Upper- and Lower-body Pain Perception during DOMS: The Effect of Menthol-based Topical Analgesic Application

By

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COVID 19 statement.

As outlined by the School of Graduate Studies, this document will discuss the changes and impacts that the COVID-19 pandemic had on my research. Prior to COVID-19, I had planned to conduct research on changes in muscle EMG and cranks forces when performing upper-body Wingate tests with hands pronated and supinated and cycling in a forward and backward directions. I had ethical approval and all other research related activities completed in order to start my original thesis research. Because I could not get into my lab for a long period of time nor have access to participants, my supervisor and I have decided to change my thesis to a different topic and base it on data that was previously collected. The data is original and has not been published anywhere. I feel that this data set is enough to be worthy of a master's thesis.

Abstract

Menthol-based analgesic gels have been proven to alleviate delayed onset muscle soreness (DOMS) pain perception. The difference in pain pressure threshold (PPT) between the lower vs. upper extremities is not well understood nor is there much research illustrating the effect of the menthol-based analgesic on pain perception between these extremities during DOMS. Thus, the purpose of this study was to compare the upper- and lower-body's responses to pain 48 hours after DOMS induction and following menthol-based analgesic gel application. Fourteen participants were placed into two groups (placebo and experimental) and each performed 5 sets of 10 repetitions of eccentric contractions of the knee extensors and elbow flexors on an isokinetic dynamometer. PPT measurements were taken via a handheld algometer at baseline, immediately after and 48 hours after the DOMS protocol. After 48 hours the PPT was measured prior to and 15 minutes following gel application and every 5 minutes for 45 minutes thereafter. Statistical analysis revealed a significant difference for PPT in both groups between the knee extensors and elbow flexors ($p<0.001$). For experimental group both elbow flexors (48%) and knee extensors (26%) showed a decrease in PPT 48 hours after DOMS, but only the elbow flexors significantly reduced ($p=0.018$). The application of a menthol-based gel to the elbow flexors increased PPT at 30 minutes after gel application until the end of the test ($p=0.018$). The placebo group showed a significant reduction in their PPT values 48 hours after DOMS in both the elbow flexors (45%) and knee extensors (38%) ($p=0.018$). Unlike the experimental group the placebo group showed no change in PPT following its application. Pain perception is different in the lower and upper extremities and menthol-based analgesic reduced pain perception during DOMS.

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Due to the COVID-19, my thesis project has been completely changed. This thesis project could not have been completed without the help of many others. I would like to briefly acknowledge all the people who were kind enough to aid me in the completion of this thesis.

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List of Abbreviations

DOMS - Delayed onset muscle soreness

PPT - Pain pressure threshold

EIMD - Exercise-induced muscle damage

EMG - Electromyography

HP - Hydroxyproline

HL - Hydroxylysine

MDT - Mechanical detection thresholds

PT - Pain thresholds

VAS - Visual analog scale

EPT - Maximal eccentric peak torque

LEGS - Eccentric exercise of the knee extensors

ARMS - Eccentric exercise of elbow flexors

SR - Sarcoplasmic reticulum

MHC - Myosin heavy chain

ADLs - Activities of daily living

Mb - Myoglobin concentration

MVC’s - Maximum voluntary isometric contraction strength

TRP - Transient receptor proteins

MVMC - Maximum voluntary muscular contraction

Ca^{2+} - Calcium

Potassium - K^{+}

TRMP8 - Transient receptor potential member 8

POMC - Proopiomelanocortin

ROM - Range of motion

NSAIDs - Nonsteroidal anti-inflammatory drugs

ATP - Adenosine triphosphate

CPK - Creatine phosphokinase

CK - Creatine kinase

ACTH - Adrenocorticotropin

R.I.C.E - Rest, ice, compression, and elevate

Chapter 2 Review of Literature

2.0 Introduction

Following an unaccustomed physical activity, a sensation of discomfort may be experienced in the elite or novice athlete or non-athlete (Cheung, Hume, & Maxwell, 2003). This exercise-induced phenomenon is referred to as delayed onset muscle soreness (DOMS) which increases within the first 24 hours following exercise cessation, and peaks between 24 and 72 hours, subsides and ultimately disappears by 5–7 days post-exercise (Cleak & Eston, 1992, Talag, 1973). This exercise-induced phenomenon is referred to as delayed onset muscle soreness (DOMS) and is perhaps one of the most common and recurrent forms of work and sports injury (Cheung et al., 2003). DOMS is typically subclinical and is classified as a type I strain (Lewis, Ruby, & Bush-Joseph, 2012) and is associated with reduced range of motion, reduced strength, increased muscle stiffness, pain, and/or muscular tenderness (Gillis, Vellante, Gallo, & D'Amico, 2020). DOMS is most common at the beginning of the sporting season when athletes are returning to training following a period of reduced activity. It is also prevalent when athletes are first introduced to unaccustomed activities regardless of the time of year (Cheung, et al., 2003). DOMS is induced with unfamiliar, high-force muscular activity typically from an eccentric contraction as it causes micro-injury at a higher frequency and complexity (Armstrong, & Warren, 1993; Cheung, et al., 2003). Eccentric activity is described by an elongation of the muscle during force production. Therefore, the muscle is forced to lengthen, and active tension is produced if the external load exceeds the muscle's ability to actively resist the load (Stauber, 1989). The peak of soreness related to DOMS is at roughly 48 hours post-exercise (Stefanelli et al., 2019), making this an optimal time to determine perceived pain pressure.

Though the precise mechanism(s) of DOMS remains unknown, a number of theories have been proposed to explain the pain stimulus associated with DOMS including: lactic acid, muscle spasm, connective tissue damage, muscle damage, inflammation, enzyme efflux among others (Cleak & Eston, 1992; Gulick & Kimura, 1996). For example, cell membrane injury occurs during eccentric exercise by setting off an inflammatory response that causes prostaglandin (prostaglandin E2[PGE2]) and leukotriene synthesis. Prostaglandin E2 directly triggers the sensation of pain by sensitizing type III and IV pain afferents to the effects of chemical stimuli, whereas leukotrienes increase vascular permeability and attract neutrophils to the site of damage. The respiratory burst of the neutrophils produces free radicals, which can aggravate damage to the cell membrane. Swelling results from the movement of cells and fluid from the bloodstream into the interstitial spaces with inflammation and can contribute to the sensation of pain (Cheung, et al., 2003; Connolly, Sayers & McHugh, 2003). Based on the multiple theories of mechanisms underlying DOMS pain, various treatment methods for pain subsidization have been proposed.

Although there is no definite treatment for DOMS there are various type of treatment that have been presented to relieve DOMS symptoms (Cheung, et al., 2003). DOMS can be remedied using anti-inflammatory drugs, local anesthetics, and topical counterirritants, etc. (Stanos, 2007). Some of these treatments can be topical, including home cures such as ice application or menthol-based analgesic creams or gels. To be more precise, Johar et al (2012) compared the effect of the topical menthol to ice on pain, evoked tetanic, and voluntary force during DOMS. The results showed the topical menthol-based analgesic compared to ice, decreased perceived discomfort to a greater extent and permitted greater tetanic forces to be produced. The mechanism of action of menthol products such as Biofreeze™ is based on a cryotherapy effect. Cryotherapy using ice, cooling sprays, or topical analgesics, is often applied for alleviating

musculoskeletal injuries and/or pain (Swenson, Swärd & Karlsson, 1996). Overall, there are three mechanisms for localized cooling of the body, which are as follows: physical cooling, evaporative cooling, and chemically mediated cooling. Each mechanism includes a decrease in skin temperature stimulating thermoreceptors (transient receptor proteins; TRP) due to the cold source utilized on the skin (Page & Alexander, 2017).

The common muscles that are studied to assess DOMS are the knee extensors (Brown et al., 1997; Hortobagyi et al., 1998) and elbow flexors (Newham et al., 1988; Nosaka et al., 1991; Paddon-Jones and Abernethy, 2001). Various studies have found evidence showing the elbow flexors experience more DOMS compared to knee extensors. For example, Saka et al. (2009) showed male participants (mean age 25) who were actively inexperienced showed greater DOMS in the elbow flexors compared to knee extensors after completing maximal eccentric exercise. In comparison, Jamurtas and colleague (2005) showed there was no significant differences between DOMS of the elbow flexors and knee extensors in healthy untrained males (mean age of 21.2) following sub-maximal eccentric exercise. The possible reason could potentially be clarified by the intensity of the exercise being done, or by the fact that DOMS was evaluated in a different way in both studies. Although some research exists regarding DOMS in upper- and lower-body muscle groups, it is not understood if there is a difference in perceived pain sensation between elbow flexors and knee extensors following tha application of a menthol-based analgesic during DOMS and in addition the research is conflicting on pain perception differences between upper and lower body limbs in the absence of a topical analgesic. The following review will provide a deeper understanding of DOMS, topical analgesic, and perceived pain.

2.1 Delayed Onset Muscle Soreness

The benefits of regular exercise to improve health and prevent chronic disease are well known. However, unaccustomed exercises result in ambiguous skeletal muscle pain within hours or days during recovery after exercise cessation (Coombes & McNaughton, 2000). This muscular pain is associated with structural damage to contractile tissue and manifests as DOMS, which is a symptom of exercise-induced muscle damage (EIMD) and may be experienced in the elite or novice athlete (Smith, 1991). DOMS is defined as post-exercise muscle soreness; muscle pain and tenderness (Armstrong, 1984; Clarkson, Nosaka & Braun, 1992). DOMS is commonly an unpleasant feeling and can adversely affect muscle efficiency from reduction of voluntary effort, decreased range of motion, increased sensation of pain, as well as from the muscles' intrinsic loss of capacity to generate force (Clarkson & Hubal, 2002; Nosaka, 2007). Therefore, it is favorable to reduce exercise-induced DOMS not only in athletes but also in untrained individuals. The intensity of soreness and discomfort increase within the first 24 hours after exercise cessation, peaks between 24 and 72 hours, decreases and ultimately diffuse by 5–7 days post-exercise (Cleak & Eston, 1992; Talag, 1973). Usually DOMS is subclinical and designated as a type I strain and does not require treatment (Lewis, Ruby, & Bush-Joseph, 2012; MacIntyre et al., 1995). Exercise involving primarily eccentric muscle actions has the potential to produce greater DOMS to muscles than that involving mainly isometric or concentric actions, especially if the exercise is unfamiliar (Gillis, Vellante, Gallo, & D'Amico, 2018). An eccentric activity is the motion of an active muscle while it is lengthening under load (Bubbico & Kravitz, 2010), and it is specified by an elongation of the muscle during forceful contraction (Cheung et al., 2003). Therefore, if the load applied externally exceeds the muscle capability to resist then the muscle lengthens and tension increases (Stauber, 1989). During the eccentric contraction, cross bridges

are forced to be ruptured from higher tensile force (Stauber, 1989). Consequently, there is a higher tension per muscle fiber increasing the risk of injury to the muscle and myotendinous junction.

2.2. Mechanisms of delayed muscle soreness

Several theories have been suggested to clarify the pain stimulus associated with DOMS including lactic acid, muscle spasm, connective tissue damage, muscle damage, inflammation, and enzyme efflux among others (Cleak & Eston, 1992). Although, it is beyond the scope of this study to give a comprehensive discussion for each theory, a brief description of the mechanisms underlying DOMS will be discussed.

2.2.1 Lactic Acid Theory. Accumulation of lactic acid and other toxic metabolic waste products in the muscle is commonly believed to be one of the reasons of pain sensation during DOMS (Armstrong, 1984). Schwane and colleague (1983), examined the hypothesis that DOMS following running is related to the lactic acid production during the exercise. Concentration of blood lactic acid was assessed before and during a 45-minute treadmill run, at 0% and -10% incline. Blood lactic acid concentration and individual sensations of muscular soreness were also measured at intervals for 72 hours after the runs. Results showed lactic acid concentration was significantly increased during running at the 0% incline, but participants reported no significant post-exercise muscular soreness. In addition, lactic acid was never raised in downhill runners, but individual experienced significant delayed onset soreness, indicating that lactic acid is not associated with exercise-induced DOMS. Consequently, lactic acid may contribute to the acute pain related to acute muscle pain and fatigue following intense exercise, however, it can not be ascribed to the delayed pain which is reported 24–48 hours after exercise cessation (Dick & Cavanagh, 1987; Cazorla, Petibois, Bosquet & Léger 2001).

2.2.2 The Muscle Spasm Theory. This hypothesis, initially defined by de Vries (1961), suggested that exercise could result in the compression of local blood vessels, ischemia and the accumulation of the pain-related substances. In fact, resting muscle activity increased after eccentric activity when studied with unipolar electromyography which indicated a tonic localised spasm of motor units (De Vries, 1961). This muscle hyperactivity sustained by a vicious cycle as additional stimulation of pain nerve endings triggered continuous reflex muscle spasms and long-lasting ischaemic conditions (Bobbert, Hollander & Huijing, 1986; De Vries., 1967; Graven-Nielsen & Arendt-Nielsen, 2008). However, to understand muscle spasm theory, several studies have been conducted, with conflicting results. For example, in one study it has been postulated that individuals with DOMS showed higher electrical activity of the muscle as recorded by surface electromyography (EMG) (de Vries, 1966). Another study indicated an increase in EMG activity in muscles following eccentric exercise, however the activity magnitude was not associated to muscle soreness perception (McGlynn et al., 1979). Conversely, when a bipolar EMG technique (decreased artifact) was applied, the increased activity was not detected (Abraham, 1979). The application of unipolar or bipolar EMG techniques may proved more information as to the effect of muscle spasms on DOMS.

2.2.3 Connective Tissue Damage Theory. It is obvious that DOMS result from muscle overuse. In other words, any activity in which muscle produce greater forces than normal, or exerts force over a longer period of time than normal, is able to induce DOMS. Muscle is comprised of various sheathes, endomysium, perimysium and epimysium, which help maintain the muscle structure. The endomysium is possibly the most crucial in relation to the idea of damage theory, as it surrounds each myofibril and may be linked with nearby myofibrils (Stauber, 1989). This elevation of muscular activity could increase tension in contractile and

elastic elements making physical damage to the structural components (Tiidus & Ianuzzo, 1983). The connective tissue content and composition is different among muscle fibre types. Type I (slow twitch) fibres show stronger structure than type II (fast twitch) fibers (Stauber., 1989). As a result, type II muscle fibers could be more vulnerable to connective tissue damage as a result of stretch-induced exercises (Stauber, 1989) and extreme stretch which can lead to muscle soreness (Hough, 1902). By examining the urinary excretion, more specifically hydroxyproline (HP) and hydroxylysine (HL), in humans after exercise cessation reveals an elevated catabolism of collagen. It is an evident that the presence of HP and HL amino acids, which are distinctive components of mature collagen, can only happen when there is a dilapidation of collagens. Thus, the presence of collagen components from muscle connective tissue, in urine, is believed to be adequate proof of overuse or strain damage to the muscle (Stauber, 1989). However, excretion of HP and HL can indicate either increasing of collagen synthesis or its degradation. As a result, the exact mechanism resulting in an increase in HP and HL remains unclear (Stauber., 1989).

