (TMS) in the non-stretched muscle. The intervention involved six sets of 45-s static stretching (SS) (15-s recovery) or control (345-s rest) in the dominant leg's (DL) plantar flexor muscle.

Results: The DL and NDL-MVIC force reduced significantly (↓10.87%, 112.62±39.73 to 100.37±31.2 Nm, p=0.027)(d=0.34) and near significantly (p=0.06) with SS. The SS also significantly improved the DL (6.5%, $F_{(1,10)}= 22.80$; $p<0.001$, $p\eta^2= 0.69$) and NDL (5.35%, $F_{(1,10)}= 18.53$; $p= 0.002$, $p\eta^2= 0.65$) ROM. MEP/M_{Max} ratio did not change significantly in the NDL ($F_{(3,30)} = 0.96$; $p= 0.42$, $p\eta^2= 0.35$). The H_{Max}/M_{Max} ratio was also unchanged in the NDL ($F_{(3,30)} = 2.22$; $p= 0.13$, $p\eta^2= 0.59$).

Conclusion: Static stretching improved the ROM in the stretched muscle. However, the stretched limb's force was negatively affected for several minutes following the stretching protocol. The ROM improvement and force impairment (near significant) were transferred to the contralateral muscles. The lack of significant changes in spinal and corticospinal excitability may confirm that the afferent excitability of the spinal motoneurons and corticospinal excitability may not play a substantial role in non-local muscle's ROM or force output.

Keywords: non-local muscle stretching; ROM; force; TMS; Hoffman reflex

2-1 Introduction

Static stretching (SS) as a principal warm-up activity during the task-specific warm-ups is applied to increase range of motion (ROM) (Behm and Chaouachi 2011, Behm et al. 2016, 2021), decrease injury incidence and pain (Behm et al., 2021b), and improve athletic performance (Young et al., 2006). It has been shown that stretching a muscle group may also have crossover and/or global effects (Behm et al., 2021b, 2021d, Chaouchi et al., 2017). Research shows an improvement in the non-stretched limb's ROM (Behm et al. 2021b). Clark et al. (Clark et al. 1999) demonstrated a 14.4% improvement in the straight leg raise ROM following unilateral hamstrings static stretches (6x6s). Chaouachi et al. (Chaouachi et al. 2017) reported 6-8% increases in the contralateral non-stretched hip flexors ROM after a unilateral static and dynamic hip flexion stretch. Additionally, Behm's recent meta-analysis showed that unilateral passive static stretching induced moderate-magnitude increases in passive ROM with non-local, non-stretched joints (Behm et al., 2021b).

Additionally, the non-local impairments of performance due to stretching has been elucidated that stretching a muscle may impair the strength, power, endurance, sprint, balance, and other performance measures of the non-stretched limb (Behm et al., 2021d, Caldwell et al., 2019). Marchetti (Marchetti et al. 2017) showed muscle activation reduction in both non-stretched pectoralis major (32.6%) and triceps brachii (12.6%) following a prolonged unilateral SS (6x45-s) on the pectoralis major muscle. Jelmini et al. (Jelmini et al. 2018) used three SS repetitions of 45-s each in the finger flexors and reported no change in handgrip force or EMG activity. However, the rate of force generation decreased by 10%. Chaouchi et al. (Chaouchi et al. 2017) showed no significant impairments of isokinetic leg flexion torque following eight repetitions of 30-s each of unilateral hip flexion SS. Behm's recent meta-analysis showed the strength impairments in the contralateral non-stretched limbs (with small magnitude) for the first time (Behm et al. 2021d).

Thus, with this conflict in the literature, it is not clear whether there is sufficient evidence to substantiate that unilateral SS generally has global (contralateral or non-local) consequences (i.e., positive, negative, or no significant effect) on performance (Behm et al. 2021d).

The mechanisms involved in non-local ROM improvements have been attributed to psychophysiological influences (i.e., increased stretch or pain tolerance) (Behm et al. 2016) and the reduction in the stimulation of sympathetic excitation (Behm et al. 2021d). It has been suggested that the musculotendinous unit can tolerate substantial stress without a change in tension for a given length (Magnusson et al., 1995 & 1997). The stretching causes the muscle-induced sympathetic nervous activity to be elevated (Cui et al., 2006). This activity facilitates stretch reflexes (Kamibayashi et al., 2009), influencing both the stretched and non-stretched muscles (Ray and Mark, 1995). The joint ROM is highly influenced by the muscle resistance caused by tonic reflexes (Guissard and Duchateau, 2006). There is evidence for increases in motor unit discharge frequency and twitch ½ relaxation time reduction due to sympathetic activity, demonstrating adrenergic stimulation of muscle fiber contractility (Roatta et al., 2008). Hence, sympathetic-induced (fight or flight) reflexive and motor unit-induced effects on muscle tonicity could increase resistance to global muscle extensibility (Behm et al., 2021b). It has been speculated that sustaining the stretch at the point of discomfort would be sensed as a non-harmful manipulation, and the sympathetic response would be downregulated over time to allow the individual to relax and globally extend the stretched and non-stretched muscles (Behm et al., 2021b).

The non-local impairments of the stretching were initially attributed to CNS-inhibiting effects when isokinetic torque deficits were found in the contralateral quadriceps following a unilateral quadriceps stretch (Cramer et al., 2004). The reduction of the muscle spindles excitatory reflexive activity following prolonged SS (>30-s) has been suggested (Guissard et al. 2001). Type I and II muscle spindle afferents innervate spinal motoneurons (Prochazka and Ellaway, 2012). These afferents also project to the somatosensory and primary motor cortex (Phillips et al. 1971). Therefore, they may have global consequences. Motor-evoked potentials (MEPs) have been tested in research studies through transcranial magnetic stimulation (TMS) over the cortex (Pulverenti et al., 2019; Pulverenti et al., 2020). However, there is a conflict in the studies. When it comes to stretching, two bouts of maximum dorsiflexion stretching (30-s) on the plantar flexors left the relaxed muscle's corticospinal excitability unchanged (Budini et al., 2017).

The Hoffman (H-) reflex measures presynaptic inhibition at the Ia- motoneuron synapse and the excitability of the neural reflex arc (Misiaszek, 2003). It assesses motor units activated by the afferent pathway (Duclay et al. 2005). There is insufficient information about the H-reflex changes with non-local stretching effects, with no studies examining crossover H-reflex responses.

Therefore, the present study aimed to investigate possible ROM and force (torque) changes in the DL and non-DL following the DL-SS. We aimed to investigate the MEPs, and H-reflex changes following the dominant leg's stretching as well. We hypothesized that the stretching would improve the non-dominant leg's ROM and reduce the maximal force output. Moreover, we hypothesized that the static stretching might decrease supraspinal and spinal excitability in the contralateral plantar flexors.

2-2 Materials and Methods

2-2-1 Participants

A statistical power analysis (G Power software version 3.1) based on a related article (Killen et al. 2019) revealed that approximately 12 participants were necessary to achieve an alpha (α) = 0.05, power= 0.8 and effect size (f) = 0.42. Hence, fourteen (n=14) (healthy recreationally active) participants consisting of males (n=9) and females (n=5) between the ages 18-30 years (age= 30 ± 1.64; BMI= 25.69 ± 1) were recruited. Exclusion criteria included no previous surgery on the lower extremities and no history of injury with remaining symptoms in the lower limbs within the last year (da Silva et al. 2015). Verbal and written consent was attained from the volunteers before the study. Every participant was required to read and sign a physical activity participation questionnaire (PAR-Q) and an approved informed consent form. They were asked to fill-out a TMS Safety Checklist to be eligible to participate in the study. The study was approved by Memorial University's Interdisciplinary Committee on Ethics in Human Research (ICEHR) before the beginning of the study (ICEHR#: 20221983-HK).

