Effects of Instrument Assisted Soft Tissue Mobilization on Physiological and Structural Properties of Human Skeletal Muscle

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William Hawkins

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M.Ed., Wichita State University, 2012

B.A., Wichita State University, 2010

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Chair: Dr. Phillip Gallagher

Dr. Trent Herda

Dr. Joseph Weir

Dr. Phillip Vardiman

Dr. Brian Ackley

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The dissertation committee for William Hawkins certifies that this is the approved version of the following dissertation:

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Chair: Dr. Philip Gallagher

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Abstract

INTRO Instrument Assisted Soft Tissue Mobilization (IASTM) is a popular treatment technique to reduce pain, help improve functional range of motion, and corresponding functional task completion. It has been reported both anecdotally and through controlled-clinical trials to evoke acute changes in skeletal muscle physiology through a variety of proposed mechanisms. However, the efficacy of IASTM has been called into question particularly as it pertains to its ability to improve skeletal muscle and connective tissue pathologies relative to traditional therapies including: stretching, light exercise, and therapeutic ultrasound. The purpose of this three-study investigation was to elucidate the effects of IASTM on human skeletal muscle as well as to examine possible mechanisms of change. **METHODS** To examine the efficacy of IASTM we designed three experiments. The first experiment tests the effects of IASTM on IL-6 and TNF-α cytokine expression in human skeletal muscle using Bergstrom needle muscle biopsies. Pro-inflammatory cytokines were of interest as they have been suggested to mediate positive outcome measures associated with IASTM. The second investigation was designed to examine the dose response in the presence of two different forces being administered. This study was largely designed as a follow up of an IASTM dose response experiment that was carried out in rodents. The final investigation was designed to examine the effects of IASTM on the architecture of skeletal muscle using diagnostic ultrasound. For this investigation both hamstrings range of motion restricted and age appropriate controls were used to examine if IASTM only elicits benefit in pathological tissue. **RESULTS** Results from this multi-study examination of the effects of IASTM have suggested that IASTM may not be the most αefficacious treatment available for degenerate soft-tissue. Our three investigations found no

changes in MTS, PROM, MVC-PT, myokine expression, perception of functional ability as measured by the PFAQ, muscle quality (echo intensity), pennation angle or hip ROM.

DISCUSSION The results from these three investigations suggest IASTM may not be efficacious especially when compared to more cost effective self-therapies including stretching and light exercise. However, the current investigations at hand were limited by sample size and the fact that two of the investigations were carried out in non-pathological tissue. Literature review reveals that IASTM can elicit change in degenerate muscle tissue through a fibroblast mediated pathway. Future investigations should use larger sample sizes and special populations including older adults and adults suffering from chronic tendinopathy.

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Chapter 1: Introduction

Injury to human skeletal muscle tissue is a common occurrence and is caused by a variety of mechanisms. Soft tissue pain and corresponding loss of function often lead to missed work, sports participation, and other lifestyle disturbances. For this reason, it is vital to understand the pathophysiology of injured skeletal muscle and connective tissue as well as the mechanisms by which healing can best be facilitated.

It is generally believed that multiple pathophysiological factors are at play in acute or chronically damaged skeletal muscle. First, in instances of muscle damage there is the presence of over-stretched sarcomeres within the myo-fibril structure. Second, there appears to be damage to the excitation coupling system. It remains controversial, which of these two phenomena represents the point of primary damage. Some researchers believe that excessive sarcomeric stretching is the primary cause of loss of function, still others have declared that decreased passive muscle tension and other markers of dysfunction following muscle damage is caused by disturbance of the excitation-contraction coupling system. In addition to sarcomeres being stretched and disruption of the excitation-contraction, coupling system it has also been hypothesized that muscle damage entails micro-tears to the contractile proteins of the sarcomere. Damage to actin, myosin, titin, or any of the proteins associated with the sarcomeric scaffolding could obviously disturb normal physiological function. With these three pathophysiological considerations, it is logical that the aim of post injury rehabilitation should be to return sarcomeres to normal, resting length, to return excitation-contraction coupling to normalcy and to initiate skeletal muscle protein synthesis. These outcome measures all require muscle biopsy and subsequent molecular biology experimentation to observe but in-vivo

derivatives of these do exist and would include variables associated with whole muscle force production.

Due to the fact that skeletal muscle injury is a leading cause of missed work. For this reason, the health sciences community is perpetually developing and evaluating new techniques designed to aid in expedited return of function and decreased pain perception. Traditionally these rehabilitation efforts are broken up into two broad subcategories. First, there is the acute injury phase of treatment where the general focus of treatment is to decrease pain, swelling and inflammation. This is generally carried out by rest, cryotherapy, compression, elevation of the injured region and pharmacological interventions. These pharmacological treatment regimens generally include the use of non-steroidal ant-inflammatory medications (NSAID's) and occasionally opiates depending on the level of perceived pain.