2.2.4 The Muscle Damage Theory. Unaccustomed exercise causes a disruption to the muscle's homeostasis. Although muscle tissue is tremendously plastic, devastating changes to the fine muscle structure could happen through atypical demands (Jockusch & Pette, 1990; Hoppeler, 1986). Muscle damage theory first proposed by Hough (1902) stated that delayed discomfort and pain is strongly related to mechanical tension in active muscles. The pain and discomfort after eccentric exercise completion can rise from contractile component rupture of the muscle tissue, more specifically the z-line (Armstrong, 1984; Jones, Newham, Round & Tolfree, 1986; Fridén, Seger & Ekblom, 1988; Friden, Sjöström & Ekblom, 1981; Newham, Jones & Edwards, 1983). At the microscopic level, most of the structural abnormalities occur at the z-line (Fridén, et al., 1988) and sarcomere architecture (Newham, Jones & Edwards, 1986), as a result

of increasing tension per myofibrillar. Mechanical disturbance to the structural components rises, especially among the fast twitch fibres which have the thinnest and weakest z-lines (Cheung et al., 2003). During sensation of pain, nociceptors located in the muscle connective tissue as well as arterioles, capillaries and the musculotendinous junction are stimulated. In addition, Creatine kinase (CK) is considered as an excellent indicator of muscle damage, because this enzyme is observed almost entirely in both skeletal and cardiac muscle (Clarkson, Byrnes, Gillisson & Harper, 1987). Therefore, z-lines disruption and sarcolemma damage will enable the diffusion of soluble muscle enzymes, like CK, into the interstitial fluid (Cheung et al., 2003). However, it is questionable that such indicators of muscle damage truly result in production of pain, as the vast majority of researchers have reported a discrepancy between the time of the damage and the pain occurrence, with delayed peak enzyme efflux happening 4-7 days after exercise, and DOMS peak 24-72 h post-exercise (Komi and Viitasalo, 1977; Clarkson et al, 1986b; Newham and Jones, 1985; Newham et al, 1983a, 1986). Consequently, the muscle damage theory may be accepted only as an incomplete justification for DOMS onset.

2.2.5 The Inflammation Theory. Studies have shown that DOMS may occur as a result of inflammation responses following repetitive eccentric muscle activity (Evans et al., 1986; Francis & Hoobler, 1987). Proteolytic enzymes, which are available in the muscle, are released following an injury initiating the degradation of lipid and protein structures of cells (Hasson et al., 1987). The rapid breakdown of injured muscle fibres and connective tissue in addition to the accumulation of bradykinin, histamine and prostaglandins, attracts monocytes and neutrophils to the injured site (Hasson et al., 1987). The inflammatory response is believed to start within the first few hours and remain up to 24-48 hours after exercise cessation (Armstrong, 1990). In the meantime, by increasing permeability of small blood vessels following eccentric exercise, an

influx of fluid into the injured muscle would occur (Smith, 1991). Eventually, the osmotic pressure is elevated, and pain is produced when group IV sensory neurons are activated (Friden et al., 1986). However, muscle edema progression and increased intramuscular pressure could be associated with the delayed onset response of muscle soreness perception (Friden, 1984; Friden, Kjörell & Thornell, 1984). Some evidence showed that only peak edema levels seem to coincide with peak muscle soreness (Gulick & Kimura, 1996; Lightfoot, Char, McDermott & Goya, 1997). This might possibly explain why some researchers prefer to name this theory as the Tissue Fluid Theory instead (Gulick & Kimura, 1996). Nevertheless, Smith (1991) and Armstrong (1984) have both asserted that monocytes which turn to macrophages, accumulate at the injury site, and produce substances which stimulate the type III and IV nerve endings. Whether it is edema formation or inflammatory cell infiltration underlying DOMS remains debatable.

2.2.6 The Enzyme Efflux Theory. The enzyme efflux theory is based on the assumption that several collagen and protein metabolites are released into the extracellular spaces (Armstrong, 1984). The eccentric exercise intensity and duration affect the permeability of membrane and also the amount of fiber degeneration. One study showed that an indirect marker of muscle damage, Creatine phosphokinase (CPK), increases significantly following eccentric muscle activity (Schwane, Johnson, Vandenakker & Armstrong, 1981). However, it has been postulated that there is mismatch between peak CK levels and peak DOMS which do not always occur at the same time (Schwane et al., 1981). Calcium, which is usually stored in the sarcoplasmic reticulum, accumulates in the damaged muscles following sarcolemma injury (Armstrong, 1984). Although, calcium has been known to activate proteases and phospholipases which is the reason for the protein degradation (Fridén, Seger, Sjöström & Ekblom, 1983), the

application of a calcium antagonist like verapamil as an example has been rejected to substantially reduce DOMS (Lane, Turnbull, Welch, & Walton, 1986). In addition, inhibition of cellular respiration at the mitochondrial level results in slowing down the regeneration of adenosine triphosphate (ATP), which is necessary for returning calcium back into the sarcoplasmic reticulum (Cheung, et al., 2003). Consequently, degradation of muscle protein at the z-lines rises causing chemical stimulation of pain nerve endings (Cheung, et al., 2003). Besides muscle soreness, structural damage may happen in eccentrically activated muscles and connective tissue resulting in reduction in muscle function, performance and/or a less optimal training intensity (Rowlands, Eston & Tilzey, 2001).

The overall conclusion among researchers is that one particular theory cannot explain the onset of DOMS. There is overwhelming evidence which shows agreement or disagreement for each theory. It is feasible that DOMS is initiated by a combination several hypothesized mechanisms. Hence, treatments could also consist of a combination of techniques. These treatments have focused on reducing the inflammation, or edema, tissue damage, and/or reducing these factors which are thought to contribute to tonic muscle spasm or pain.

2.3 Treatments for Delayed Onset Muscle Soreness

Although there is no prevalent treatment for DOMS, there are various suggested treatment techniques which have been introduced to alleviate DOMS symptoms (Cheung et al., 2003). The purpose of treatment is to reduce pain and regain the maximum function of the muscles that were activated during the eccentric portion of the exercise as fast as possible. The treatments that have been taken into consideration involve anti-inflammatory medications, submaximal concentric activity, stretching, electrical stimulation, homeopathic herb, cryotherapy, and a local anesthetic (Gulick & Kimura, 1996; Stanos, 2007).

2.3.1 Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). Nonsteroidal anti-inflammatory drugs (NSAIDs) act to inhibit prostaglandin synthesis via the cyclo-oxygenase pathway at the site of inflammation (Searle, 1993). Several studies have indicated that the consumption of anti-inflammatory medication has not been effective on either alleviating exercise-induced myalgia or muscle soreness perception (Donnelly, Maughan, & Whiting, 1990; Gulick, Kimura, Sitler, Paolone, & Kelly, 1996; Headley, Newham, & Jones, 1986; Kuipers, Keizer, Verstappen, & Costill, 1985). However, studies including ibuprofen (Donnelly et al., 1990; Donnelly, McCormick, Maughan, Whiting, & Clarkson, 1988; Hasson et al., 1993), dexamethasone (Hasson, Wible, Reich, Barnes, & Williams, 1992) and aspirin (Riasati, Moghadasi, Torkfar, Shirazinejad & Arvin, 2010), have exposed contradictory results. For example, Riasati et al. (2010) have examined the effect of aspirin supplementation on DOMS after an eccentric exercise on sixteen healthy female participants. The subjects were ascribed to either an experimental (200 mg of aspirin; n=8) or a placebo group (Same dosage of lactose; n=8) applying a double-blind research design. Results indicated no difference between groups for thigh circumference and range of motion (ROM) before, immediately, 24 and 48 hours after the eccentric exercise. Serum CK levels and pain increased ($p<0.05$) in both groups immediately after the eccentric exercise and increased to maximum at 48 hours after the eccentric exercise. The aspirin supplementation reduced ($p<0.05$) the serum CK levels and pain in comparison to the placebo group at 24 and 48 hours following the eccentric exercise, indicating that aspirin supplementation can be influential to reduce DOMS induced by eccentric exercise.

2.3.2 Submaximal Concentric Muscle Exercise. The common believe that muscle soreness can be treated by "working it out" has been around for many years (Hasson, Barnes, Hunter, & Williams, 1989). Submaximal concentric muscle exercise does not produce tissue

damage and cause considerably lower intramuscular pressure than eccentric muscle activity (Friden, 1984). On the contrary eccentric muscle exercise increases pressure and transfer intravascular fluid into the interstitial spaces within the exercised muscle fibers (Burovykh, Samtsova, & Manuilov, 1989). Previous research showed conflicting results using concentric exercise as treatment. Hasson (1989) found a reduction in muscle soreness and an increase in muscle function after submaximal concentric activity. This indicated that limb edema acts as a limitation factor, however neither limb volume nor limb girth was measured. In addition, Gulick et al. (1996) assessed limb edema and reported a preliminary increase in girth volume that may be due to the local metabolic response, nevertheless the edema abated over the next 72 hours whereas DOMS increased. Consequently, edema and DOMS do not seem to be related in cause and effect (Burovykh et., 1989; Hill & Richardson, 1989).

2.3.3 Electrical Stimulation. The implementation of small electrical stimulation has been clinically employed to accelerate the healing process of wounds and fractures (Real, 2019). However, the efficiency of this kind of treatment on musculoskeletal damages is less recognized as only a few investigations have examined the effect of microcurrent (Kim, Hwang, Lee, Kim & Cho, 2019), interferential electrotherapy (Motto, 1997), high-volt pulsed current electrical stimulation (Butterfield, Draper, Ricard, Myrer, Schulthies & Durrant, 1997) or transcutaneous electrical nerve stimulation (TENS; Denegar, Perrin, Rogol & Rutt, 1989) on DOMS. Butterfield et al. (1996) stated that high voltage pulsed current electrical stimulation applied for 30 minutes at 125 pps was ineffective for decreasing DOMS, or increasing the ROM and strength loss related with DOMS. On the contrary, in 1988, Denegar found a significant decrease in pain perception and an increase in elbow extension ROM in eight female subjects 48 hours after DOMS-inducing exercise (elbow flexors, repeated eccentric contractions). It has been suggested

that TENS with low frequency and long pulse duration can result in the release of beta-endorphin from the anterior pituitary gland (Weerapong, Cheung, Hume & Maxwell, 2004). Beta-endorphin releases the pre-cursor hormone propiomelanocortin (POMC) with adrenocorticotropin (ACTH; Schmitz, Martin, Perrin, Iranmanesh, & Rogol, 1997). Consequently, cortisol synthesis and release from the adrenal cortex stimulates gluconeogenesis, increases glucose consumption, protein synthesis, mobilisation of fatty acid, and reduces acute and chronic inflammatory responses (Denegar, 1988; Baxter, 1979).

2.3.4 Stretching Exercise. Pre or post static stretching exercise has been suggested as a preventive method for DOMS because it is supposed to alleviate the muscle spasm explained in de Vries' muscle spasm theory (Wessel, & Wan, 1994) as well as disperse the edema accumulatation following tissue damage (Bobbert, Hollander & Huijing, 1986). De Vries (1961, 1967) displayed a reduction in pain after static stretch exercise, but later studies have failed to replicate a decrease in pain following stretching. Abraham (1977) demonstrated a small decrease in pain lasting only 1-2 min after stretch exercise, however, McGlynn and colleagues (1979) observed no reduction in pain. Two other studies, exploring stretch exercise as a preventive DOMS strategy, failed to show any changes in perceived pain in either intervention or control group (Buroker & Schwane, 1989; High, Howley, & Franks, 1989). In addition, studies have examined the effect of stretching prior to (Johansson, Lindström, Sundelin & Lindström, 1999; Wessel, & Wan, 1994), following (Buroker, & Schwane, 1989; Maxwell, Kohl, Watson, & Balnave, 1988) and prior to and following eccentric contraction (Lund, Vestergaard-Poulsen, Kanstrup, & Sejrsen, 1998) that resulted in no preventive outcome on DOMS. The possible reason might be due to the fact that stretching less than 30 seconds could be restricted by the stretch reflex response (Cheung, et al., 2003). In fact, by employing stretch in muscle, the muscle

spindles are also stretched resulting in sensory impulses being sent to the spinal cord to notify that a muscle is being stretched. Meanwhile the spinal cord is sending efferent feedback signals to the muscle to be contracted. For stretches over 6 seconds, the Golgi tendon organs send out sensory impulses to the spinal cord triggering a relaxation reflex to the antagonist muscle. This relaxation reflex allows the agonist muscle to stretch while its antagonist is relaxed, minimizing the risk of muscle damage. However, a short duration of stretch exercise would not give enough time to the Golgi tendon organs to respond to the length change as well as tension of the muscles.

2.3.5 Homeopathy. Homeopathy is based on the idea that "like cures like." That is, if a substance causes a symptom in a healthy person, a very small amount of the same substance, may cure the illness to the given person. In theory, a homeopathic dose enhances the body's normal healing and self-regulatory processes (Cheung, et al., 2003). The most popular homeopathic medicine of choice is Arnica (which can be administered by tablet, massage oil, or cream) due to its analgesic, antibiotic, and anti-inflammatory properties (Gulick et al., 1996, Vickers, Fishe, Smith, Wyllie, & Lewith, 1997). However, recent investigation, which have studied the effect of arnica on DOMS, have indicated minimum benefit for it (Gulick et al., 1996; Vickers et al., 1997). For example, in a randomised double-blind study, results showed no statistical differences in DOMS between the control group and experimental group following bench stepping exercise (Vickers et al., 1997). Gulick and colleagues (1996) stated comparable results between participants, who took three pellets (50 g) of arnica (sublingual form) every eight hours for three days following DOMS induced eccentric contractions of the forearm extensor muscles. Similarly, when arnica was administered in an ointment form (4%, 0.5g dose, rubbed into the skin every 8 hours for 3 days) the findings were again inconclusive when considering the

amount of muscle soreness compared with a placebo group (Gulick et al., 1996). Some experiments have been performed to measure the effects of various substances on prevention of muscle damage, like fish oil (Lenn et., 2002) ethanol (Clarkson, 1992), herbs (Vickers et al., 1997) and pollen extract (Krotkiewski, Brzezinska, Grimby, & Palm, 1994) and all were unsuccessful for reducing muscle damage (Weerapong et al., 2004).