2-2-2 Experimental Design

In a quasi-experimental, repeated measures study, the subjects were required to identify the preferred leg for kicking a ball to determine the dominant leg (Behm et al., 2016b). The participants were asked to attend the laboratory for two sessions with a random allocation of the following experimental sessions: (1) Dominant leg's 270-s (six sets of 45-s of passive stretch with 15-s of rest between the stretches) stretching and testing the non-dominant leg's force, ROM, EMG, spinal and supraspinal excitability before and after the stretching protocol; (2) Dominant leg's 270-s of rest (Control) and testing the non-dominant leg's force, ROM, EMG, spinal and supraspinal excitability pre- and post-test. Two MVICs in both legs (dominant and non-dominant leg) were performed before the intervention, and a 3rd MVIC was performed if the difference between the two previous MVICs was more than 5% (Aboodarda et al., 2015). Before the measurements, the participants were asked to warm up their plantar flexor muscles with 2-3 submaximal intensity voluntary isometric contractions (40-50% of their maximum force). All the testing was conducted on the non-dominant leg before the intervention and immediately after the intervention's completion (Figure 1). The spinal (H-reflex) and cortical excitability (TMS) were measured 10-s and 30-s post-intervention as well.

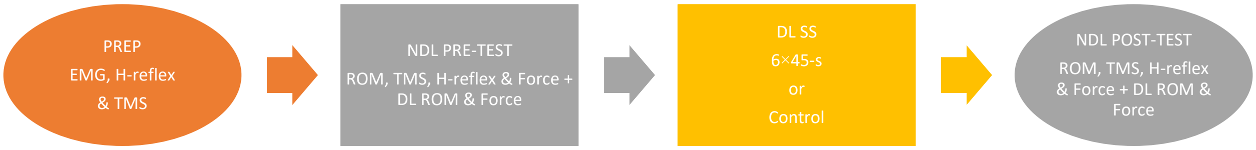

Figure 1. Experimental Design. *PREP: The preparation of the skin and the attachment of the electrodes for EMG (Electromyography), H-reflex (Hoffmann reflex), and TMS (Transcranial Magnetic Stimulation) recordings as well as the head preparation for TMS stimulation; NDL PRE-TEST: The non-dominant leg's testing before the intervention; ROM: the range of motion; DL SS: the passive stretching intervention in the dominant leg for 6×45-s (six sets of 45-s with 15-s of rest between the two consecutive repetitions); NDL POST-TEST: the repetition of the PRE-TEST measurements after the intervention (the stretching protocol or Control).*

During the stretch intervention, the participants were verbally reminded to relax the non-dominant leg. The relaxation of the non-dominant leg was controlled via EMG as well.

2-2-3 Measures

Dorsiflexion Ankle Range of Motion (ROM)

The subjects were required to lean their back to the wall while both legs were aligned and extended forward. The ankle joints were positioned at a neutral alignment (90° to the ground). Then a researcher passively moved the foot to the maximal ankle dorsiflexion ROM. Another researcher took pictures of the stretching leg when the participant confirmed their maximal dorsiflexion ROM. Two pictures were taken from each leg before and after the intervention. The landmarks on the lateral fifth metatarsal, lateral malleolus and, fibula head were used to analyze and report the ROM. The photographs taken from the legs were analyzed using the Kinovea app version 0.9.5 to read ROM (Figure 2).

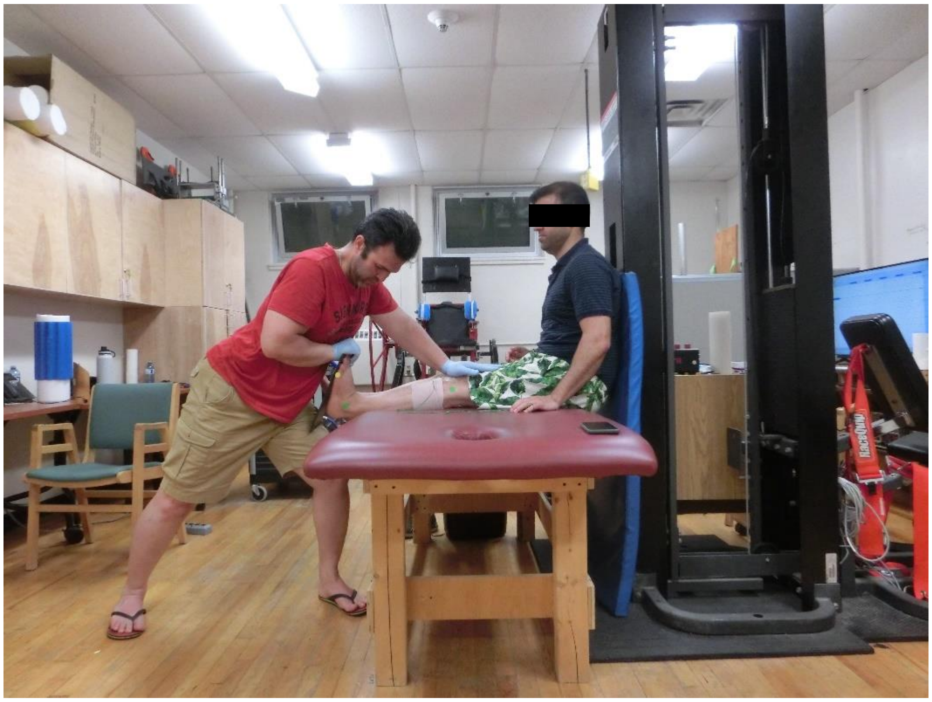

Figure 2. A typical set up for the ankle range of motion (ROM) measurement. *A researcher moved the ankle to the maximum range of motion twice with 60-s of rest between the two consecutive measurements. The camera was adjusted carefully, and the camera's position was marked for the whole research process.*

Maximal Voluntary Isometric Contraction (MVIC)

The MVICs were performed on a modified and reliable "Boot" Apparatus (Technical Services, Memorial University of Newfoundland) with the thigh horizontal to the floor while the knee and the ankle joints flexed at 90° (Halperin et al. 2014b) (Figure 3).

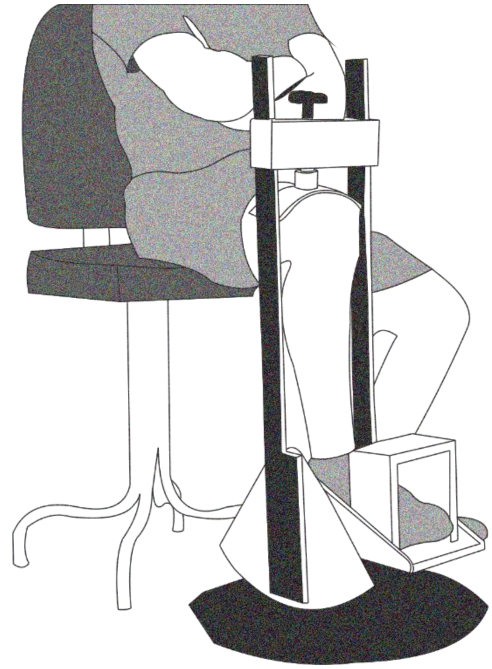

Figure 3. Boot apparatus *for measuring the maximal voluntary isometric contraction with the plantar flexors*

One strap fixed the contracting leg to prevent extraneous movement of the lower leg. At the same time, a thigh fixture securely restrained the foot so that any attempt to plantar flex the ankle joint resulted in an isometric contraction (Halperin et al. 2014). The device was calibrated before each session by hanging known weights off the footplate. The participants were asked to perform 2-3 plantar flexion warm-ups equal to 50% of their maximum force for 5-s before the principal MVICs (Aboodarda et al., 2015). Two MVICs were initially conducted, and if the second MVIC force exceeded the first MVIC force by more than 5%, then a third MVIC was attempted. The peak-

peak amplitude of the torque between the baseline and the maximum torque was considered and reported as the maximum force of each leg.