The second general phase of rehabilitation begins when signs of acute injury are diminishing or have completely passed. During this phase of rehabilitation the general treatment goal is to return the affected region to its normal, pre-injury functional ability when possible or to minimize permanent loss of function. This includes therapy techniques and corrective exercises centered on increasing muscular strength, increasing muscular endurance increasing range of motion. At this stage in recovery techniques like instrument assisted soft tissue mobilization (IASTM) are often used. IASTM is an umbrella term for techniques that feature clinicians using specialized instruments to mechanically load degenerate soft tissue in a massage like manner.

It has been reported both anecdotally and through a small body of controlled, clinical trials that IASTM alters the chemistry of soft tissue. However, it is unclear which recovery signaling pathways IASTM is capable of initiating as well as whether or not IASTM can alter pro-inflammatory signaling. Further, there is controversy as to whether or not altering the body's natural inflammation pathways is beneficial or deleterious to long term growth and repair. More controlled, clinical trials are needed to address these research questions.

Mechanical loading of soft tissue whether by injury, massage or IASTM affects the extracellular matrix (ECM) of skeletal muscle. This phenomena is most simply demonstrated by coordinated ECM fibroblast proliferation. Fibroblasts are specialized cells that are present in the extracellular matrix of skeletal muscle as well as other cells. Skeletal muscle fibroblasts that become mechanically stimulated produce vital structural proteins including collagen and elastin as well as signaling molecules including cytokines and growth factors. It has been demonstrated that IASTM can cause up-regulated fibroblastic activity. Both injury and IASTM increase the number of fibroblasts in skeletal muscle fascia leading to downstream signaling cascade activation. In cases of acute injury up-regulated pro-inflammatory cytokine expression has been observed. The downstream signaling effects of IASTM are less clear. It has been hypothesized that IASTM initiates similar signaling cascades to that of an acute injury just on a smaller, more beneficial scale. However, it has also been hypothesized that IASTM causes anti-inflammatory signaling following injury. These theories are apparently contradictory and though many clinicians have found IASTM treatments efficacious the exact mechanism by which positive outcome measures have been observed is poorly understood.

To this end we found it prudent that our first study examine how IASTM effects whole muscle performance as well as cytokine expression. We believed that by procuring –pre and –post IASTM muscle biopsies we could determine if benefits reported concerning IASTM are initiated via a cytokine mediated pathway. Upon completion of this study we saw that a possible confounding component of our study design and most study designs in the literature is that there are currently no research groups quantifying or controlling IASTM pressure volume administered to human subjects. Further, no researchers were exploring IASTM dosage parameters in human subjects. Upon completion of this study we determined to begin working in populations in which IASTM is clinically indicated. In order to carry this out we found it more prudent to recruit subjects with existing loss of muscle function rather than inducing it through a muscle damage protocol.

1.1 Study 1- IASTM and Cytokine IL-6 signaling

Prior to investigating the effects of IASTM on muscle damage or other chronic pathologies we found it prudent to study the effects of IASTM in healthy tissue. To carry this out we used a repeated measures study with a within subject control. In addition to measuring both active neuromuscular and strength properties, biopsies were taken to examine pro-inflammatory signaling.

First, this allowed us to make sure our treatment wasn't detrimental to skeletal muscle tissue prior to investigations in populations that are already suffering from chronic or acute loss

of function. Secondly, this allowed us to see if IASTM evokes physiological changes through cytokine mediated pathways. Of particular interest was Interleukin 6 (IL-6) as it is commonly studied in rehabilitation sciences research due to its pro-inflammatory signaling properties. We found it vital to study the effects of IASTM on cytokine expression as it has been proposed that IASTM may cause benefit by evoking beneficial inflammation. Upon completion of this study it became evident to us that proper control and study of dosage parameters was a common gap in the literature that needed to be addressed.

1.2 Study 2- IASTM Graded Pressure

Further complicating the questions surrounding the mechanism by which IASTM treatments allegedly improve skeletal muscle function is the inexact nature of current dosage parameters. As previously described, IASTM is an umbrella term for a wide variety of treatments that mechanically load skeletal muscle using instrumentation. These treatments are generally carried out by certified athletic trainers or licensed physical therapists. Generally speaking, clinicians abide my manufacturer instructions concerning dosage parameters for treating a given injury. Most IASTM tooling manufacturers have their own protocol recommendations depending on intended use. Even if frequency of treatment and volume dosage parameters are followed by the clinician there isn't currently a way for clinicians to know how much pressure is being applied to the affected area. For this reason, if there is a dose/response relationship between IASTM and positive outcome measures it is entirely possible that some clinicians are within the optimal dosage parameters while others could be causing sub-optimal healing or even evoking deleterious results.