2.3.6 Cryotherapy. The primary treatment advised for traumatic soft tissue injuries is R.I.C.E (rest, ice, compression, and elevation). In fact, the superficial use of ice reduces the temperature of the skin, muscle and other tissue structures surrounding the joints (Brukner, Bradshaw, Khan, White & Crossley, 1996). A reduction in tissue temperature evokes cutaneous receptors to excite the sympathetic adrenergic fibres resulting in the local arterioles and venules constriction (Cheung et al., 2003). Cryotherapy (cold therapy) has been known to be beneficial in amending soft tissue injury through its ability to reduce the circulatory response, inflammation, swelling, and intravascular pressure (Bierman, 1955; Knight, 1976; Lehmann, Warren & Scham, 1974). The primary decrease in ROM after cryotherapy has been ascribed to an increase in intensity of collagen stiffness (Fox, 1961; Lehmann, 1990), and the temporary decrease in muscle soreness after cryotherapy is assumed to be due to the reduction in excitability of the free nerve ending (Gulick & Kimura, 1996; Lehmann, 1990). However, studies to date have shown conflicting results on the magnitude of decreased muscle soreness or the acceleration of soreness recovery following cryotherapy application (Gulick, et al., 1996; Gulick & Kimura, 1996; Kokkinidis, Tsamourtas, Buckenmeyer & Machairidou, 1998; Verducci, 2000). Paddon-Jones & Quigley (1997), examined whether a post-exercise cryotherapy protocol could facilitate recovery of the elbow flexor strength and reduce DOMS severity following eccentric exercise in eight college aged resistance-trained males. The participants performed 64 eccentric elbow flexions on

each arm. One arm was exposed to five, 20-minute immersions in a 5 ± 1 °C ice-water bath interspersed by 60-minute rest periods. The non-immersed arm acted as the control. No significant difference between the immersed and control arms was noted for any variable. Results indicated that the application of cryotherapy immediately after traumatic eccentric exercise may not have the same therapeutic effects generally ascribed to cryotherapy following damaging muscle injury. On the contrary, another study examined the effectiveness of cryotherapy in the prevention and treatment of DOMS. Twenty-one participants were instructed to perform eccentric, free-weight curl exercises until fatigue using a 10-lb dumbbell at a tempo of one second for the concentric phase and three seconds for the eccentric phase to induce DOMS. The experimental group undertook 30-minute ice application, which were processed instantaneously after exercise, 2, 4, 6, 24, and 48 hours afterward. There was a significant difference between experimental and control groups in perceived pain. Additionally, no significance difference was reported between groups for the recovery of isometric force production of the biceps. The pattern of data implies that the application of ice to alleviate symptom of DOMS has been effective in reducing perceived pain 24-96 hours after activity (Day & Ploen, 2010). Also, Cryotherapy was reported to be ineffective in reducing the functional deficits associated with DOMS and may affect the adaptation and repair of the tissue responsible for the symptoms of DOMS (Isabell, Durrant, Myrer & Anderson, 1992). However, Cryotherapy other than its analgesic effect, is an accepted modality for acute injury management for recovery from DOMS, but limited research evidence is available to guide management of the condition (Snyder, Ambegaonkar, & Winchester, 2011).

2.3.7 Menthol. Local anesthetics applied topically can alleviate pain of neuropathic origin by decreasing ectopic discharges of superficial somatic nerves in areas of localized pain

(Heyneman, Lawless-Liday & Wall, 2000). It binds to atypical sodium channels, which are excessively regulated in injured peripheral nerves, thus controlling unusual instinctive activity that may initiate or maintain neuropathic pain status. This nonsurgical treatment, which contains menthol, results in a cooling sensation and are reported to act as a counterirritant to decrease the pain sensation (Yosipovitch, Szolar, Hui & Maibach, 1996). Menthol is a terpene compound that when employed to the skin in different doses triggers anti-nociceptive and counterirritant sensations, causing in reducing sensation of burning discomfort, muscle soreness, and joint pain (Galeotti, Mannelli, Mazzanti, Bartolini, & Ghelardini, 2002). Menthol generates feelings of cold via transient receptor potential member 8 (TRMP8). TRMP8 are found mostly within thermosensitive neurons, which respond to reductions in temperature, are also sensitive specifically to menthol (Behrendt, Germann, Gillen, Hatt, & Jostock, 2004). Normally, TRPM8 allows the flow of ions, such as calcium (Ca^{2+}) or potassium (K^{+}), through cell membranes where they are mainly located (Reid, 2005). TRPM8 acts as a neuronal sensor of cold temperatures and is necessary for receiving input regarding innocuous cool and noxious cold sensations (Bautista et al., 2007; Dhaka, Murray, Mathur, Earley, Petrus, & Patapoutian, 2007). Operation of calcium imaging techniques has revealed that in accordance with the application of menthol to cloned TRPM8 cells, a heavy intracellular influx of calcium ions triggered neural depolarization as a result of the opening of non-selective calcium permeable cation channels (Bautista et al., 2007; Reid, 2005). This escalation in sensitization of the thermosensitive neurons leads to the experiences of coldness with application of topical menthol. In addition, incitement of these thermosensitive neurons has been linked to an analgesic effect. Stimulating of afferent thermosensitive neurons by mild cooling or the utilization of menthol have been discovered to inhibit the nociceptive afferent neurons and the dorsal-horn neurons which are responsible for

conducting pain impulses and noxious stimuli to the thalamus (Proudfoot et al., 2006). Haeseler et al. (2002) studied the analgesic effect of menthol by applying electrical stimulus to human skeletal muscle tissue after application of menthol on selected the tissues. At several menthol application strengths, inactivated sodium channels were evaluated to show the effect on depolarization. It was revealed that the menthol blocked the alpha subunit of voltage gated sodium channels, resulting in the hyperpolarization of the neuron cell membrane and blocking the pain stimuli. Consequently, this study determined that the utilization of menthol may possibly have an analgesic effect through employing an inhibitory gate control over nociceptive inputs. In support of their work Johar and colleagues (2012) have shown that a menthol based topical analgesic was more effective than ice for lessening DOMS-induced symptoms of pain in the elbow flexors. There have not been many studies determining the effect of different dosages of topical menthol application on pain. However, Topp et al. (2014) examined the effects of two different doses of menthol (3.5% menthol gel, 10% menthol wipe) on blood flow and arterial diameter before and after an acute bout of three isokinetic maximum voluntary muscular contractions (MVMC) of the quadriceps and hamstrings. The results showed that the application of either 3.5% menthol gel or 10% menthol wipe to the thigh decreases blood flow in the ipsi- and contra-lateral popliteal arteries after completion of a high intensity-short duration bout of exercise. The application of 3.5% menthol gel or a 10% menthol wipe decreased arterial popliteal diameter on the side receiving these treatments but not in the same vessels on the contra-lateral side. A possible explanation for the current result was that the enhanced adrenergic stimulation caused by the sympathetic reflex which might be associated with menthol application stimulation of TRPM8 thermosensitive neurons comparable to the effect of tissue cooling (Bailey, Eid, Mitra, Flavahan & Flavahan, 2004; Hodges, Zhao, Kosiba & Johnson, 2006).

2.4 Biofreeze™

Biofreeze® topical analgesic (Performance Health, Akron, OH) is a form of popular topical analgesic normally used to decrease the musculoskeletal pain like sprains, strains, and bruises (Page, & Alexander, 2017). Ever Since 1991, Biofreeze has been suggested to customers through healthcare providers (physical therapists, chiropractors, etc.). The mechanism associated with Biofreeze pain reduction is deemed to be through the cryotherapy method. As mentioned earlier, cryotherapy is possible through application of ice, cooling sprays, or topical analgesics, in order to alleviate musculoskeletal damage and pain. Broadly speaking, there are 3 mechanisms for confined cooling of the body, which are named: physical cooling, evaporative cooling, and chemically mediated cooling. A reduction in skin temperature and/or stimulation of cold-sensitive receptors are common with each mechanism as a result of applying cold substances on skin (Page, & Alexander, 2017). Thermal receptors or transient receptor proteins (TRP) which are placed in the skin on subcutaneous nerves and blood vessels, are being activated in response to a cold stimulus and then send a "cold" signal to the thalamus via the spinothalamic tract where a cold sensation is perceived. This cold sensation provokes a sympathetic response in order to preserve tissue temperature and protect tissues from extreme cold. Different subtypes of TRP receptors react to different temperature ranges and TRPM8 is one of them. TRPM8 is sensitive to cold temperatures that are felt during utilization of ice or menthol to the skin and it responds to temperatures fluctuating between 30°C and 8°C. Alongside thermo sensitivity, the TRPM8 channel is also sensitive to menthol, which is an active ingredient in Biofreeze (Bautista et al., 2007). The local impacts of cryotherapy are as follows: reduced nerve conduction velocity; reduce sensation; reduced pain threshold; reduced skin temperature, arteriolar vasoconstriction, superficial vasodilation; and reduced tissue metabolism. When the skin is subjected to prolonged

low temperatures, it can lead to side effects, such as pain, numbness, nerve damage, and frostbite, that are related with directly applied ice on target area (Galeotti et al. 2002).. The cryotherapy mechanism of Biofreeze can be achieved by stimulating these certain cold receptors in the skin (Patel, Ishiuji, & Yosipovitch, 2007). The localized cooling by Biofreeze can be also achieved via the evaporation of alcohol and menthol. The reason behind it is that alcohol has a lower heat point evaporation and consequently it would rapidly reduce the skin temperature, resulting in stimulating the cold receptors (McLeay, 2011).

Several studies have demonstrated Biofreeze™ as an effective method in pain reduction, both clinically and statistically, in numerous musculoskeletal disorders (Page, & Alexander, 2017). For instance, Olive and colleagues (2010) determined a blood flow reduction in brachial artery when the efficiency of the Biofreeze application was compared to an ice pack over the forearm. Results indicated that both modalities significantly decreased blood flow by approximately 35% within the first 60 seconds of application. Two other studies showed that the superficial cooling with application of ice to the knee can decrease arterial blood flow by 38% in less than 5 minutes (Ho, Coel, Kagawa, & Richardson, 1994; Ho, Illgen, Meyer, Torok, Cooper, & Reider, 1995). Consequently, the decline in blood flow with Biofreeze is quantitatively comparable to the decrease in blood flow with ice application. In addition, patients have reported less discomfort with application of Biofreeze in comparison with ice (Bishop, Greenstein & Topp, 2011; Topp, Ledford, & Jacks, 2013). Traditionally direct application of ice packs on damaged areas to reduce pain may result in painful side effects such as numbness, burns, frostbite, and decreased performance, however, Biofreeze provides the advantages of cryotherapy without the side effects of ice (Topp et al., 2013; Topp, Winchester, Mink, Kaufman, & Jacks 2011). The dose of Biofreeze®, is usually based on the estimation that the

average skin surface area for example over the biceps brachii is approximately 400 cm^2 and the recommended dosage of Biofreeze® of 1 ml per 200 cm (Johar et al., 2012). To be more precise, in 2016, Craighead & Alexander sought to illustrate the dose-response curve between menthol concentration and changes in cutaneous vascular conductance (CVC) and skin blood flow (SkBF). as a part of their study. Seven different gels including 0.04%, 0.4%, 1%, 2%, 4%, 7%, and 8% menthol were applied on 10 participants. Four 15 cm^2 regions for gel application have been identified on the ventral forearm with volumes of 0.25 ml of each gel. The result indicated that topically applied menthol based gel, dose-dependently increases blood flow in the cutaneous microvasculature. This increase in blood flow is mediated, in-part by sensory nerves and endothelium derived hyperpolarizing factors (EDHFs; Craighead & Alexander, 2016).

2.5 Measuring Delayed Onset Muscle Soreness

There are numerous reliable and valid methods to measure DOMS (Connolly, Sayers, & McHugh, 2003). However, to quantify the level of muscle soreness is a challenge due to the subjective nature of pain (O'connor & Cook, 1999). The main methods for identifying and quantifying DOMS are pressure pain threshold (PPT), ROM, and self-reported perceived muscle soreness through different pain scales such as a visual analog scale [VAS] (Zainuddin, Newton, Sacco & Nosaka, 2005), verbal rating scale (Abraham, 1977), numerical rating scale (Jones, Newham & Clarkson, 1987), and descriptor differential scale (DDS; Hilbert, Sforzo & Swensen, 2003) graphic rating scale (GRS; Allen, Mattacola & Perrin,1999). DDS provides a more complete assessment of pain than simple scales, such as visual analog, numerical, or verbal, because it measures both the sensory and emotional aspects of pain. For example, Hilbert et al (2003) investigate the physiological and psychological effects of massage on delayed onset muscle soreness (DOMS) using DDS which contains two sets of 12 descriptor items that

measure both the intensity and unpleasantness of soreness. Results showed massage did not have an impact on the unpleasantness of soreness, which boosts the supposition that this dimension of the DDS scale does not figure prominently in DOMS. Additionally, the use of VAS to assess musculoskeletal pain has been reported to be reliable (Boonstra, Preuper, Reneman, Posthumus & Stewart, 2008; Price, McGrath, Rafii & Buckingham, 1983). VAS is a single-item measure, that is, an instrument measuring the whole construct at once. A VAS is easy to use and therefore applicable to a variety of practice and research.

While bias can devalue self reported measures, PPT – the minimal pressure (force) required to induce pain had been extensively studied to assess pain (Fischer, 1987). Connolly et al. (2003), provided a table in a review of DOMS treatment literature outlining the damage information, cost, difficulty of measure, and reliability of nine different indices of muscle damage. PPT has been shown to be reliable and valid when measuring perceived pain during DOMS (Connolly, Sayers, & McHugh, 2003).