Electromyography (EMG)

The surface of skin was prepared by the target area shaving, followed by cleaning and abrading with an abrasive pad and alcohol swabs to remove dead epithelial cells (Young et al., 2018), obtain low resistance between the electrodes (< 5-kOhms), and adequate signal-to-noise ratio (Gondin et al., 2006). The point of 5 cm distal to the insertion of the gastrocnemii on the Achilles tendon was measured and marked carefully to attach a pair of surface electrodes (Kendall 130 foam electrodes, Conductive Adhesive Hydrogel, Covidien IIc,) on the soleus muscles in both legs (Gondin et al., 2006) with an inter-electrode distance of 2-cm (Young et al., 2018). The reference electrode was attached to the lateral malleolus of the dominant leg (Halperin et al., 2014). The noise of the EMG signals was monitored to be less than 0.05mV (Konrad, 2005). The root mean square (RMS) of the EMG activity was collected 500ms before and after the maximum amplitude of the torque during the MVICs (Behm et al., 2019). RMS EMGs of the non-dominant leg during the 1st and the last 5-s of the dominant leg stretching protocol were monitored to determine the extent of relaxation/activation of the non-dominant leg during the intervention. The baseline (pre-test) peak RMS EMG of the non-dominant leg was normalized to the average RMS EMG of pre-intervention MVIC. The EMG signals were filtered with a 10-500 Hz bandpass filter and amplified (×1000 bipolar differential amplifier, input impedance = 2MΩ, common-mode rejection ratio > 110dB min (50/60 Hz), noise > 5 µV) and analog-to-digitally converted (12 bit). EMG was collected at a sampling rate of 2000 Hz (Biopac System, Inc., DA 100: analog-digital converter MP150WSW;

Holliston, Massachusetts, USA) and dedicated software (AcqKnowledge 4.1, Biopac System Inc.) (S. Aboodarda et al., 2016).

Spinal and Supraspinal Motoneuron Excitability

The H-reflex and compound muscle action potential wave (M-Wave) were evoked in the non-dominant leg by stimulating the soleus muscle through percutaneous stimulation. A single rectangular pulse (1 ms) was delivered to the posterior tibial nerve by a Digitimer stimulator (model DS7AH, Hertfordshire, UK). The stimulator cathode probe (MYO004 Stimulator Probe, SS; Neurospec. Research Neuroscience, Switzerland) was held in the popliteal fossa, and the anode electrode (Kendall 130 foam electrodes, Conductive Adhesive Hydrogel, Covidien IIc) was placed over the patella for the optimal site identification and testing. The optimal site of the stimulation, giving the greatest amplitude of the evoked potentials, was carefully located by a probe and marked. The subjects were required to close their eyes and refrain from moving their hands or heads. This was to keep consistent cortico-vestibular influences on the motor pool excitability. The consistent head and hand position would limit afferent feedback from other peripheral receptors as well. An H-reflex and M-Wave recruitment curve was produced (Young et al. 2018). The recruitment curve involved testing under a resting condition with an initial evoked stimulation at 2mA, with four stimuli provided at each intensity interspersed with approximately 10-s rest, and 2mA raised the intensity to the point of no further increase in evoked peak twitch torque and concomitant peak-to-peak M-wave amplitude (M_{max}). At this point, the stimulation intensity was increased by 10% to ensure that M_{Max} was elicited throughout the study (Lockyer et al. 2019). Following the identification of the peak H-reflex amplitude (H_{Max}), four stimulations at this intensity were evoked to measure the H-reflex. An H_{Max}/M_{Max} normalization ratio was calculated and used for statistical analysis. The participants were tested for afferent excitability of the spinal motoneuron (H-reflex) under resting conditions in the non-dominant leg's soleus muscle before and after the stretching protocol (6×45s interspersed with 15s rest) or control period (270-s).

A submaximal plantar flexion intensity (20% Soleus MVIC) was calculated during the non-dominant leg's MVIC, and the desired rate of force during TMS was set on a visual feedback screen. During TMS measurements, the participants were instructed to perform the plantar flexion on the boot apparatus until soleus EMG activity matched the 20% target and then hold the EMG as steady as possible for the duration of the TMS testing (Pulverenti et al. 2020). Transcranial magnetic stimulation (TMS) over the cortex elicited motor-evoked potentials (MEPs) in the non-dominant leg's soleus muscle (Hahn et al. 2012). TMS (Magstim 2002, The Magstim Company Ltd, UK) stimulations were applied during submaximal voluntary isometric plantar flexions (i.e., 20% Soleus MVIC) in the non-dominant leg using a double-cone coil positioning over the motor cortex, with monophasic current pulses running through the center of the coil from anterior to posterior (Hahn et al. 2012). The vertex was measured and marked on the participant's scalp with a permanent felt-tip marker (Lockyer et al., 2019). One investigator ensured proper and consistent coil placement directly over the vertex throughout the experiment (Lockyer et al. 2019). The coil was held firmly against the participants' skull, parallel to the floor. The current flow direction was oriented to preferentially activate either the left or right motor cortex, depending on leg dominance (Lockyer et al. 2019). The stimulation intensity for the test was determined with respect to the active motor threshold (AMT) for MEPs from the soleus muscle (Oya et al. 2008). AMT was determined in a trial consisting of eight TMS stimulations. AMT was considered a stimulation intensity that elicited an average of four to five MEPs with an amplitude of approximately 0.1-0.2 mV when the subject produced an isometric plantar flexion torque of their 20% MVIC (Oya et al.

2008). The subsequent test intensity was determined as AMT intensity × 1.2, and the resulting maximal stimulator output was set for the rest of the testing process (Oya et al. 2008).

2-2-4 Intervention

The stretching protocol was performed in the dominant leg sitting with the knees extended. The participants were asked to lean their back against the wall and extend both legs forward. The force was applied to the sole of the foot at the level of the metatarsal heads while the researcher secured the participant’s knee with one hand and stretched the leg with the other hand. Dependent upon the condition (Stretch (SS) or Control), the participants performed six stretches of 45 seconds with 15 seconds of rest periods or rested for 345 seconds. The SS protocol was performed at 70-90% of the point of discomfort (POD) based on the participant's repeated feedback. The subjects were informed that 0= “no stretch discomfort” and 10= “the maximum stretch discomfort.” The same researcher applied and controlled the SS protocol.

2-2-5 Statistical Analyses

Data were analyzed using SPSS-28 software. The assumption of normality was tested with the Shapiro-Wilk test, and the sphericity was assumed for all the dependent variables with the Mauchly test. In the case that the sphericity was violated, the corrected value for non-sphericity with Greenhouse-Geisser epsilon was reported.

A two-way repeated measures ANOVA involving two conditions (SS vs. Control) × two times (Pre-test and Post-test) was used to compare the ROM and force mean differences between groups at the designated testing times. Another two-way repeated measures ANOVA involving two conditions (SS vs. Control) × four times (Pre-test, Immediately Post-test, Post-10-s, and, Post-30-s) was used to compare the H_{Max}/M_{Max} and MEP/M_{Max} mean differences between groups at the designated testing times. Bonferroni post-hoc test was used to locate the possible significance. The effect sizes were calculated (Cohen, 1988) and reported (small = 0.01, medium= 0.06, large= 0.14). The reliability of the pre-intervention measures was also reported with an intraclass correlation coefficient (ICC).

2-3 Results

2-3-1 Reliability

Table one (Table 1) below shows reliability measures (ICC) from the two ROM and MVICs pre-tests for the DL and NDL. The measurements were consistently excellent, except for the NDL-ROM pre-static stretching (0.74) and DL-MVIC pre-SS (0.73).