Further, inexact dosage parameters make comparison of one research study to the next difficult. Though controlled clinical trials featuring IASTM usually do report IASTM dosages used in-vivo, many of the research papers published by clinicians do not. Unfortunately, most of the studies that have controlled for or at minimum quantified pressure forces during IASTM treatments have occurred in in-vivo rodent models. While these rodent models are highly controlled and IASTM dosages are precisely administered the carry over between rodents and humans will always be questioned.

Not until recently has a research cohort attempted to quantify pressure forces administered during an in-vivo IASTM controlled trial. This tooling developed at the University of Kansas is essentially a retro-fitting in line, load cell device that could potentially integrate with most commercially available IASTM tools. This development should set the bar as far as what is expected of laboratory methods for lab groups who wish to publish in-vivo IASTM controlled. Upon completion of this paper it became evident that we needed to begin clinical trials in special populations including those who have chronic musculo-skeletal restrictions.

1.3 Study 3- IASTM as a Treatment for ROM Restriction

Another common application of IASTM can be observed in ROM restricted populations. ROM restriction is generally caused and perpetuated by sedentary living. The traditional treatment for such conditions is increased activity and possibly a stretching program. While this decreased muscular pliability may initially appear innocuous it is highly correlated with and potentially the cause of many forms of chronic pain including low back and joint pain.

It remains unclear if IASTM or even massage alter range of motion in acute applications. For this reason it is vital that acute study designs be utilized. Further, in addition to tracking changes in ROM caused by IASTM it is vital that muscle-tendon stiffness properties, muscle length and pennation angle data be collected to speak to possible mechanisms of change. It is for this reason that our most recent investigation examined markers of skeletal pliability using both passive ROM and torque data as well as in-vivo imaging data collect sonographically.

Chapter 2: Literature Review

2.1 Introduction:

Instrument assisted soft tissue mobilization (IASTM) is a technique commonly used in the field of physical therapy and is generally carried out by using metal or plastic tools with smooth edges to mechanically compress the affected muscle and tendon in a massage-like motion (Hammer, 2008). IASTM is clinically indicated when muscular adhesions and scar tissue lead to restricted range of motion. Irrespective of its popularity, the efficacy IASTM has largely been supported by anecdotal findings rather than through controlled research. Further, most published research featuring human subject model designs are case studies written by clinicians in the field. Thus, it is difficult to draw application from these models because they are purely observational.

The alleged benefits of IASTM range from increased blood flow, increased venous return, decreased cortisol production and increased ROM due to decreased scar tissue (Hammer, 2008). Unfortunately, the small body of literature that does exist in human skeletal muscle do not explore mechanism(s) of change. The few randomized, controlled, clinical trials that do exist are generally featuring animal models that explore the mechanism of change, but are not necessarily directly translational. For this reason. It is difficult to draw conclusions concerning the efficacy if soft tissue mobilization and further research should be done.

2.2 Origins of IASTM

In ancient Greece and Rome, the use of small, curved metal tools known as strigils were used to scrape dirt and sweat from the body but anecdotally helped with generalized muscle soreness as well (Hammer, 2008). Similarly, Gua Sha a healing technique that originated in Asia was being developed around the same time (Braun et al., 2011; Hammer, 2008; Lee, Choi, Kim, & Choi, 2010). Gua Sha was generally carried out by use of animal bone and horns for instrumentation (Braun et al., 2011; Lee et al., 2010). Despite the fact that IASTM has been around since ancient civilizations, it is still unclear if measurable benefit is derived from IASTM treatment. Further, the mechanism by which IASTM changes the physical and chemical properties of human skeletal muscle is unknown. IASTM is a technique that was invented based on the anecdotal benefits of massage therapy. However, further research is needed to explore IASTM and its effect on human skeletal muscle.

Instrument assisted soft tissue mobilization uses specifically designed solid instruments that are generally made out of aluminum alloys. These instruments are often preferred because they allow for more pressure to be applied for longer periods of time without the clinician becoming fatigued. This is particularly helpful in the treatment of tendinopathy as many tendons are difficult to manually palpate. It has been anecdotally reported that IASTM ultimately allows the clinician to cause micro-trauma in a more precise and localized fashion when compared with manual massage. It is generally believed that this micro trauma initiates Type-1 collagen synthesis and re-alignment via a prostaglandin mediated pathway (Langberg et al., 2007).