2.6. Eccentric Exercise

It is well stablished that eccentric muscle activities are related to muscle damage (Clarkson, 1992). An eccentric contraction is defined as a contraction in which a muscle elongates as it exerts force (Balnave & Thompson, 1993). Eccentric muscle contractions create more tension per cross-sectional area of active muscle than concentric contractions (Armstrong, 1984). Negative work is performed by a muscle when it attempts to stop the joint movement, as it reduces speed of the body during walking or running. Eccentric muscle activity is an essential factor in activities of daily living (ADLs) like downhill walking or stairs, squatting, lowering an object, and overall coordinated activity (Gregory, Morgan & Proske, 2004). More generally during a movement the joint is controlled by an agonist /antagonist muscle pair, in which one

will be shortening and the other will be lengthening. Athletes utilize eccentric muscle force for performing different tasks such as limb deceleration when throwing a ball and for control in lowering a barbell. Talag (1973) revealed the association between muscle soreness and different type of muscle contractions (Figure 1). The results indicated that there was no significant difference observed between concentric and isometric muscle contraction; however, eccentric muscle activity caused significant muscle soreness that peaked 48 hours following exercise.

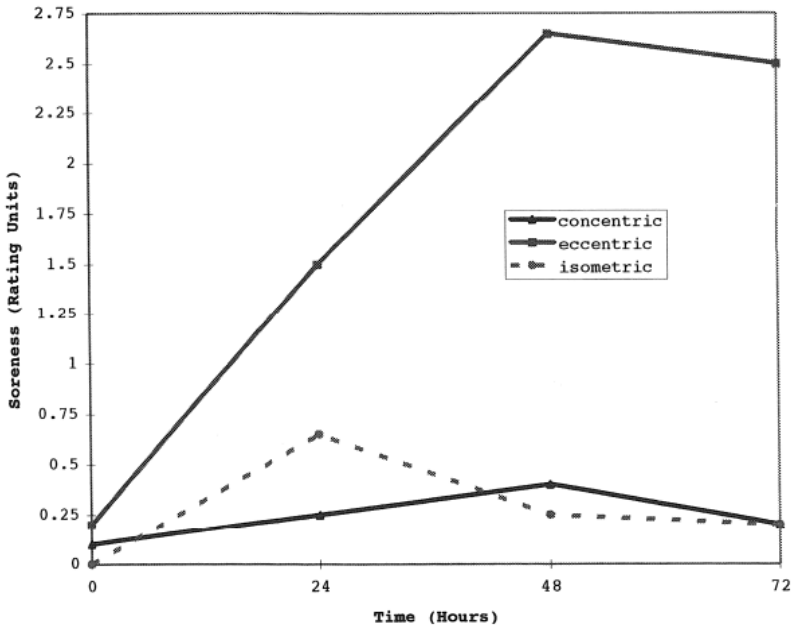

Figure 1: The effect of different types of muscle activity on muscle soreness over time (Talag, 1973)

As discussed, DOMS is normally related to unaccustomed, high-force muscular work and is precipitated by eccentric actions (Armstrong & Warren, 1993). Eccentric contraction is identified as the type of muscle contraction that induces the highest DOMS in the entire body, but the reason for this is not well understood (Vila-Chã, Hassanlouei, Farina & Falla, 2012). Various studies have indicated that maximal force (Prasartwuth et al., 2005; Crameri et al., 2007), power (Sargeant & Dolan 1987; Crameri et al., 2007), and force steadiness (Bachrathy & Mészáros, 2010; Saxton et al., 1995; Semmler et al., 2007) are decreased after severe eccentric

activities. The changes in these components have generally been related to morphological alterations of the muscle such as muscle fibre degeneration (Armstrong et al., 1983; Friden et al., 1983; Newham et al., 1983), muscle fibre necrosis (Nosaka and Clarkson, 1996; Friden and Lieber, 1998), inflammation (Jones et al., 1986; Round et al., 1987) and repair (Ebbeling and Clarkson, 1989; Clarkson and Sayers, 1999), which results in DOMS and increased muscle stiffness (Jones et al., 1987; Howell et al., 1993; Yu and Thornell, 2002).

The first proposed evidence of the morphological changes in eccentric muscle damage was provided by FrideÂn et al. (1981). They investigated muscle biopsies from humans who had run down a flight of stairs resulting in DOMS. Electron microscopy results showed high tensile forces generated during eccentric muscle activity induced disruption and disorganization of structural proteins in muscle fibres, particularly at the weakened z-lines. These changes could be restricted to a single myofibril or could include many neighboring myofibrils of affected muscle biopsies. In a few hours, there was a significant increase in neutrophils circulation at the damaged site (FrideÂn et al. (1981). Following repeated eccentric activities, it is hypothesized that the number of disturbed sarcomeres develops, until a maximal point where membrane damage happens (Talbot & Morgan, 1996). Damage to elements of the excitation-contraction coupling machinery also becomes apparent. In a study done by Takekura and colleagues (2001), rats that were exercised by downhill running, displayed a number of ultrastructural abnormalities including more longitudinal t-tubule segments, changes in disposition of triads, caveolar clusters and disarrangement of multiple t-tubule segments resulting in changes in Ca^{2+} release in their forelimb muscles. Warren et al. (2001) support the damage of the excitation-contraction coupling phase and report that 75% or more of the decrease in force production following eccentric

exercise is due to the excitation-contraction coupling, whereas Proake and Morgan (2001) found that only very small component occurs at the level of the sarcomere.

In addition, it has been accepted that the occurrence of overstretched sarcomeres raises series compliance, which leads to a shift in the muscle's active length–tension relation in the direction of longer muscle lengths (Morgan, 1990). Such a shift was first explained by Katz (1939) and since then it has been studied in single frog fibres (Morgan et al. 1996), whole amphibian muscle (Wood, Morgan & Proske, 1993; Talbot & Morgan, 1996) and human muscle (Jones et al., 1997; Wise, Morgan, Gregory & Proske, 2001). Moreover, cytoskeletal proteins like titin, nebulin, desmin and dystrophin are responsible with stabilizing the structure of the sarcomere and transferring the forces laterally across the fibre and from fibre to fibre (Patel & Lieber 1997). For example, Lieber and Friden (1994), demonstrated that loss of desmin staining of fibres was an indicator of eccentric damage and could appear in cells whose membrane was undamaged and whose contractile proteins appeared normal. Consequently, they indicated that desmin loss could be found as early as 5 min following the eccentric activity implying that it could be an early sign of eccentric damage. Numerous of these desmin negative cells later demonstrated an increase in titin staining and ultimately degenerated (Lieber et al., 1996). As a result, the following was hypothesised: 1) overstretched sarcomeres generate a local increase in (Ca^{2+}) by some specific mechanisms; 2) elevated (Ca^{2+}) triggers activation of proteases such as calpain which hydrolyses desmin; and 3) the loss of the structural support of desmin contributes to the development of the sarcomere disorder (Lieber & Friden 1999).

On the other hand, Connolly et al. (2003) looked at pain sensation associated with DOMS after eccentric activity at the cellular level. Connolly et al. (2003) stated that eccentric exercise resulted in cell membrane damage, putting off an inflammatory response that led to

prostaglandin and leukotriene synthesis. Prostaglandin E2 directly affects the sensation of pain, while leukotrienes boost the vascular permeability and attract neutrophils to the site of damage. Moreover, cells and fluid start to navigate through the bloodstream in the interstitial fluid, initiating swelling, which keeps contributing to the pain sensation and soreness all over the body 24-72 hours after eccentric exercise (Connolly et al., 2003). The degree of injury or pain sensation depends on the trained aspect of the muscle and how often the individual is performing the exercise.

2.7. Elbow Flexors vs. Knee Extensors

It appears that responses to eccentric exercise are different between leg and arm muscles. Differences in the use of daily muscles (repeated bout effect), muscle architecture and muscle fiber composition, makes arm muscle more susceptible to muscle damage and DOMS than leg muscles. Skeletal muscles are comprised of motor units, including muscle fibers with the same specific features (Canepari et al., 2010). Generally, muscle fibers differentiate from each other according to the following principles: 1) the contractile apparatus [myosin heavy chain (MHC) or ATP^{ase} isoforms]; 2) contractile characteristics (fast vs. slow twitch); 3) Ca^{2+} handling properties and metabolic profile (oxidative or glycolytic), with the golden standard being the MHC-isoform (Schiaffino & Reggiani, 2011). The functional importance of the MHC isoform for its contractile characteristics is well known (Schiaffino & Reggiani, 2011), even for hybrid fibers co-expressing MHC isoforms (Talmadge, Roy & Edgerton, 1999). The metabolic capacity of the muscle fiber is reliant on the degree of capillarization, availability of substrate and mitochondrial content, while the Ca^{2+} control elements are reliant on sarcoplasmic reticulum (SR) content as well as its properties (Stephenson et al., 1998; Olsson et al., 2020; Gejl et al., 2014). The metabolic and Ca^{2+} control elements are generally considered as being linked with

contractile fiber type characteristics. Human muscle fibers expressing MHC-1 have the highest oxidative capacity while having slow shortening velocity (including excitation–contraction coupling) and slower Ca^{2+} handling, whereas MHC-2 fibers have the contrasting characteristics. Yet, metabolic differences within each fiber type and between fibers in arm and leg muscle is not well-understood, either with respect to the extent and effect on the metabolic response of the fiber. A few studies that investigated the difference the difference between muscle fibers of arm and leg muscles, suggest that arm muscles are less oxidative and less able to extract oxygen from the circulation, regardless of training level, with larger dependability in blood flow during the training session (Van Hall et al., 2003; Calbet et al., 2005). Moreover, it is evident that training arm muscle has a lower fat oxidation in comparison with the leg muscle (Calbet et al., 2005; Helge, 2010).

Previous findings regarding which muscle group experiences greater DOMS following eccentric contractions indicate that the elbow flexors are more susceptible to DOMS than knee extensors (Chen, Lin, Chen, Lin, Nosaka, 2011; Jamurtas et al., 2005; Saka et al., 2009). The total amount of increase in CK activity in the blood is higher after elbow flexors eccentric exercise (Nosaka and Newton 2002) than that observed in leg eccentric exercises such as downhill running (Sorichter et al., 2001) and knee extensor eccentric exercise (Prou et al., 1999). Following a bout of eccentric exercise, DOMS appeared to be greater in arm exercises (Clarkson & Tremblay, 1988; Newham et al., 1988) than leg exercises (Dolezal et al. 2000; Prou et al. 1999). One study compared the leg and arm eccentric exercises of the same relative intensity for muscle damage indices in eleven healthy untrained males. The experimental protocol consisted of six sets of 12 repetitions of submaximal (75% of the predetermined maximal eccentric peak torque (EPT) of each muscle) eccentric exercise of the knee extensors and the elbow flexors,

separately. Significant (p<0.05) changes in DOMS and ROM were seen up to 96 hours following both exercise bouts. Increases in CK and myoglobin were considerably (p<0.05) greater in the elbow flexors than the knee extensors at 72 and 96 hours post-exercise. Decreases in muscle strength were significantly (p<0.05) larger at 48, 72, and 96 hours post-elbow flexor than knee extensor eccentric exercises.

These findings suggest that the magnitude of muscle damage is larger, and the recovery of muscle function was slower following eccentric exercise in elbow flexors than the knee extensors (Jamurtas et al., 2005). Possible causes for such a different response to eccentric exercise, may rise from different modes of exercise (e.g. downhill running vs. weightlifting), different types of muscle contraction (e.g. isotonic vs. isokinetic), different intensities and number of muscle actions, and different groups of subjects (Nogueira et al., 2014). Variations in muscle structural design were considered as an important reason for muscle damage following eccentric exercise (Friden and Lieber, 2001). It has been postulated that, in eccentric exercise, the ability to produce tension rises and a greater load is being distributed among the equal amount of fibers, which leads to a greater load per fiber ratio (Clarkson and Hubal, 2002). In addition, maximal eccentric exercises induce more noticeable muscle damage opposite to submaximal eccentric exercise (Nosaka and Newton, 2002; Paschalis et al., 2005). This implies that the greater the eccentric load, the greater the mechanical stress per muscle unit, and this could affect additional muscle damage. Based on different structural design of arm and leg muscles, it is plausible that mechanical stress per muscle unit varies between these two muscle groups, while doing eccentric exercises at a similar intensity (Lieber and Friden, 2000). According to Jamurtas and colleagues (2005), fusiform muscles are more prone to eccentric muscle damage than penniform muscles, therefore, this can possibly be a clarification why the

biceps brachii experience more DOMS than the vastus lateralis. In addition, the dominant muscle fiber type is completely different in either muscles; type II fibers can be found mainly in the biceps brachii, while the quadriceps have more type I fibers (Jamurtas et al., 2005). Evidently, type II muscle fibers are more vulnerable to muscle damage because these fibers have the weakest z-lines leading to extreme mechanical disruption, therefore DOMS is expected to appear more in the biceps brachii muscles (Cheung et al., 2003; Jamurtas et al., 2005).

2.8. Relief of Pain

Recently, topical analgesics have been broadly used as an effective treatment for various pain conditions such as neuropathic pain (Zhang et al., 2008). Wasner et al. (2008) stated that topical menthol had an analgesic effect amongst patients with peripheral and central neuropathic pain. Similarly, in another study done by Kraemer et al. (2005), functional ability of knee arthritis patients improved after application of a topical menthol gel. In another study which a 1% menthol cream was administered on the painful area of cancer patients twice a day, pain scores improved by 82% (Fallon et al., 2015). Furthermore, both walking cadence and velocity of participants were improved after treatment (Fallon et al., 2015).

The topical application of menthol gels has also been proven to decrease arterial blood flow in the upper (Hunter, Grigson & Wade, 2018), and lower (Stevens et al., 2016) limbs at the application site. For instance, one investigation, reported intramuscular temperature, blood flow responses and subjective temperature sensation following application of menthol-based cooling gel to their anterior thigh. Ice and gels reduced intramuscular temperature by 5.7 and 1.9 °C, respectively. Menthol gel cold sensation was also individually reported to be cooler than the other two other treatments. Menthol and cold therapy provide cold sensations as well as an analgesic effect (Babes, Cristian Ciobanu, Neacsu & Babes, 2011). In contrast with traditional

ice therapy which decreases cutaneous blood flow (Ho, Coel, Kagawa & Richardson, 1994), menthol has been demonstrated to increase cutaneous blood flow (Craighead & Alexander, 2016). This increased cutaneous flow occurs through activation of TRMP8 receptors in the vascular cells (Johnson, Melanaphy, Purse, Stokesberry, Dickson & Zholos, 2009). which in turn increase Nitric Oxide production from the endothelial cells resulting in localized vasodilation.