Table 1. Reliability test results. *Reliability was assessed with Intraclass Correlations (ICC) for all the outcomes, ROM and MVIC. DL: the dominant leg; NDL: the non-dominant leg; ROM: Range of Motion; MVIC: Maximal Voluntary Isometric Contraction; Pre-Control: testing at the pre-test in the control session; Pre-SS: testing at the pre-test in the SS session; SS: static stretching session; Control: control session; CV: Coefficient of variation (%).*

	DL	**CV**	**NDL**	**CV**
		ROM		
Pre- Control	0.98	8.35	0.93	5.73
Pre- SS	0.98	7.79	0.74	6.52
		MVIC		
Pre- Control	0.95	47.06	0.95	26.94
Pre- SS	0.73	38.9	0.99	31.81

2-3-2 Dominant Leg Maximal Voluntary Isometric Contraction Force

The main effects of the time (Pre-test vs. Post-test), conditions (Control vs. SS) and interaction (time × condition) in the ANOVA repeated measures test has been shown in the table 2. There were significant effects of the condition (*p*= 0.030) and interaction (Time× Condition) (*p*= 0.027) on the DL-MVIC reduction. The interaction effect showed when the subjects rested (control condition), from pre- to post-MVIC, the DL MVIC force slightly and insignificantly increased (↑5.15%, 118.16 ± 43.26 to 124.25 ± 40.61 Nm) (d = 0.14). In contrast, the DL MVIC force decreased significantly (↓10.87%, 112.62 ± 39.73 to 100.37 ± 31.2 Nm) (d= 0.34) pre-to-post during the stretching session (*p*= 0.027) (Figure 4).

Table 2 DL-MVIC force changes (Nm) has been shown with the main effects of the time (Pre-test vs. Post-test), condition (SS vs. Control) and interaction (time × interaction). "": Significant effect of the condition; "**": Significant effect of the interaction.*

			SD	F	*p*	pη^2
Time	Pre-test	115.39	12.27	0.39	*0.54*	0.03
	Post-test	112.31	10.11			
Condition	SS	106.5	10.4	6.41	*0.03**	0.39
	Control	121.21	12.23			
Interaction (Time × Condition)	Pre-test (Control)	118.16	13.04	6.7	*0.027***	0.4
	Post-test (Control)	124.25	12.24			
	Pre-test (SS)	112.62	11.98			
	Post-test (SS)	100.37	9.4			

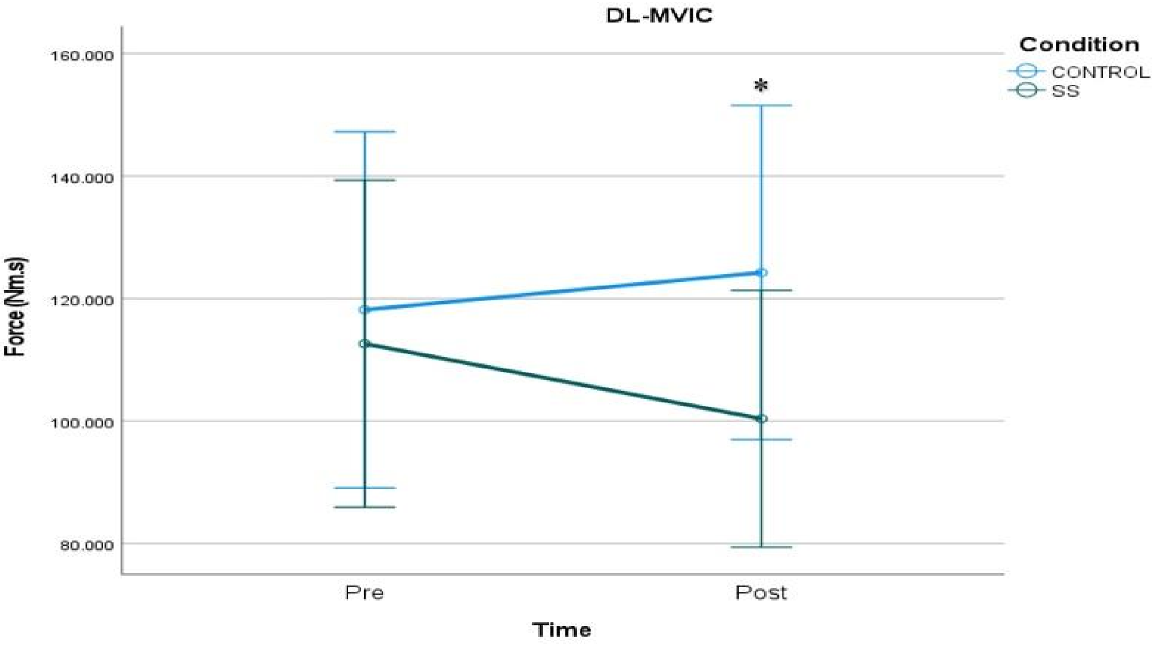

Figure 4. Dominant leg's (DL) Maximal Voluntary Isometric Contraction (MVIC) *Force (Nm.s) Changes pre-to-post-test in conditions (Control) and (SS). Times Pre: Pre-test and Post: Post-test. "*": The DL-MVIC force significantly (p=0.027) decreased following the static stretching protocol.*

2-3-3 Non-Dominant Leg Maximal Voluntary Isometric Contraction Force

There was no significant effect of time (pre-to-post-intervention) but there was a large effect size (pη^2= 0.16) on the non-dominant leg's MVIC force ($F_{(1,10)}$= 1.93; *p= 0.19*). Although large effect sizes were also found with the condition main effect and condition x time interaction, there was a near significant effect of the condition (Control vs. SS) ($F_{(1,10)}$= 4.27; *p= 0.06* ; pη^2= 0.29) and a non-significant interaction ($F_{(1,10)}$= 2.35; *p= 0.15;* pη^2= 0.19) on the NDL-MVIC force (Figure 5).

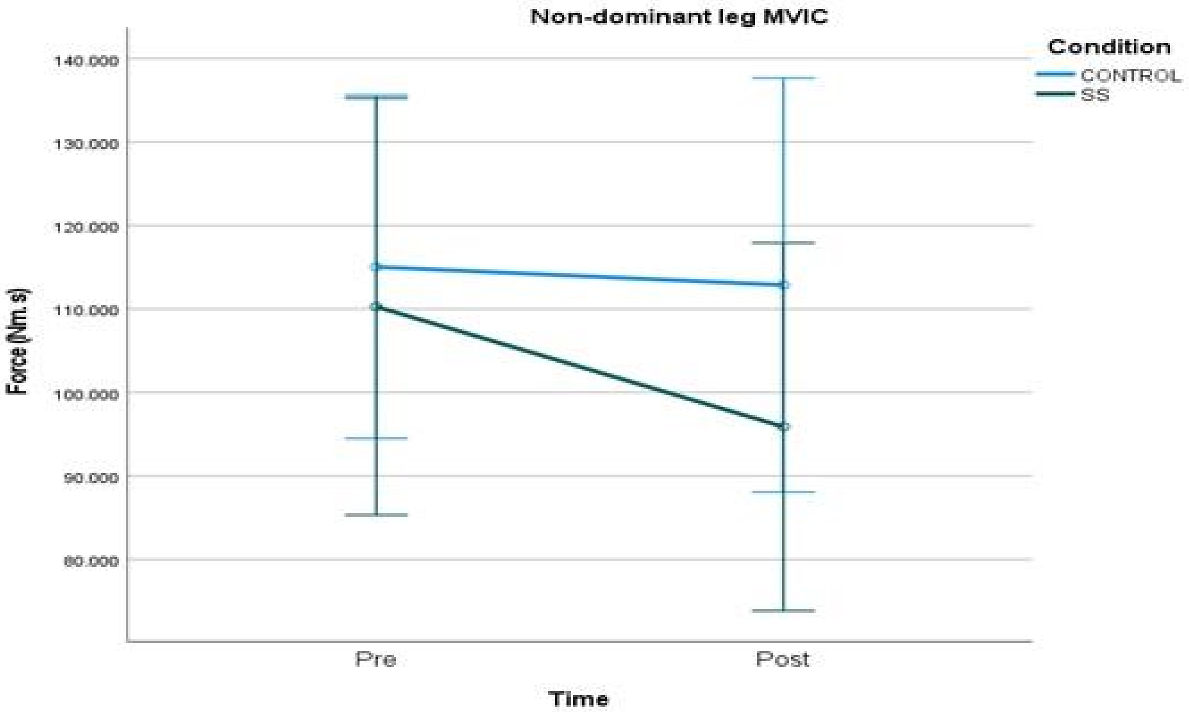

Figure 5. Non-dominant leg's Maximal Voluntary Isometric Contraction *Force (Nm. s) Changes pre-to-post-test following the Control and SS sessions. There was no significant effect of time, condition, or interaction on the force reduction in the non-DL. Time Pre: Pre-test; Time Post: Post-test; Conditions: Control and static stretch (SS).*

2-3-4 Dominant Leg Range of Motion

The DL-ROM increased significantly pre-to-post-test as there were significant main effects with large magnitude effect sizes for time (Pre-Post) (↑ 3.23%) ($F_{(1,10)}$= 16.95; *p*= 0.002, $p\eta^2$= 0.62), condition (Control vs. SS) (↑ 4.38%) ($F_{(1,10)}$= 7.95; *p*= 0.018, $p\eta^2$= 0.44) and interaction (time × condition) (↑ 6.5 %, 99.9˚ ± 7.95 to 92.8˚ ± 8.59) ($F_{(1,10)}$= 22.80; *p*<0.001, $p\eta^2$= 0.69) (Figure 6).

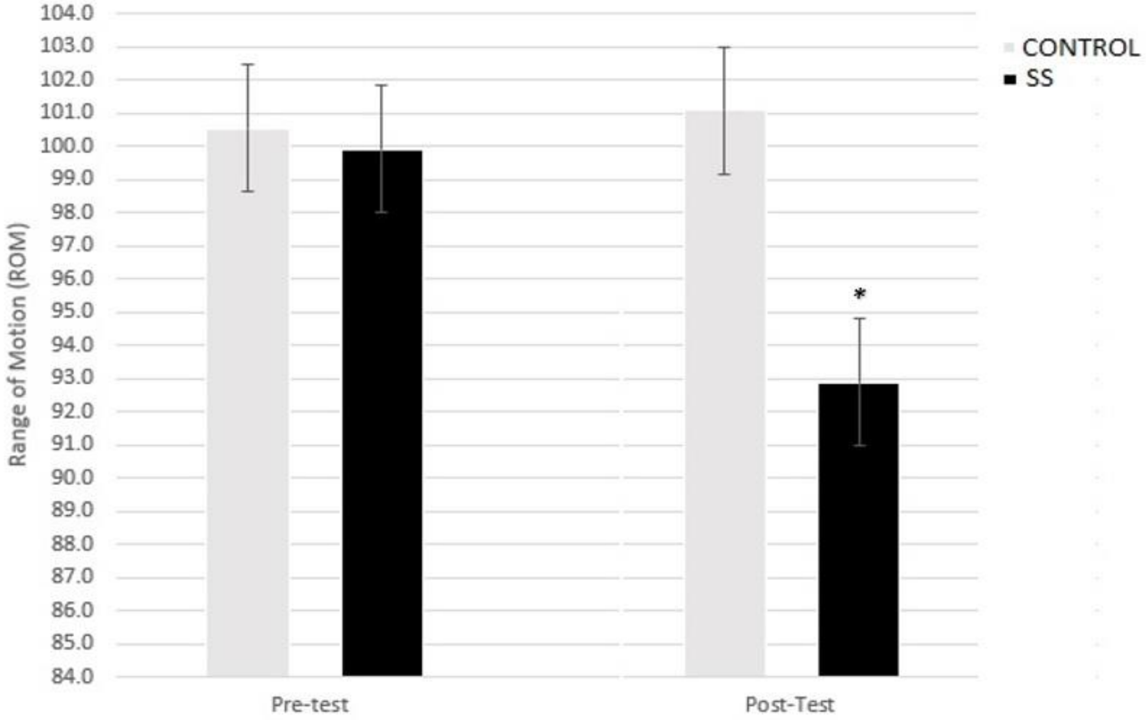

Figure 6. DL-ROM improvement. *The figure shows a significant "*" (p=0.002) improvement in the Dominant leg's range of motion (DL-ROM) pre-to-post following the static stretching. The lower numbers represent increases in ROM. DL ROM (Dominant leg range of motion); Conditions (Control vs. SS); Times Pre-test and Post-test.*

2-3-5 Non-Dominant Leg Range of Motion

A main effect for time displayed a significant, large effect size improvement in the non-dominant leg's ROM ($F_{(1,10)}= 12.75$; *p= 0.005,* $p\eta^2= 0.56$). The main effect for condition was not significant ($F_{(1,10)}= 1.56$; *p= 0.24*) but exhibited a medium effect size ($p\eta^2= 0.13$). There was a significant, large effect size interaction ($F_{(1,10)}= 18.53$; *p= 0.002,* $p\eta^2= 0.65$). The NDL-ROM increased 5.35% significantly in the SS session (97.34 ˚ ± 6.66 to 92.09 ˚ ± 8.47), while there was a slight decrease in the Control session, from 96.15 ˚ ± 5.76 (Pre-test) to 97.01 ˚ ± 5.71 (Post-test) (Figure 7).

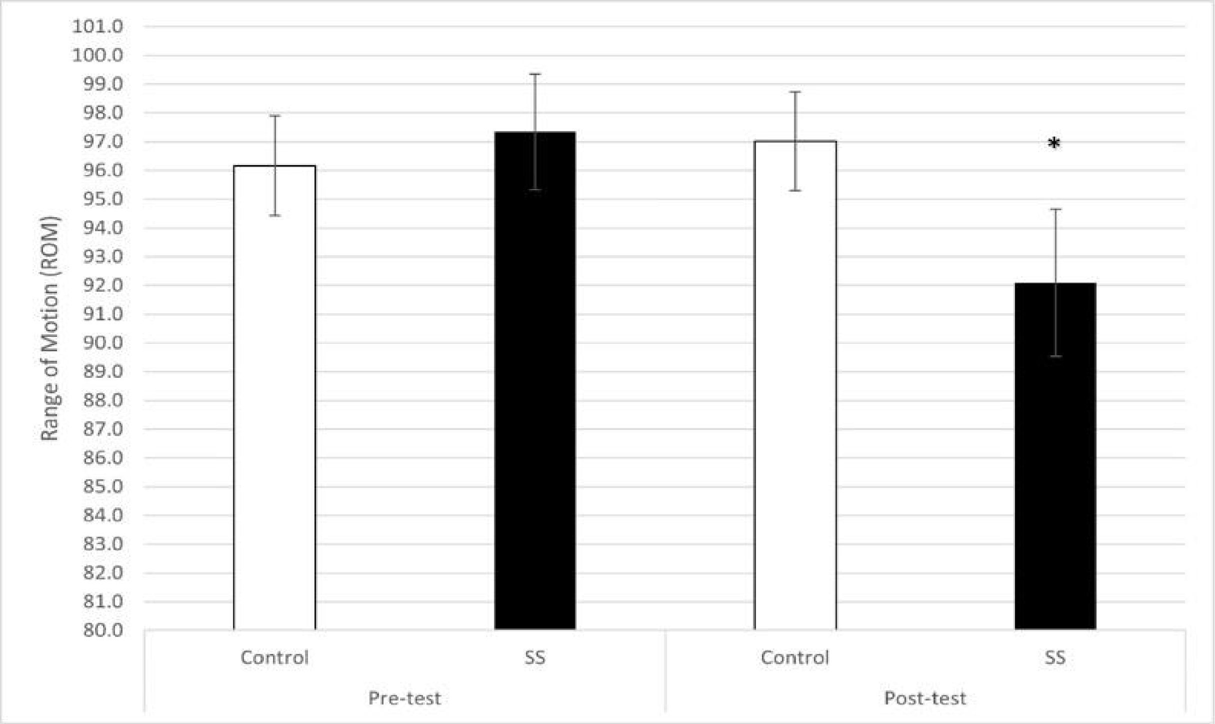

Figure 7. NDL-ROM Changes. *Non-dominant leg's ROM changes pre-to-post-test during the Control and the stretching (SS) sessions. The NDL-ROM was improved significantly "*" (p= 0.005) following the DL-SS and has been reported in angular degrees (˚). The lower values represent increases in the ROM.*