Also, it has been proven that following eccentric exercise, the influx of calcium ions into the muscle fibres and a subsequent disruption of the calcium homeostasis may be restored by increasing the amount of oxygenated blood flow to the injured area (Cheung et al., 2003). It has been suggested that an increased blood flow after application menthol-based gel, the margination of neutrophils and reduces subsequent prostaglandin production, thus reducing any further damage associated with the inflammatory process.

In a study of decerebrate cats the implementation of topical menthol gel decreased the exercise blood flow within an activated muscle without an observable impact on heart rate (Ragan et al., 2004). The researchers examined established peak mean arterial pressure responses, induced by static muscle contraction and it was found that they were significantly reduced after application of the gel (Ragan et al., 2004, Ichiyama et al., 2002) which led to a decline in total blood flow to the area. It was assumed that the decrease in the pressure response is because of the suppression of the small-diameter sensory nerve fibers that are identified as group III and IV afferents which synapse with the central nervous system (Ragan et al., 2004). These authors determined that topical menthol could decrease blood flow to the fundamental tissues through stimulation of cold receptors of the skin.

In another research study low back pain (LBP) subjects were instructed to employ Biofreeze™ to the lower back 3 times a week and used a visual analog scale (VAS), amongst

other evaluation tools to assess pain reduction (Zhang, Enix, Snyder, Giggey, & Tepe, 2008). A significant pain reduction was reported following each week of treatment with the Biofreeze™ group, indicating Biofreeze™ in combination with chiropractic adjustment presented a decrease in LBP (Zhang, Enix, Snyder, Giggey & Tepe, 2008). Overall, topical analgesics such as menthol has been shown to be an effective method for treatment of pain. It has multiple physiological effects on injured tissue decreasing temperatures of skin and muscle reduces blood flow to the cooled tissues by activating a sympathetic vasoconstrictive reflex (Paine, DeLee, Drez & Miller, 2010). Besides, decreases in blood flow reduce edema and slow the delivery of inflammatory mediators (eg, leukocytes), reducing inflammation of the affected area (Deal, Tipton, Rosencrance, Curl, & Smith, 2002). Decreasing tissue temperature also reduces the metabolic demand of hypoxic tissues, potentially preventing secondary hypoxic damage in injured tissue resulting in pain relief (Merrick, Rankin, Andres & Hinman, 1999).

2.9. Pressure Pain Threshold (PPT):

To quantify the level of muscle soreness is challenging as, pain is by nature, subjective (O'connor & Cook, 1999). Various pain scales like the VAS (Zainuddin, Newton, Sacco & Nosaka, 2005), verbal rating scale (Abraham, 1977), numerical rating scale (Jones, Newham & Clarkson, 1987), and descriptor differential scale (Hilbert, Sforzo, & Swensen, 2003) are employed to assess the level of DOMS. Amongst them the VAS is the most popular method to assess DOMS (Bajaj, Graven-Nielsen, & Arendt-Nielsen, 2001; Zainuddin et al., 2005), comprising of a specific length of line (i.e. 100 mm) in which one end of the line implies no pain and the other end implies worst pain. Due to the fact that DOMS is not felt without mechanical stimulus, quantifying muscle pain requires a standardized palpation, stretching, or muscle-

contraction protocol (O'connor & Cook, 1999, Slater, Thériault, Ronningen, Clark & Nosaka, 2010). The application of VAS to evaluate musculoskeletal pain has been demonstrated to be a reliable tool (Boonstra, et al., 2008; Price, et al.,1983), however, the soreness palpation evaluation is usually criticized due to the ambiguity in the palpation procedure (Bendtsen, Jensen, Jensen & Olesen, 1994). An additional way to quantify muscle pain is the usage of a pressure algometer that evaluates the point where a sensation of pressure changes into a sensation of pain in the muscle, which is known as the pressure pain threshold (PPT) (Greenspan & McGillis, 1994; Jones, Kilgour & Comtois, 2007). In fact, mechanically induced pain (i.e. PPT) is a common model for evoking severe experimental pain (Chesterton, Sim, Wright & Foster, 2007). Treatment induced changes in PPT observed in laboratory settings are intended to be associated with changes in clinical status of pain, and as such the use of PPT is deemed to be a valuable experimental model (Fischer, 1987).

Algometry is an effective technique in evaluating PPT by manually employing increasing force at a predetermined rate until the perception of pain is reported by the participant (Chesterton et al., 2007). It has been utilized broadly in both clinical and laboratory settings (Fischer, 1987; Walsh, Lowe, McCormack, Willer, Baxter & Allen, 1998), however it has been suggested that 2 to 5 assessments should be used at each testing point, and then averaged to a better illustrate the value (Chesterton et al., 2007). The PPT has been proven to be reliable for measuring pain threshold (Chesterton et al., 2007; Nussbaum & Downes, 1998), as well as assessing DOMS (Binderup, Arendt-Nielsen & Madeleine, 2010; Fernández-Carnero et al., 2010). Previous investigation indicated that muscle soreness measured by VAS peaked at two days, and PPT reduced mostly at one day after exercise and no additional reduction was observed at two days post eccentric exercise of the elbow flexors (Lau & Nosaka, 2011). Fleckenstein et

al. (2017) aimed to identify underlying mechanical thresholds in an experimental model of DOMS using various tools [e.g. PPT, secondary thresholds included mechanical detection (MDT) and pain thresholds (MPT)] on twenty volunteers. Results show that functional impairment after DOMS seems to be related to the increased excitability of high-threshold mechanosensitive nociceptors. The PPT was the most valid mechanical threshold to quantify the extent of dysfunction. Therefore, rather than pain intensity, PPT could be considered a possible marker to indicate the athletes' potential risk of injury. Additionally, in another study the PPT was assessed between different areas of the elbow flexors before, immediately after, and 24-72 hours after eccentric exercise, and it was reported that the medial region of the elbow flexors had the lowest PPT before exercise, however, the lowest PPT values were recorded from the central and distal regions of the biceps brachii 24-72 hours post-eccentric exercise (Lau, Blazevich, Newton, Wu & Nosaka, 2015).

As we discussed earlier, measurement of PPT has been employed comprehensively to assess the perception of pain, and the effectiveness of therapeutic interventions for alleviating pain (Kosek and Ordeberg, 2000; Hong, Chen, Pon & Yu, 1993; Fischer, 1987). Treatment induced alterations in the PPT detected in laboratory settings are intended to correlate with alterations in clinical status of pain, and as such PPT is accepted as an effective experimental model to quantify perceived pain (Fischer, 1987). A great deal of literature investigating PPT quantification recommends that there are significant differences between genders, with females displaying lower thresholds in response to typical experimental pain stimuli (i.e., electrical, pressure, hot, and cold; Fillingim, 2000; Fillingim and Maixner, 1995). Clinically, however, it is well documented that women are more probable than men to report a wide range of recurrent

pains, in different body parts, which are frequently identified as being more severe and frequent compared to men (Berkley, 1997). However, experiments comparing responses to noxious heat among females and males have reported conflicting results. For instance, some researchers have stated greater pain threshold among males (Arendt-Nielsen & Bjerring, 1988; Meh & Denišlič 1994), whereas others have claimed no sex difference in thermal pain threshold (Clausen and King, 1950; Kenshalo, 1986; Fillingim et al., 1996). One possible reason for these inconsistent findings is the superiority of one-dimensional pain assessment methods operated in earlier studies, which cover only the magnitude of pain (Fillingim et al., 1996). Another study done by Fleckenstein and colleagues (2017) also reported significantly lower PPT in the biceps for females compared to males, post-eccentric exercise. This difference is shown to be independent of the anatomical measurement site, despite the fact that there is a tendency for larger discrepancy in more elaborately innervated anatomic regions (Fillingim et al., 1999). A meta-analysis exploring gender differences responding to induction of mechanical pain, supported the presumed gender differences in pain perception and stated that females present lower pain thresholds (Riley et al., 1998). In Fact, the meta-analysis has shown that mechanical stimuli revealed the greatest gender differences in pain threshold in comparison with other forms of experimental stimuli (Riley et al., 1998). For example, a study examined the temporal summation to noxious mechanical stimulation and also examined gender differences in temporal summation of mechanically induced pain (10 healthy men and 10 healthy woman). The results demonstrated that temporal summation of mechanically evoked pain is higher in females in comparison to males, and is dependent on stimulation frequency which is centrally mediated (Sarlani & Greenspan, 2002). Some experiments have addressed sex differences in the sensation of DOMS after unaccustomed eccentric exercise (Evans, Haller, Wyrick, Parkey & Fleckenstein,

1998; Rinard, Clarkson, Smith & Grossman, 2000). However, Evans et al. (1998) and Newham et al. (1987) concluded that there was no significant relationship between genders in response to DOMS induced eccentric exercise. In contrast, MacIntyre and colleagues (2000) determined a significant sex difference in the DOMS immediately following strength testing. More precisely, females' pain reports rose across different time periods over 24 hours whereas males' pain reports rose immediately following the "maximal" eccentric isokinetic contractions and remained elevated.

Numerous mechanisms have been recommended to clarify sex differences in the experience of both clinical and experimental pain, and these mechanisms are mostly categorized as psychosocial versus biologic (Chesterton, Barlas, Foster, Baxter & Wright, 2003). One possible explanation for sex differences in pain is based on the idea that feminine sex models is encouraged to express pain while masculine sex models discourage pain behavior. In spite of its widespread agreement, evidence advocating this idea is limited, and this problem has mainly been investigated in connection with the experimental rather than clinical pain. To be more precise, in 1985, one study demonstrated that for males, high masculinity scores were related to greater pain thresholds, while neither masculinity nor femininity was reported to be related to pain threshold for females. Nevertheless, even after evaluating for masculinity-femininity scores, the sex difference in pain threshold continued to be significant, but after evaluating for the sex of the subject, sex models scores were not associated with pain measures (Otto & Dougher, 1985). In a study done by Levine & De Simone (1991) to determined sex role expectations, male and female participants rated cold pressor pain for either a female or a male individual, both sexes were instructed to dress up nicely to emphasize the feminine and masculine stereotypes,

respectively. Males showed significantly lower pain compared to a female, while females' ratings did not change as a function of participant sex.

2.11 Measurement Sites

After exercise, DOMS is normally experienced in the musculotendinous junction, and then the feeling progresses to the muscle belly (MacIntyre et al., 1995). PPT measurements are commonly taken 48 hours following exercise at measurement sites in the center of the muscle belly of both the upper- and lower-body muscles. Lau and colleague (2015) evaluated the proper location of PPT measurement in the biceps brachii by a grid of 50 squares (2 cm x 2 cm) placed on the upper arm. The location of grid square was positioned approximately 9 cm above the elbow crease, being the centre of the muscle belly of the biceps brachii. Furthermore, Stefanelli et al. (2019), quantified PPT for each participant's dominant biceps brachii during eccentric elbow flexor contraction in each session via a pressure pain algometer and the PPT measurement site was selected according to the literature to be the mid belly of the muscle 9 cm above the antecubital space. In a study done by Pearcey and colleagues (2015), the measurement site for PPT of the quadriceps was determined to be midway down the quadriceps, between the iliac crest and the superior border of the patella. Similarly, the PPT measurement site for the vastus lateralis was selected to be 10 cm lateral to the midpoint of the superior edge of the patella (Martin-Alguacil et al., 2019).

2.12 Conclusion

According to previous findings, eccentric exercise is known as an effective exercise to induce DOMS. Elbow flexors experience larger muscle damage in comparison to knee extensors because of its differences in daily use, muscle architecture and muscle fiber composition. The induction of DOMS results in a reduction in PPT. Following DOMS induction, PPT has

demonstrated to peak nearly 2 days post-exercise, with the elbow flexors indicating a larger reduction in PPT than the knee extensors. Furthermore, both measurement sites of 9 cm above the antecubital space and midway between the iliac crest and border of the patella for the biceps brachii and the vastus lateralis, respectively, have been previously utilized broadly in the PPT literature. However, there is no agreement on where the best anatomical location is to take PPT measurements. Topical menthol analgesics, such as Biofreeze™, is an effective treatment for reducing perceived pain during DOMS.

To the best of our knowledge there are no studies investigating if there is a difference in perceived pain during DOMS, utilizing PPT, between the biceps brachii and vastus lateralis after prior to and following the application of a topical menthol analgesic, Biofreeze™. Often times comparison of the literature needs differentiates intensities and exercise type with different participant groups, making it challenging to accurately compare results. Consequently, additional research is required to include more studies which use the same intensities and types of exercises on the same participant group to look at DOMS and PPT in several muscle groups concurrently. The hope is that the results of our future study will shed light on how menthol topical analgesics affect different muscles, which has practical implications in terms of prescribing the use of menthol products, such as Biofreeze™, as a treatment of pain.

2.13 References

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Chapter 2: Co-authorship Statement

My contributions to this thesis are outlined below:

Foley, Herat, Fudge, and Murrin we recruited all participants for data collection.

I prepared the manuscript and thesis with the help and guidance of my supervisors, Drs. Duane Button and Shahab Alizadeh.

Drs. Button and Alizadeh provided constructive feedback on the whole thesis.

Chapter 3

Title: Upper- and Lower-body Pain Perception during DOMS: The Effect of Menthol-based Topical Analgesic Application

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3.0 Abstract

Both athletic and nonathletic populations when subjected to any unfamiliar or unaccustomed exercise will encounter pain 24-72 hours following the exercise bout. This exercise particularly eccentric in nature triggered mainly by muscle damage is known as delayed-onset muscle soreness (DOMS). Topical application of menthol is a popular form of cold therapy which known to be effective with alleviation of DOMS. To date, the difference in pain pressure threshold (PPT) reduction in the lower vs. upper extremity prior to and following the application of these gels is not well understood. Therefore, the purpose of this study was to compare the upper- and lower-body's responses to pain following menthol-based analgesic application 48 hours after an unfamiliar bout of eccentric exercise induced DOMS. Fourteen participants were randomly selected into two groups (placebo and experimental) and each completed 5 sets of 10 repetitions of eccentric contractions of the knee extensors and elbow flexors on an isokinetic dynamometer. PPT was measured via a handheld algometer at baseline, immediately after and 48 hours after the DOMS protocol from the biceps brachii and vastus lateralis. After 48 hours the PPT measurements were taken prior to and 15 minutes after application of gel and every 5 minutes for 45 minutes afterwards. Statistical analysis showed a significant difference for PPT in both groups between the knee extensors and elbow flexors ($p<0.001$). Experimental group revealed a decrease in PPT 48 hours after DOMS both elbow flexors (48%) and knee extensors (26%), however, only the elbow flexors indicated a significant decreased ($p=0.018$). The menthol-based gel application on the elbow flexors increased PPT at 30 minutes after application of gel until the test was over ($p=0.018$). in contrast, the placebo group demonstrated a significantly reduced in their PPT values 48 hours after DOMS in both the elbow flexors (45%) and knee extensors (38%) ($p=0.018$). Contrasting the experimental group,

the placebo group indicated no change in PPT after gel application. Pain perception was differed in the elbow flexors and knee extensors, also a change in pain perception after the application of the menthol gel is muscle specific during DOMS.

Keywords: elbow flexors, knee extensors, topical analgesic, eccentric exercise.

3.1 Introduction

DOMS is referred to muscular pain that occurs 24-72 hours following unaccustomed activity and remains 5-7 days post-exercise (Cheung, Hume, & Maxwell, 2003; Cleak & Eston, 1992). DOMS can be described as the feelings of tenderness, stiffness, and overall discomfort of dull pain post-exercise. Normally, DOMS is subclinical and assigned as a type I strain and does not need a specific treatment (Lewis, Ruby, & Bush-Joseph, 2012; MacIntyre et al., 1995). However, at times DOMS can also be severe and have different resemblance to pain (MacIntyre et al., 1995). Although, DOMS is not a new occurrence, the treatment methods to provide long-lasting relief still remain unclear (Cheung et al., 2003). The sensation of pain as a result of DOMS can disperse during the day or can continue being severe and restrict an athlete's ability (Lewis et al., 2012).

Exercise with increased amounts of eccentric activity tend to trigger the greatest amount of DOMS and the greatest reported muscle soreness usually occurs 48 hours post-exercise (Stefanelli et al., 2019; Vila-Chã, Hassanlouei, Farina, & Falla, 2012). Connolly and colleagues (2003) reported that eccentric exercise initiate an injury to the cell membrane, setting off an inflammatory response that leads to prostaglandin and leukotriene synthesis. Prostaglandin E2 directly result in the sensation of pain, whilst leukotrienes improve vascular permeability and magnetize neutrophils to the damage site. Furthermore, cells and fluid start to move through the

bloodstream in the interstitial fluid, causing swelling, which remains to contribute to the sensation of pain and soreness all over the body 24-72 hours following eccentric exercise (Connolly et al., 2003). The degree of damage or sensation of pain is related to the muscle training level and how often the person repeats the exercise.

One way to determine an individual perceived pain during DOMS is by measuring pain pressure threshold (PPT) through the use of a handheld algometer. Essentially, PPT is a pressure value when the individual perceives the amount of pressure being displaced on their muscle as being painful (Jones, Kilgour, & Comtois, 2007). PPT is often employed to examine muscle tenderness in numerous circumstances and disorders (Chesterton, Sim, Wright, & Foster, 2007; Jones et al., 2007). Furthermore, using a pressure algometer to determine PPT has been shown to be both a valid and reliable technique for measuring pain perception and for measuring pain perception from multiple locations around the body over consecutive days (Jones et al., 2007; Park, Kim, Park, Kim, & Jang, 2011).

Despite the fact that there is no one treatment for DOMS, there are various types of treatments that have been reported to alleviate DOMS symptoms, such as menthol topical analgesics (Cheung et al., 2003). Menthol functions to decrease pain via cryotherapy. Page & Alexander (2017) describe cryotherapy as the use of cold therapy for musculoskeletal pain treatment. Three mechanisms for localized cooling include physical cooling, evaporative cooling, and chemically mediated cooling. These include a decrease in skin temperature result in stimulation of thermal receptors (Page & Alexander 2017). Transient receptor proteins (TRP), specific thermal receptors, are activated when there is a cold sensation applied to different parts of the body (Pérez de Vega, Gómez-Monterrey, Ferrer-Montiel, & González-Muñiz, 2016). There are many different subtypes of TRP, including the TRP melastatin 8 (TRP-M8). This

receptor is sensitive to cold temperatures that are felt when ice or menthol is applied to the skin. Consequently, the activation of TRP-M8 leads to a decrease in the nociceptive response to pain (Pérez de Vega et al., 2016). Menthol has been shown to be more efficient for alleviating DOMS associated pain perception compared to ice, as it facilitates to improve production of force and reduce perception of pain (Johar, Grover, Topp, & Behm, 2012). Thus, menthol application during DOMS can reduce pain perception and increase performance.

In the area of research regarding eccentric muscle contractions to induce DOMS, the elbow flexors and knee extensors are the two most frequently used muscle groups. Numerous investigations have reported evidence demonstrating the elbow flexors experience more DOMS in comparison to the knee extensors (Jamurtas et al., 2005). The possible reason could be based on variation in structural design of arm and leg muscles, it is conceivable that mechanical stress per muscle unit differs between arm versus leg muscle groups, whilst performing eccentric exercises at a similar intensity (Lieber and Friden, 2000). As stated by Jamurtas and colleagues (2005), fusiform muscles are more susceptible to eccentric muscle damage than penniform muscles, consequently, this can probably be an explanation why the biceps brachii experience more DOMS than the vastus lateralis. Additionally, the dominant muscle fiber type is entirely different in both muscles; type II fibers could be found primarily in the biceps brachii, whereas the quadriceps have more type I fibers (Jamurtas et al., 2005). It is well understood, type II muscle fibers are more susceptible to muscle damage for the reason that these fibers have the weakest z-lines which leads to severe mechanical disruption, this is why the DOMS is likely to experience more in the biceps brachii muscles (Cheung et al., 2003; Jamurtas et al., 2005). According to the aforementioned, menthol gel appears to be more effective on arm in compare to

leg muscle. Studies showed that higher vasoconstriction in arm exercising resulting in higher oxygen delivery to and utilisation by the working muscles (Volianitis & Secher 2002).

Although, there are few studies comparing the perceived pain during DOMS in the elbow flexors and knee extensors. Furthermore, it is not known if there is a difference in the reduction of PPT between the elbow flexors and knee extensors following the application of menthol to those muscles during DOMS. The purpose of this study was to compare perceived pain via PPT in the elbow flexors versus the knee extensors 48 hours after the induction of DOMS and following the application of a menthol-based analgesic. We hypothesized that there will be greater pain associated with DOMS in the elbow flexors compared to the knee extensors and as a result there would be a greater decrease in elbow flexor perceived pain (i.e., increased PPT) following menthol application.

3.2 Material and methods

3.2.1 Participants. Based on previous studies (Robinson-Lane & Vallerand, 2018; Stefanelli, 2017), fourteen college aged participants were recruited for this study. An equal number of male and female subjects were recruited with no prior acute or chronic health conditions or history of injury that would prevent participation in the experimental protocol. All participants were recreationally active (self-reported aerobic and/or resistance exercise ≥ 2 times per week). Prior to testing, all participants filled out the Physical Activity Readiness Questionnaire (PAR-Q) along with a motivational scale, the Waterloo Footedness Questionnaire, and the Edinburgh Handedness Inventory Scale. Participants were also instructed to refrain from physical activity 24 hours before testing, as well as in between test days, and to follow the Canadian Society for Exercise Physiology instructions before testing. Participants were briefed regarding the benefits and potential risks, both written and verbally, and if willing gave informed

consent according to the Declaration of Helsinki. This study was approved (**20211026-HK**) by the University's Interdisciplinary Committee on Ethics in Human Research.

3.2.2 DOMS Protocol. For the induction of DOMS, all participants performed both eccentric elbow flexor and knee extensor contractions using a HUMAC NORM dynamometer (CSMi Medical Solutions, Stoughton MA). For the elbow flexors, participants completed a warm-up consisting of 5 minutes of arm cycling (SCIFIT ergometer, model PRO2 Total Body, Tulsa, OK) at 60 rotations per minute (RPM). For the knee extensors, the warm-up included 5 minutes on a cycle ergometer (Monarch Peak Bike Ergomedic 894E) at 70 RPM. Participants were seated upright with their back supported at a 90° angle and strapped in by shoulder, chest, and leg straps to prevent excess movement. The lateral epicondyle of the humerus was aligned with the rotational axis of the dynamometer and participants were asked to move their arm in a comfortable range of motion between full extension (0° flexion) and 180° flexion to set the range or motion. Likewise, the lateral epicondyle of the femur was aligned with the rotational axis of the dynamometer and the participants were asked to move their leg in a comfortable range of motion between full extension (0° flexion) to 90° flexion to set the range of motion. Before beginning the protocol, all participants were given 3 passive practice trials for elbow flexion and for knee extension to familiarize themselves with the dynamometer. The DOMS protocol for both muscle groups consisted of 5 sets of 10 repetitions at a velocity between 21°/S - 24°/S. During the contractions, participants were instructed to resist the eccentric force maximally and relax as the dynamometer passively brought the tested limb to extension (elbow flexors) or flexion (knee extension). Participants were given 90 seconds rest between each set. Following each set participants were asked to give their rate of perceived exertion on the modified Borg

scale. Participants were asked to pick music of their liking to play during the protocol for motivation.

3.2.3 Pain Pressure Threshold (PPT). PPT measurements were taken for the participant's biceps brachii (BB) and vastus lateralis (VL) via a pressure-pain algometer with a probe tip of 1.7 cm^2 (Lafayette Manual Muscle Test System TM, Model 01163, Lafayette Instrument Company, IN, USA). Measurement sites were based on a percentage of the distance between anatomical sites of both the upper- and lower-body to ensure individual differences were considered. For the biceps brachii, the distance between the coracoid process and antecubital space was measured and a mark was placed on the muscle belly above the antecubital space based on 30% of this distance (Nussbaum & Downes, 1998). Participants laid supine on a mat and with their arm abducted to 90° for biceps brachii measurements. A mark was placed on the muscle belly of the vastus lateralis based on 50% of the distance between the anterior superior iliac spine (ASIS) and the lateral superior border of the patella (Bohannon & Bass, 2017). For vastus lateralis measurements, participants were seated in a chair with their knees at 90° flexion. For PPT measurements, the pressure was gradually applied to the marks on the biceps brachii and vastus lateralis. Participants were instructed to verbally indicate when the pressure changed from discomfort to pain, signifying the researcher to stop applying pressure. Each PPT value throughout the study was taken three times.

3.2.4 Gel Application. Either topical analgesic gel (5% menthol; Biofreeze®) or a placebo gel (all ingredients are the same except menthol) was applied over entire elbow flexors and knee extensors to the dominant side. The amount of gel used was based on the muscle sizes of each participant. Surface area of the muscle was measured in centimeters and obtained via palpation of proximal and distal insertions for length, and medial and lateral insertion points for

width. Using this value, 1 mL of gel was applied for every 200 cm^2 of skin surface area based on the recommended Biofreeze® dosage. For each participant, the researcher applied the gel using a glove and lightly rubbed it in over the span of 30 seconds.

3.2.5 Experimental Protocol. Participants completed two sessions, a pre- and post-test with 48 hours between the sessions. For all participants, randomization was used to determine the order of DOMS induction (elbow flexors versus knee extensors first) and which sides of the upper- and lower body that would be tested (left versus right). In the first session prior to the DOMS protocol, baseline PPT measurements were taken for the biceps brachii and vastus lateralis. Participants then completed the DOMS protocol detailed above. PPT measurements were taken again for both muscles after 90 seconds post-DOMS protocol completion. Participants were then told to return to the laboratory 48 hours later for the second session. During the second session, baseline PPT measurements were taken again for the biceps brachii and vastus lateralis before applying gel. Each participant was randomly assigned to one of the two groups: topical analgesic (n = 7) or placebo (n = 7). Both the researcher and participant were blinded to treatment type, as the bottles of gel were labelled with a code. For each participant, gel was applied to the muscle tested first during DOMS protocol and 2 minutes later gel was applied to the other muscle to allow time in between for PPT measurements. Following gel application, there was a 15-minute activation period then PPT measurements were taken every 5 minutes for 45 minutes, for a total session of 60 minutes (Figure 1)

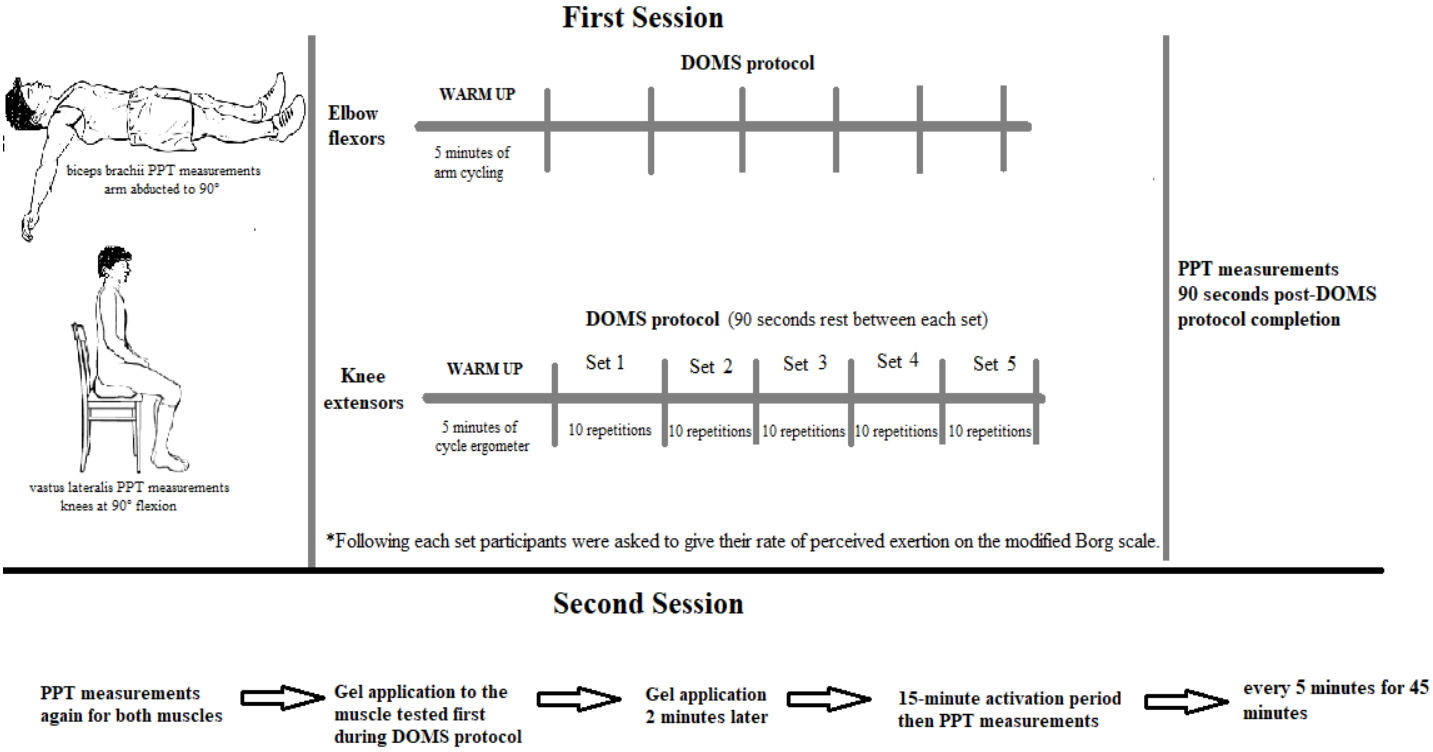

Figure 2 shows the experimental protocol.