2-3-6 Motor Evoked Potential. M_{Max}

There was no significant effects on time ($F_{(3,30)}$= 1.35; *p= 0.27,* pη^2= 0.25), condition ($F_{(1,10)}$ = 2.04; *p= 0.18,* pη^2= 0.17) or interaction (*$F_{(3,30)}$ = 0.96; p= 0.42,* pη^2= 0.35) but all displayed large effect sizes on the MEP/M_{Max} ratio. The MEP/M_{Max} increased 53% immediately after the SS condition (0.047 ± 0.027 to 0.072 ± 0.053) and the ratios were 10.64% (0.052 ± 0.046) and 23.4% (0.058 ± 0.044) higher than the pre-test values after 10-s and 30-s (Post10 and Post30). The MEP/M_{Max} ratios were 4.76% (0.044 ± 0.03), -2.38% (0.041 ± 0.039) and -9.52% (0.038 ± 0.021) of the pre-test ratio (0.042 ± 0.027) immediately, after 10-s and 30-s in the Control condition (Figure 8).

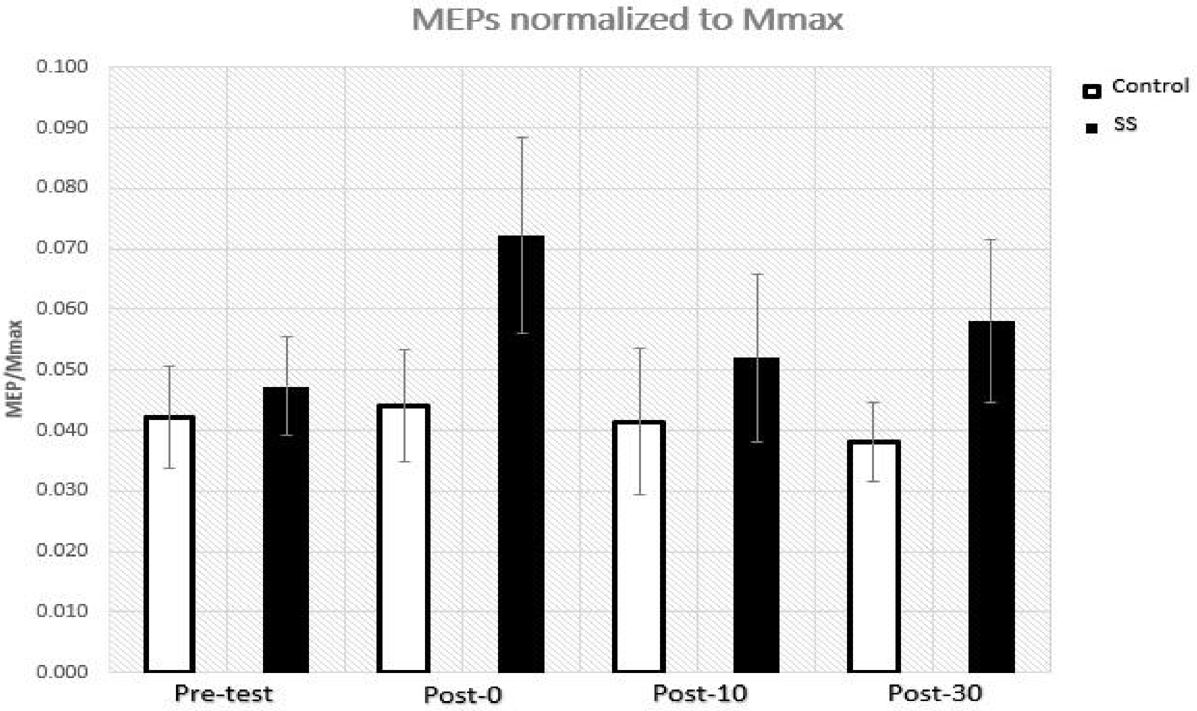

Figure 8. MEP changes. *MEP values normalized to M_{Max} (MEP/M_{Max}) ratio changes during the control and SS sessions; Times Pre- (Pre-test), Post0 (Immediately after the intervention), Post10 (ten seconds after the intervention), Post30 (30 seconds after the intervention).*

2-3-7 Hmax. Mmax

There was a near significant, large magnitude main effect for time ($F_{(3,30)}$= 3.5; *p= 0.06,* $p\eta^2$= 0.72), but no significance for condition ($F_{(1,10)}$ = 0.027; *p= 0.87,* $p\eta^2$= 0.003) or time x condition interaction (*$F_{(3,30)}$ = 2.22; p= 0.13,* $p\eta^2$= 0.59) on the H_{Max}/M_{Max} ratio. The H_{Max}/M_{Max} ratio in the SS condition decreased 26.9% (0.52 ± 0.18 to 0.38 ± 0.23) immediately post-test (Post0). The rates were 15.38% (0.44 ± 0.24) and 5.77% (0.49 ± 0.29) lower than the pre-test at the times Post10 and Post30, respectively. The H_{Max}/M_{Max} ratio in the control session decreased 11.54% (0.52 ± 0.2 to 0.46 ± 0.2) immediately post-test. The ratios were 23.1% (0.4 ± 0.22) and 19.23% (0.42 ± 0.21) lower than the pre-test at the times of Post-10 and, Post30, respectively (Figure 9).

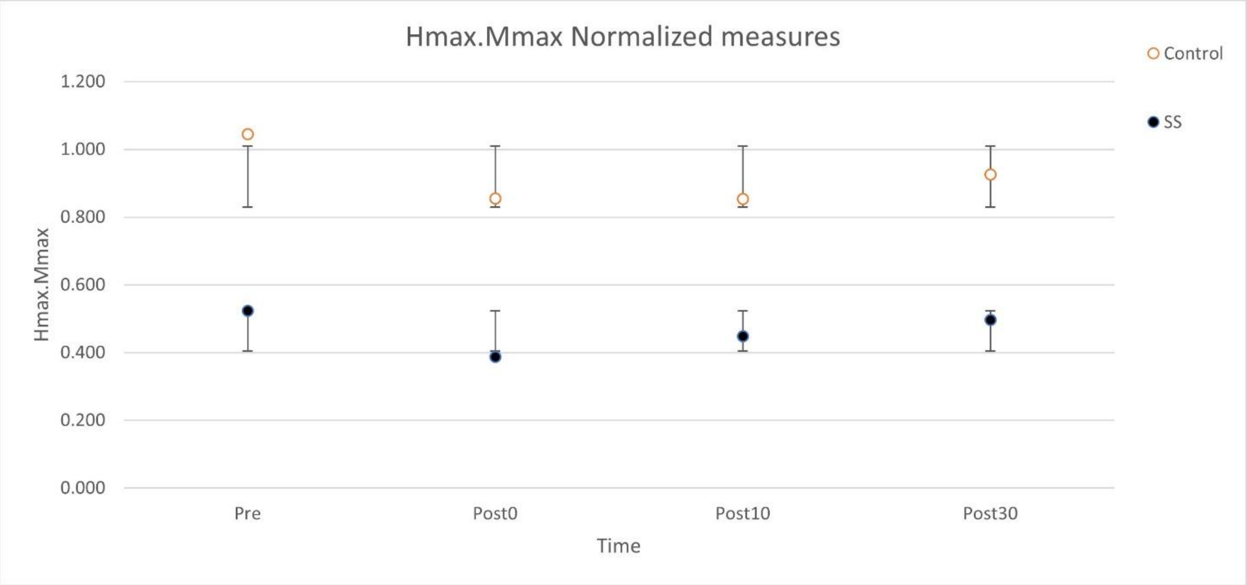

Figure 9. HMax changes. *H_{Max} normalized to M_{Max} (H_{Max}/M_{Max} ratio) changes during the SS and control sessions. Times Pre (Pre-test), Post0 (Immediately after the intervention), Post10 (ten seconds after the intervention), Post30 (30 seconds after the intervention).*

2-3-8 M_{Max}

There was no effect of time (Pre-Post) ($F_{(1,13)}= 1.36$; *p= 0.26;* $p\eta^2= 0.095$), condition (Control vs. SS) ($F_{(1,13)}= 3.40$; *p= 0.088;* $p\eta^2= 0.2$), or interaction ($F_{(1,13)}= 0.26$; *p= 0.87;* $p\eta^2= 0.002$) on M_{Max} amplitude changes (Figure 10).