3.2.6 Statistical Analyses. All statistics were performed using IBM's SPSS software (IBM SPSS, version 20.0; IBM Corp., Armonk, N.Y., USA). Assumptions of sphericity were tested using Mauchly's test and if violated, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. Two separate two-way repeated measures ANOVAs were performed to compare muscle groups (elbow flexors versus knee extensors). Repeated pairwise comparisons using Bonferroni post hoc tests were used to determine where significant differences existed when significant interaction effects were found. F-ratios were considered statistically significant at the $p < .05$ levels. Descriptive statistics in text and Tables 1, 2, and 3 include mean ± SD and the figures include mean ± SE.

3.3 Results

3.3.1 Pain Pressure Threshold. The lowest recorded PPT values were obtained pre-gel application, 48 hours after the DOMS protocol (see Table 1 for all PPT values). The biceps brachii had an overall lower PPT than the vastus lateralis. PPT of both the biceps brachii and vastus lateralis increased immediately post-DOMS but decreased 48 hours pre-gel application for all groups. The PPT of the topical analgesic group increased for both the biceps brachii and vastus lateralis greater than the placebo group (Table 1).

The normalized mean PPT of the vastus lateralis was greater than biceps brachii for 15 and 20 minutes post-topical analgesic application (Figure 2). However, the normalized mean PPT of the biceps brachii remained higher after 20 minutes post-topical analgesic application compared to the vastus lateralis (Figure 2). The maximum normalized mean PPT for biceps brachii reaches 75.4% of the post-DOMS maximum PPT value at 45 minutes for the topical analgesic. However, vastus lateralis reached 63.7% of the post-DOMS maximum value at 20 minutes for the topical analgesic (Figure 2). On the other hand, the normalized mean PPT of the vastus lateralis for the placebo group remained higher than the normalized mean PPT of the bicep brachii except at 50 minutes post placebo gel application. The maximum normalized mean PPT for the biceps brachii (24.4%) occurred 45 minutes post-placebo gel application. Similarly, for the vastus lateralis, the maximum normalized mean PPT (31.1%) occurred 60 minutes post-placebo gel application (Figure 2). There was a significant difference ($p < .05$) in PPT between the topical analgesic and placebo groups for the vastus lateralis for all time intervals. For the biceps brachii there was a significant difference ($p < .05$) in PPT between topical analgesic and placebo groups for some time intervals (15, 20, 25, 45, and 60 minutes post-gel). In figure 2, significance was not shown for clarity purposes.

3.3.3 Rate of Perceived Exertion (RPE). RPE scores continuously increased as participants progressed through the DOMS protocol for the biceps brachii and vastus lateralis (see Table 2 for all RPE values). RPE recorded for the biceps brachii was higher than for the vastus lateralis. There was a significant difference ($p < .05$) between RPE recorded for biceps brachii and vastus lateralis.

3.3.4 Peak Torque. The mean peak torque (Nm) for the biceps brachii and vastus lateralis fluctuated throughout the sets. The greatest mean peak torque production for the biceps brachii occurred in set 1 compared to set 3 for the vastus lateralis (see Table 4 for all mean peak torque values). There was a significant ($p < .05$) decrease in peak torque between set 1 and set 5 for the biceps brachii and vastus lateralis.

3.4 Discussion

This study investigated the differences in PPT of the biceps brachii and vastus lateralis 48 hours post-DOMS and following the application of a topical analgesic (Biofreeze®) or placebo gel. The aforementioned results indicate menthol-based topical analgesic significantly affect PPT post-DOMS by boosting the threshold at which pain is experienced. In addition, PPT of the biceps brachii show a greater increase when compared to vastus lateralis following application of topical analgesic gel, and that PPT values increased over time for biceps brachii and decreased for vastus lateralis. As we hypothesized, biceps brachii had a total lower PPT in comparison with vastus lateralis, which could be associated to eccentric activity resulting additional damage in upper-body muscle groups (biceps brachii) versus lower-body muscle groups (vastus lateralis).

Comparing PPT between different muscle groups indicated that the distal region of the muscle is more susceptible to pain and soreness following eccentric contraction-induced muscle damaged (Mense & Meyer, 1988). It could be due to the fact that the majority of group III

nociceptors and mechanoreceptors served by group III axons are located in distal region of the muscle (Kumazawa & Mizumura, 1977; Skarpsno, 2014). Consequently, since our study evaluated PPT at half of the total muscle length where concentration of the nociceptor probably is lower in compared to distal of the muscle, this might have had an impact on the PPT values.

Moreover, it has been documented that type IIb fibers can be found mostly at the distal regions in comparison with more proximal regions, also type IIb fibers has been stated to be more susceptible to damage following eccentric contraction (Travnik, Pernus & Erzen, 1995). This has been associated to their unavailability of oxidative capacity or enhanced strain and damage as a result of their shorter fiber length (Fridén & Lieber, 1998), their higher tension (Appell, Soares & Duarte, 1992), and shorter optimum length for tension (Brockett, Morgan, Gregory & Proske, 2002), compared to type I fibers. On the contrary, the Hennemans size principle claims that low threshold motor units are the main contributors to muscle activity in situations with low muscle force demands (Henneman, Clamann, Gillies, & Skinner, 1974; Henneman, Somjen & Carpenter, 1965). The fast-twitch fibers have been known to not be recruited during typical physical activities, and fast-twitch fibers only demonstrates altered activity during rapid walking or running (Grimby, 1984; Samani, Holtermann, Søgaard & Madeleine, 2009). One can argue that even the muscle fibers that are not damaged to badly were still altered by the eccentric exercise intervention and led to increased perceived pain.

Additionally, it has formerly been observed that PPT is compressed by subcutaneous tissue and muscle thickness (Fischer, 1987). Apparently, the vastus lateralis has a larger muscle thickness than biceps brachii, enhancing its capability to tolerate more pressure before the sensation becomes pain. On the other hand, the biceps brachii is a smaller muscle with less thickness which may possibly boost its pain susceptibility (Dartnall, Nordstrom & Semmler,

2008). Furthermore, Lau and colleagues (2015), indicated that the sensitivity of medial region to pressure is higher prior to exercise than other regions, however, the central and distal regions are more susceptible to postexercise. Our measurement of PPT for the biceps brachii had happened at 30% of the muscle's length, close to the middle of the muscle belly where the muscle junction along with the brachial artery, vein and medial antebrachial cutaneous nerve runs (Lau, Blazevich, Newton, Wu, & Nosaka, 2015). As a result, this could be a potential explanation as to why the vastus lateralis has a higher overall PPT than biceps brachii.

Comparable to the present study, Jamurtas et al. (2005) revealed that there was a tendency for the elbow flexors to show larger DOMS than the knee extensors in healthy untrained males. They suggested the difference in DOMS of the two target muscle groups could rely on the daily use of the muscles. It has been well documented that previous damage to muscle produces a subsequent prophylactic effect on muscle damage that lasts for at least a few weeks (Jamurtas et al. 2000; McHugh 2003; Nosaka et al. 1991; Nosaka et al. 2001). This effect is commonly referred to as a repeated bout effect, and the characteristic presentations of this effect are smaller reductions and faster recovery of muscle function, smaller increases in muscle proteins in the blood, and less progress of muscle soreness and swelling (McHugh, 2003). It is assumed that the knee extensors had already acquired an adjustment to the effect against eccentric-exercise-induced muscle soreness through daily physical activities such as downhill walking, going downstairs, which includes the submaximal eccentric muscle contractions (Jamurtas et al., 2005). Even though the knee extensors had not undergone the absolute eccentric load asemployed in the exercise, it is likely that anti-gravity muscles containing most of the leg muscles are less prone to eccentric-exercise-induced muscle soreness when compared to arm muscles that are less often subjected to eccentric load in daily activities (Jamurtas et al., 2005).

Brown and colleagues (1997) stated that even after maximal eccentric exercise, leg muscles indicated less decreases in muscle strength and small increases in muscle proteins in the blood. In the meantime, the elbow flexors become less sensitive to eccentric-exercise-induced muscle soreness after completing maximal eccentric exercise (Newham et al. 1987).

In agreement with this statement, a study reported significantly higher increases in plasma creatine kinase (CK) and myoglobin (Mb) of the elbow flexors and extensors in comparison to the knee flexors and extensors. As previously mentioned, both CK and Mb are indirect markers that are known to ascertain muscle damage, which reveals that the arm muscles experience more muscle damage and therefore will experience more severe DOMS (Chen, Lin, Chen, Lin, Nosaka, 2011). Another conceivable explanation is differences in muscle structure and muscle fibre type between two aforementioned muscles. The biceps brachii is structured in a fusiform manner, whereas the vastus lateralis is structured in a multi pinnated manner (Jamurtas et al., 2005; Friden & Lieber, 2001). It seems that fusiform muscles are more sensitive to eccentrically induced muscle soreness than pinnate muscles. In addition, it has been documented that type I muscle fibres are less prone to eccentric exercise-induced muscle damage in compared to type II fibres therefore greater DOMS will occur (Friden, 1984; Lieber and Friden, 1988). Biceps brachii is confirmed to have a high percentage of type II fibres (Klein et al. 2003), whereas the knee extensors contain a higher percentage of type I fibres (Travnik et al. 1995). These reasons may provide some insight as to why the biceps brachii experienced more damage and loss of self-reported function than the vastus lateralis.

PPT demonstrated that participants in both the topical analgesic and placebo groups, had an decrease in perception of pain as the second session progressed. A potential explanation is that all the measurements were taken from the same spot every 5 minutes for 45 minutes overall,

causing an increase in sensitivity around the skin, thereby possibly increasing the VAS scores. Likewise, for either the biceps brachii or the vastus lateralis the topical analgesic group reported lower perceived pain (lower VAS) and higher PPT values when compared to the placebo group. This can be associated to the topical analgesic, whose function is to provide pain relief, which is done by stimulating thermoreceptors in the skin to cause vasoconstriction and reduced blood flow to the area (Page & Alexander, 2017). Both Johar et al. (2012) and Stefanelli et al. (2019) have also reported the perception of soreness is positively affected following application of the topical analgesic menthol, as perception of pain is decreased.

In our study, the lowest recorded PPT values for both muscle groups were found pre-gel application (48 hours post-DOMS), Interesting to note that, 60 minutes post-gel application, PPT of the placebo group increased quite the opposite to our assumption, while PPT for the topical analgesic group decreased (Table 1). Participants were advised when the last PPT measurement was to be recorded; this may have motivated participants to tolerate more pressure. However, if this was the case, the last PPT measurement of the topical analgesic group 60 minutes post-gel application should have also increased. This warrants further investigation.

In the current study, the rate of perceived exertion increased with each set however, the mean peak torque values fluctuated. It was expected mean peak torque would constantly decrease, as participants were instructed to execute maximal contractions. Surprisingly, the findings do not provide support for this as the highest mean peak torque output for the knee extensors was detected during set three. While the highest mean peak torque output for the biceps brachii was detected in set one there was still an increase in mean peak torque output in set three that was incompatible with what was expected. Since RPE is a score measured from participants' perceived exertion, there can be a number of various factors that could change the

expected result of peak torque decreasing as participants' RPE increases. Moreover, motivation plays a big role in the total amount of effort that a participant spends during physical activity, therefore if motivation levels were not high, there may be a mismatch between peak torque and perceived exertion.

3.5 Limitations

There were several limitations in this study which may have affected the outcomes. First and foremost, based on the verbal response of participants, the level of DOMS experienced was considerably different when it is compared between upper- and lower-body. To some extent, the upper-body may have been exercised differently than the lower-body during the DOMS protocol, which could have potentially been for several reasons. Participants reported the leg pad caused discomfort as it did not conform to the lower leg and tended to dig into the participant's leg. Because of the lack of comfort, participants could have been unable to maximally contract against the arm of the dynamometer. Motivation also plays a substantial role in the DOMS protocol; participants might have been using pacing strategies to distribute their energy despite the instructions given to produce maximal force for each repetition. Understanding instructions may also have been a limitation within this study when it came to taking PPT measurements, as some participants may have tolerated pain rather than reporting the point when pressure changed to pain. This in turn could have led to greater PPT values, thereby affecting the results. Finally, some participants reported the strap used to secure the knee prevented full knee extension, which may have hindered their ability to produce force.

3.6 Conclusion

To the best of our knowledge there have been no studies examining whether there is a difference in DOMS induced PPT between the biceps brachii and vastus lateralis after the

application of a topical menthol analgesic. Additionally, there is limited research illustrating the differences in PPT following the application of topical analgesics. This study shows that maximal eccentric contractions of the biceps brachii induce greater DOMS perceived pain compared to the vastus lateralis and that PPT is higher in the vastus lateralis compared to the biceps brachii, both before and after topical analgesic application. Furthermore, topical analgesic appears to have a greater positive effect on the biceps brachii, increasing post-DOMS PPT values closer to baseline compared to vastus lateralis. Oftentimes comparison of the literature involves looking at different intensities and types of exercise with different participant groups, among others, making it difficult to properly compare findings. Therefore, further research is needed using the same intensities and types of exercises on the same participant group to look at DOMS and PPT in various muscle groups simultaneously. The findings of our study, however, help shed light on how topical analgesics affect different muscles, which can have practical implications in terms of prescribing the use of menthol products as a potential treatment for DOMS.