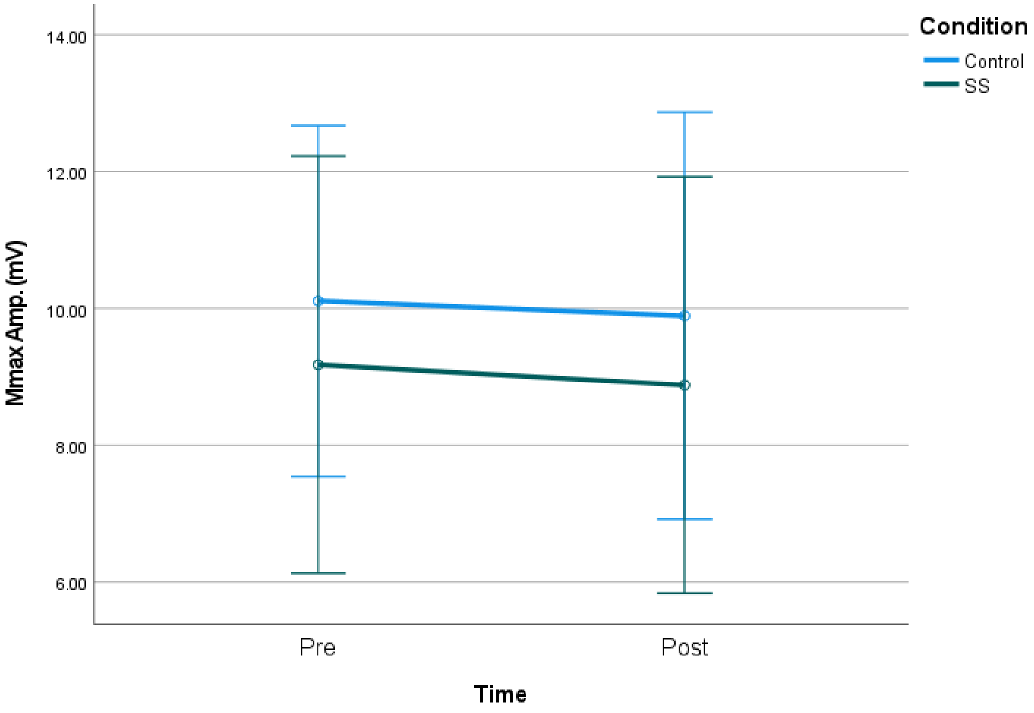

Figure 10. M_{Max} Changes. *The M_{Max} amplitude changes over time in the Control and SS groups; Amp: Amplitude; mV: milli-volts; Time: Pre (Pre-test) and Post (Post-test).*

2-4 Discussion

The major findings showed that with unilateral SS of the dominant leg, the: a) DL-ROM (6.5%) and NDL-ROM (5.35%) significantly increased b) DL-MVIC and NDL-MVIC forces significantly and near significantly (p=0.06) decreased respectively, c) MEP/M_{Max} and H_{Max}/M_{Max} ratio did not change significantly in the non-dominant leg.

The present study's DL plantar flexors ROM significant increase agreed with previous studies using similar SS durations by Lima et al. (Lima et al. 2014) (six SS of 45s), da Silva et al. (da Silva et al. 2015) (six SS of 45s) and Coratella and colleagues (Coratella et al. 2021) (five sets of 45s). Similarly, a systematic review of five studies by Radford et al. (2006) indicated that plantar flexors muscle SS induced small and statistically significant increases in ankle dorsiflexion (15 - >30 min of SS induced 2.07^0 – 3.03^0 ROM increases).

The crossover SS effects with increases in the NDL- ROM in our study (5.35%: $97.3^0 \pm 6.6$ to $92.1^0 \pm 8.4$) was in accordance with the Behm et al. results (Behm et al. 2019) that showed 4.3% ROM improvement in the contralateral non-stretched lower limb. Additionally, Coratella and colleagues (Coratella et al. 2021) reported 8% ROM increases in the non-stretched plantar flexors. Power et al. (Power et al. 2004) stretched the dominant leg’s hamstrings, quadriceps, and plantar flexors with two successive SS, each consisting of 3×45s and reported a 10% ROM increase only in hamstrings with no significant changes in quadriceps and plantar flexors ROM. The difference between our results compared to the others could be due to the stretching intensity (maximal POD in our study vs. the onset of pain in the Power study), differences in the stretching protocol (static stretching by the experimenter vs. self-stretch by the participant in Power study or warm-up prior the stretching in Lima’s study), the position of the stretching (sitting vs. standing in Power study or prone position in Coratella and Lima studies) or sex differences (male and female vs. only males in Coratella study).

Although the DL-MVIC force was slightly increased during the control session in the present study, the DL-MVIC reduction with SS in the present study was in line with previous studies (Cè et al. 2015**;** Cè et al. 2020**;** Esposito et al. 2011**;** Coratella et al. 2021). A large magnitude, near-significant (p=0.06) decrease in the NDL-MVIC force in the present study was also in accordance with a number of studies (Coratella et al. 2021, Behm et al. 2019, Marchetti et al. 2014; da Silva et al. 2015; Power et al. 2004). There are inconsistencies in this phenomenon with some other studies that also showed a reduction in maximal force (Cramer et al. 2004; 2006; Ce et al. 2020). The force-generating capacity has been attributed to mechanical and neuromuscular factors (Coratella et al. 2021). However, a recent study excluded the mechanical elements in the contralateral limb (Ce et al. 2020). They showed a net neuromuscular effect and reported a decrease in RMS EMG in the contralateral limb (Ce et al., 2020). Some other factors like the stretched muscle, stretching type (e.g., dynamic or passive stretching), duration of the passive stretching protocol, and stretching intensity (Tarajano et al. 2017; Coratella et al. 2021) have also been suggested to contribute to the discrepancies between studies. The mechanisms involved in the DL force-impairment after the SS was out of the scope of this research study.

However, previous stretching studies cannot clearly confirm the spinal or corticospinal role in the reduction of the force capacity as they showed no significant changes in H_{Max}/M_{Max} (Pensini and Martin, 2004; Tarajano et al. 2013; Coratella et al., 2021) or MEP/M_{Max} (Pulverenti et al. 2020; Pulverenti et al. 2019; Budini et al. 2017; Budini et al. 2019) ratios with SS (Coratella et al. 2021). With respect to no changes in the M-wave (peripheral mechanism) (Tarajano et al. 2017; Pulverenti et al. 2020; Ce et al. 2020; Rodriguez-Falces and Place, 2018) and the decrease in $V/M_{Superimposed}$ ratio with SS in other studies (Coratella et al. 2021), the efferent drive changes at the sub-cortical level (i.e., basal ganglia, cerebellum, ventral-anterior, and ventral lateral thalamus nuclei) have been suggested (Wiesendanger and Wiesendanger, 1985). However, this could not be confirmed in the present study. Although there was only a 5.15% increase in the DL-MVIC with control, it might be due to the post-activation potentiation from the pre-test contractions that can last for several minutes (Tillin and Bishop, 2009; Sale, 2002).

No changes in the M-waves or H_{Max}. M_{Max} ratios were observed in the present study. It was previously shown that passive stretching did not change the spinal excitability of the contralateral plantar flexors with a shorter and less intense (ramp-and-hold technique with 15s ramp and 60s hold with 5, 10 and 15 Nm torques) passive stretching protocol (Masugi et al. 2017) or an intense (90% of maximal discomfort) protocol (Coratella et al. 2021). Masugi and colleagues (Masugi et al. 2017) measured homonymous *Ia* excitation and heteronymous I*a* excitation using the tSCS (transcutaneous spinal cord stimulation) technique with similar characteristics to H-reflex (Coratella et al. 2021). They showed no changes in the spinal excitability in the contralateral plantar flexors (Masugi et al. 2017). Therefore, it seems unlikely that changes in the afferent excitability of the motoneuron is involved in the ROM improvement or MVIC force depression in the non-dominant leg. Previous studies have shown no changes in motor evoked potentials and corticospinal excitability with stretching the stretched muscle (Pulverenti et al. 2019; Budini et al. 2017). Furthermore, to the best of our knowledge, this is the first study that showed no pre-to-post-test changes in MEP. M_{Max} (corticospinal excitability) in the non-stretched plantar flexors muscle. These outcomes support the idea that passive stretching does not impair corticospinal activity (Coratella et al., 2021). Thus, non-local changes in ROM or MVIC force do not seem to be attributed to neural inhibition either arising from the periphery (afferent excitability) or the central system.

The mechanisms involving the ROM improvement have been attributed to: the cross-contact between actin and myosin filaments (Proske et al. 1999), the endo-sarcomeric non-contractile proteins (Borg et al. 1980), non-sarcomeric cytoskeleton properties (Magid et al. 1985), the changes in the viscoelastic properties related to the connective tissues (Borg et al. 1980). As the first three factors relate to mechanical stimuli, these mechanisms would not explain the non-dominant non-stretched limb's ROM improvement. Additionally, the increase in non-dominant ROM with no changes in afferent excitability of the spinal motoneuron and with no changes in the corticospinal excitability, leaves the acute increases in NDL flexibility to the psychophysiological stretch tolerance mechanism (Magnusson et al. 1997 & 1996). The stretch tolerance concept suggests the musculotendinous unit can tolerate greater stress without a change in tension for a given length (Behm et al. 2021b). The mechanisms underlying increased stretch tolerance are not fully understood. However, cortical activity may be involved through a somatosensory perception of the length and tension changes in musculotendinous tissue (Behm et al. 2019). A global pain modulatory system has been suggested (Cavanaugh et al. 2017; Aboodarda et al. 2015a). The cortical connectivity would suggest that improved pain or stretch tolerance would have global effects (Behm et al. 2019). Hence, it seems prudent that the SS in the DL promoted an increase in stretch tolerance with crossover or global consequences, resulting in an increased crossover/contralateral ROM.

Diffuse noxious inhibitory control (DNIC) has been proposed as a global pain modulatory system contributing to global stretch tolerance (Mense, 2000; Sigurdsson and Maixner, 1994; Pud et al., 2009). Stretching can induce pain (Behm et al. 2021b), and this nociceptive stimulus activates DNIC (Mense, 2000; Sigurdsson and Maixner, 1994; Pud et al., 2009). The activity of the nociceptive receptors is transmitted to multi-modal, dynamic range convergent neurons in the cortex located in subnucleus reticularis dorsalis, where it can suppress pain transmission monoaminergically (Mense, 2000; Sigurdsson and Maixner, 1994; Pud et al. 2009). This suppression reduces pain perception globally (Pud et al. 2009; Le Bars et al. 1992). DNIC suppresses pain sensitivity with the widespread distribution of monoamines such as endorphins and enkephalins (Pud et al. 2009; Le Bars et al. 1992), thus contributing to greater non-local stretch or pain tolerance (Behm et al. 2021b). This global analgesia/increased non-local stretch (pain) tolerance effect has been demonstrated by applying a painful cold pressor test to the hand and wrist, and an increase in passive knee extension ROM was found (Behm et al. 2021b).

Additionally, the muscle's nerve sympathetic activity can be elevated with stretching (Cui et al. 2006), facilitating stretch reflexes (Kamibayashi et al. 2009) and influencing both the exercised and non-exercised muscles (Ray and Mark, 1995). Muscle resistance caused by tonic reflexes has been shown to highly influence joint ROM (Guissard and Duchateau, 2006). There is evidence that sympathetic activity increases motor unit discharge frequency and reduces twitch ½ relaxation time demonstrating adrenergic stimulation of muscle fiber contractility (Roatta et al. 2008). Hence, sympathetic-induced reflexive and motor unit-induced effects on muscle tonicity could initially increase resistance to global muscle extensibility (Behm et al. 2021b). The pain perception caused by passive stretching alerts the individual to potential threats (Moayedi and Davis, 2013). This mechanism can excite the sympathetic nervous system (Berne and Levy, 1983). It has been suggested that the sustained stretching near or at the POD would soon be sensed as not a severe threat (Behm et al. 2021b). Therefore, the fight or flight response (increased sympathetic excitation) would be downregulated and let the individual relax and possibly extend the muscle further (greater stretch tolerance) (Behm et al. 2021b). The sympathetic nervous system affects the body globally. It is not muscle or region-specific (Cui et al. 2006, Donadio et al. 2002) and thus would impact non-local muscles (Ray and Mark, 1995). Decreased sympathetic (excitatory) activity could negatively impact the ability to fully activate the contralateral plantar flexors, adversely affecting NDL MVIC force output.

The psychological effects of a mentally fatiguing activity have also been suggested in NDL MVIC force decrements. It has been reported that mentally fatiguing cognitive tasks may hinder performance in subsequent physical activity (Dorris et al. 2012; Graham et al. 2014). It was shown that mental fatigue would lead participants to perceive the activity as more exhausting, thus quitting it earlier (Marcora et al., 2009). A cognitive demand accompanying every physical task may result in mental fatigue and increased rating of perceived exertion and impair performance in the subsequent task with non-local muscle (Halperin et al., 2015). It can thus be speculated that holding a stretch for 6 X 45s could be mentally fatiguing or at least boring and affect subsequent NDL force output.

Limitations

Although a priori statistical power analysis showed that 12 subjects were sufficient to achieve adequate statistical power, an increased number of participants, especially females, may have resolved the heterogenous sample issue and increased statistical power as well. Four measures in the analysis provided p values between p=0.06 and 0.19, which with more participants could have possibly decreased the variability contributing more significant results and a more in-depth examination of sex differences. Maintaining the stretching intensity for 45s and repeating the session six times was another challenge in this study. It would be very helpful if further studies used a method that was less researcher dependent to stretch the plantar flexors with a fixed intensity.

Conclusion

Static stretching improved the ROM in the stretched muscle. However, the stretched limb’s force was negatively affected for several minutes following the stretching protocol. The ROM improvement and force impairment (near significant) was transferred to the contralateral muscles. The lack of significant changes in spinal and corticospinal excitability may confirm that the afferent excitability of the spinal motoneurons and corticospinal excitability may not play a substantial role in non-local muscles ROM or force output.

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Appendix 1- Ethics approval letter

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Funding Source:	
Responsible Faculty:	Dr. David Behm School of Human Kinetics and Recreation
Title of Project:	*Possible Neurophysiological Mechanisms Underlying Non-Local Stretching Effects*

February 8, 2022

Mr. Saman Anvar
School of Human Kinetics and Recreation
Memorial University of Newfoundland

Dear Mr. Anvar:

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 the **MUN COVID-19 guidelines** (https://www.mun.ca/covid19/researchers/index.php), and 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 **February 28, 2023**. 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 R Drover

James Drover, Ph.D.
Vice-Chair, Interdisciplinary Committee on
Ethics in Human Research

JD/bc

cc: Supervisor – Dr. David Behm, School of Human Kinetics and Recreation