3.7 List of tables

Table 1: PPT values for BB and VL during tested time periods for both conditions.

Time Period	VL PPT (lbs) (Topical) (Mean ± SD)	VL PPT (lbs) (Placebo) (Mean ± SD)	BB PPT (lbs) (Topical) (Mean ± SD)	BB PPT (lbs) (Placebo) (Mean ± SD)
Pre-DOMS	28.18 ± 14.33	23.36 ± 7.64	15.49 ± 8.10	13.16 ± 4.31
Post-DOMS	29.22 ± 14.90	23.80 ± 7.57	16.44 ± 7.53	14.33 ± 4.56
Pre-gel application	20.92 ± 16.29	14.58 ± 6.60	8.34 ± 2.37	7.20 ± 2.55
15 min post-gel	25.75 ± 17.65	17.11 ± 7.00	12.53 ± 7.07	7.79 ± 2.87
20 min post-gel	26.20 ± 18.01	16.86 ± 5.88	12.90 ± 7.49	8.82 ± 4.23
25 min post-gel	24.91 ± 15.70	16.75 ± 5.78	12.49 ± 7.19	8.42 ± 3.73
30 min post-gel	24.78 ± 16.40	16.47 ± 4.45	12.84 ± 5.86	7.97 ± 3.98
35 min post-gel	24.74 ± 15.36	16.83 ± 4.99	14.12 ± 6.36	8.45 ± 3.02
40 min post-gel	25.51 ± 16.04	17.31 ± 4.98	14.11 ± 7.63	8.25 ± 2.85
45 min post-gel	24.85 ± 16.06	17.20 ± 4.06	14.45 ± 8.24	8.94 ± 3.14
50 min post-gel	24.66 ± 15.89	16.39 ±3.43	14.15 ± 7.52	8.68 ± 2.15
55 min post-gel	24.31 ± 15.93	17.01 ±4.16	14.44 ± 7.91	8.46 ± 2.17
60 min post-gel	23.70 ± 15.55	17.45 ± 3.70	13.32 ± 6.84	8.81 ± 2.09

Table 3: Mean rate of perceived exertion during the DOMS protocol for the BB and VL.

Set Number	VL RPE (Mean ± SD)	BB RPE (Mean ± SD)
Set 1	5.57 ± 1.87	5.36 ± 1.08
Set 2	6.64 ± 1.91	6.86 ± 1.23
Set 3	7.43 ± 1.55	7.57 ± 1.18
Set 4	7.86 ± 1.46	8.21 ± 0.97
Set 5	8.43 ± 1.50	9 ± 1.04

Table 4: Mean peak torque achieved during each set of the DOMS protocol for the BB and VL.

Set Number	VL Peak Torque (Nm) (Mean ± SD)	BB Peak Torque (Nm) (Mean ± SD)
Set 1	240.51 ± 88.21	55.24 ± 25.82
Set 2	241.57 ± 93.13	47.65 ± 20.28
Set 3	245.21 ± 91.61	50.35 ± 23.45
Set 4	233.78 ± 78.58	43.82 ± 18.70
Set 5	234.30 ± 79.39	43.09 ± 19.25

3.8 List of figures

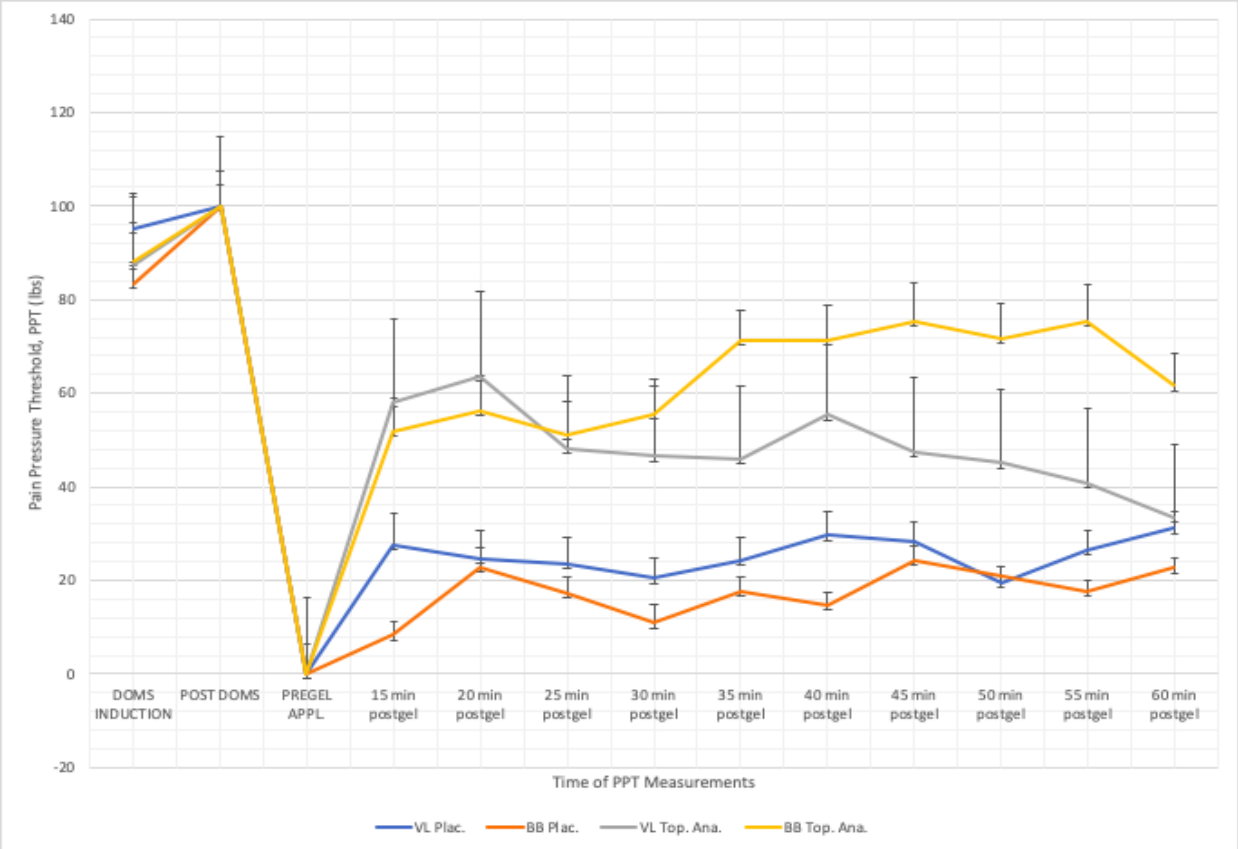

Figure 2: Normalized mean PPT values for the BB and VL pre-DOMS induction, post-DOMS, pre-gel application and all-time intervals post-gel.

3.9 References

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Appendix 1: Data Collection Sheet

DOMS INDUCTION

Participant: Date: Time:

Activity level:

Sex	Age	Height	Weight	Leg Dominance	Arm Dominance

Arm Tested: Dominant or non-dominant

Leg Tested: Dominant or non-dominant

Which was tested first upper body/ lower body? _____ Upper body_____

Pre DOMS PPT: Arm

Trial 1	Trial 2	Trial 3	VAS

Pre DOMS PPT: Leg

Trial 1	Trial 2	Trial 3	VAS

Warm-up: Leg cycling/Arm cycling

DOMS Induction

Post DOMS PPT: Arm

Trial 1	Trial 2	Trial 3	VAS

Warm-up: Leg cycling/Arm cycling

DOMS Induction

Post DOMS PPT: Leg

Trial 1	Trial 2	Trial 3	VAS
17.1	13.4	13.0	0

POST-SESSION

Participate: Date: Time:

15 minute activation period Bottle Number: _____

PRE-BIOFREEZE APPLICATION

Segment	Trial 1	Trial 2	Trial 3	VAS

(15) 0 Minute

Segment	Trial 1	Trial 2	Trial 3	VAS

(20) 5 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(25) 10 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(30) 15 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(35) 20 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(40) 25 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(45) 30 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(50) 35 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(55) 40 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

(60) 45 minutes

Segment	Trial 1	Trial 2	Trial 3	VAS

RPE SCALE

Sets	**Arms**	**Legs**
Set 1	**6**	**8**
Set 2	**7**	**10**
Set 3	**8**	**10**
Set 4	**8**	**10**
Set 5	**9**	**10**

Appendix 2 Informed Consent Form

Title: Comparison of the upper body and lower body pain perception after the application of menthol analgesic for the recovery of DOMS

Researcher(s): Sara Mirzabeigi Fini
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Supervisor(s): Dr. Duane Button, PhD
Human Kinetics and Recreation, Memorial University of Newfoundland
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You are invited to take part in a research project entitled:
*"*Comparison of the upper body and lower body pain perception after the application of menthol analgesic for the recovery of DOMS"

This form is part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. It also describes your right to withdraw from the study. In order to decide whether you wish to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. This is the informed consent process. Take time to read this carefully and to understand the information given to you. Please contact the researcher, Shahab Alizadeh, if you have any questions about the study or would like more information before you consent.

It is entirely up to you to decide whether to take part in this research. If you choose not to take part in this research or if you decide to withdraw from the research once it has started, there will be no negative consequences for you, now or in the future. This research is not a course

requirement, and that the decision to participate, or not, or to withdraw will not influence student grades or other references / recommendations.

Introduction:

I, Sara Mirzabeigi Fini, Shahab Alizadeh, Catherine Murrin, Hailey Fudge, Kelly Jewer, Melanie Lapierre, Nahara Herat, and Stephanie Foley are students at Memorial. We are part of the School of Human Kinetics and Recreation. As part of their Honours Theses and my Postdoctoral research, we are conducting research under the supervision of Dr. Duane Button, PhD.

Purpose of Study:

The purpose of this study is to compare the difference of upper body to lower body response to pain after the application of a menthol-based analgesic, 48 hours after the induction of DOMS.

What We Will Do in this Study:

You will complete two sessions with 48 hours between both sessions. In the first session, participants were required to complete the delayed onset muscle soreness (DOMS) protocol. Pain pressure threshold (PPT) measurements were obtained for the bicep brachii and quadriceps femoris prior to DOMS induction and immediately after. During the second session, PPT was obtained on arrival after which, one of the condition menthol analgesics was applied to the muscle groups. After a 15-minute activation period, PPT was measured every 5 minutes up to 60 minutes (45 minutes of measurements with a 15 minute of activation period).

Length of Time:

The first session will take approximately 2 hours and the last session will take 2 hours (a total of two sessions) with a total duration of 4 hours

Withdrawal from the Study:

You are free to withdraw from the present study at any point throughout the duration of the study and any data collected up to the point of withdrawal will be discarded and destroyed. After data collection has ended you can request the removal of your data up until four weeks after your final data collection.

Possible Benefits:

There are no direct benefits to you. The current research may benefit physicians, athletes, and the general population by increasing the pool of research.

Possible Risks:

Physical risks include strain or soreness of the quadriceps and biceps brachii. For some individuals who are not highly trained, there could be some residual muscle discomfort, which would recede after continued exercise. In order to measure the PPT a transient sense of pain

would be induced which has no serious implications. To avoid injury participants will be asked to warmup for 5 minutes. In order to minimize risk, all high intensity contractions will be supervised by an investigator all of whom are trained in first aid and CPR. Additionally, numbers for emergency services will be on hand in case medical attention is necessary and the participants would be referred to student wellness and counselling center.

Confidentiality:
The ethical duty of confidentiality includes safeguarding participants' identities, personal information, and data from unauthorized access, use, or disclosure. Certain data such as PPT and anthropometric information will be anonymized. Data will be stored in physical form and digitally in Dr. Button's office.

Anonymity:
Anonymity refers to protecting participants' identifying characteristics, such as name or description of physical appearance.

Every reasonable effort will be made to ensure your anonymity. You will not be identified in publications without your explicit permission. All data will be anonymized and kept confidential.

Use, Access, Ownership, and Storage of Data:
All data will be stored in hardcopy and password-protected digital copy in Dr. Duane Button's office at Memorial University of Newfoundland. Consent forms will be stored separately from participant data in a locked cabinet in Dr. Duane Button's office. Data access will be limited to Dr. Duane Button and investigators. Data will be kept for a minimum of five years, as required by Memorial University's policy on Integrity in Scholarly Research.

Reporting of Results:
Data potentially may be published in a thesis and online journal articles. Published data will contain no personally identifying information.

Sharing of Results with Participants:
Upon completion of the study, any study results will be available to the participants in the published work or upon request via email.

Questions:
You are welcome to ask questions before, during, or after your participation in this research. If you would like more information about this study, please contact:
Sara Mirzabeigi Fini, mmirzabeigif@mun.ca or Dr. Duane Button, dbutton@mun.ca.

The proposal for this research has been reviewed by the Interdisciplinary Committee on Ethics in Human Research and found to be in compliance with Memorial University's ethics policy. If

you have ethical concerns about the research, such as the way you have been treated or your rights as a participant, you may contact the Chairperson of the ICEHR at icehr@mun.ca or by telephone at 709-864-2861.

Consent:

Your signature on this form means that:

- You have read the information about the research.
- You have been able to ask questions about this study.
- You are satisfied with the answers to all your questions.
- You understand what the study is about and what you will be doing.
- You understand that you are free to withdraw participation in the study without having to give a reason and that doing so will not affect you now or in the future.
- You understand that if you choose to end participation **during** data collection, any data collected from you up to that **point will be destroyed**.
- You understand that if you choose to withdraw **after** data collection has ended, your data can be removed from the study up to four weeks after your final data collection.

By signing this form, you do not give up your legal rights and do not release the researchers from their professional responsibilities.

Your Signature Confirms:

☐ I have read what this study is about and understood the risks and benefits. I have had adequate time to think about this and had the opportunity to ask questions and my questions have been answered.

☐ I agree to participate in the research project understanding the risks and contributions of my participation, that my participation is voluntary, and that I may end my participation.

☐ A copy of this Informed Consent Form has been given to me for my records.

_____ _____

Signature of Participant Date

Researcher's Signature:

I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____ _____

Signature of Principal Investigator Date