2.3 Effects of Soft Tissue Mobilization on Whole Muscle Physiology

2.3.1 Soft Tissue Mobilization and joint ROM

One of the most often proposed benefit of soft tissue mobilization is that it has the ability to increase ROM of affected joints. Passive range of motion has been defined as the range of motion available to a joint or series of joints (Gleim & McHugh, 1997) and is usually measured manually with a goniometer. The majority of studies that have analyzed the effects of soft tissue mobilization on muscle and connective tissue have been based on range of motion measurement as a primary outcome measure (Crosman, Chateauvert, & Weisberg, 1984; Hernandez-Reif, Field, Krasnegor, & Theakston, 2001; Huang et al., 2010; Leivadi et al., 1999; Morien, Garrison, & Smith, 2008; Nordschow & Bierman, 1962; Wiktorsson-Moller, Öberg, Ekstrand, & Gillquist, 1983). These studies suggest that soft tissue mobilization like massage and IASTM may be beneficial for increasing joint ROM (Crosman et al., 1984; Diana Hopper et al., 2005; D Hopper et al., 2005; Huang et al., 2010; Leivadi et al., 1999; Morien et al., 2008). For example, one study found an increased joint angle and no associated increase in passive tension or EMG following massage (Huang et al., 2010). This is an interesting finding as it has been hypothesized that at least a portion of the increased ROM comes from neural responses. It was suggested that modified stretch perception, increased stretch tolerance, or increased compliance of the hamstrings as possible mechanisms for this observed increase in joint ROM (Huang et al., 2010). Unfortunately, Huang et al. and most of the other papers at hand didn't propose a definitive hypotheses in regards to the mechanism of ROM change.

Still other studies have shown conflicting data demonstrating that massage and potentially other forms of soft tissue mobilization may not effect ROM (Barlow et al., 2004;

Hilbert, Sforzo, & Swensen, 2003; Jourkesh, 2007; Vardiman et al., 2015). For example, previous reports have suggested that 15 minutes of massage elicit no significant ROM changes in adolescent soccer players (Jourkesh, 2007), or active adults (Barlow et al., 2004). Regardless of the efficacy of clinician mediated soft tissue mobilization to increase ROM, it unlikely to rival more traditional treatments if ROM restricted patients are able to improve ROM through the use of cheaper modalities like stretching and self-myofascial release.

When the effects of massage on lower extremity range of motion were compared with other pre-exercise modalities such as dynamic warm-up and stretching, massage increased only ankle dorsiflexion range of motion while stretching significantly increased all lower extremity range of motion measurements (Wiktorsson-Moller et al., 1983). Thus, the practicality of clinician directed soft tissue mobilization has been called into question due to the superior efficacy and cost effectiveness of stretching.

2.3.2 Efficacy of IASTM in treatment of muscle damage

In addition to increasing joint ROM, it has been hypothesized that IASTM may be beneficial in the treatment of post exercise muscle damage. One of the most prolific research cohorts on the topic at hand have devised a protocol for testing soft tissue mobilization in rabbit tibialis anterior (Butterfield, Zhao, Agarwal, Haq, & Best, 2008; Crawford et al., 2014; Haas, Best, Wang, Butterfield, & Zhao, 2012; Haas et al., 2013; Waters-Banker, Butterfield, & Dupont-Versteegden, 2014). One research group has set out to answer multiple research questions including how IASTM effects muscle recovery following bouts of intense eccentric exercise (Butterfield et al., 2008). The in vivo animal model allows the researchers to induce

muscle damage through an eccentric loading protocol while the rabbit is anesthetized. While anesthetized, the tibialis anterior is maximally stimulated by a surgically implanted stimulator while the muscle is being stretched through the ankle joints ROM effectively causing repeated maximal eccentric contractions (Crawford et al., 2014).

Butterfield et al. have used eccentric loading prior to IASTM and massage treatments in order to evoke muscle soreness/damage as massage and IASTM have both been indicated in such cases. They found that 30 minutes of cyclic compressive loading of the tibialis anterior has the ability to improve skeletal muscle recovery following a bout of eccentric exercise (Butterfield et al., 2008). Animals who received soft tissue mobilization demonstrated statistically significant increases in recovery of peak torque following muscle damage as well as decreased leukocyte infiltration (Butterfield et al., 2008).

2.3.3 Pathophysiology of Skeletal Muscle Damage

In order to fully understand the efficacy of IASTM to treat muscle damage one must first understand the pathophysiology of injured skeletal muscle. It is generally agreed upon that in all forms of skeletal muscle damage there are at least three prominent common pieces of cellular and subcellular dysfunction present. First, there is the presence of elongated and otherwise disrupted sarcomeres within the myofibrils. Second, muscle damage causes disruption in the excitation-contraction coupling system within the sarcomere. Third, there is damage to contractile proteins as well as cytoskeletal elements of the myofibril. It's is controversial which of these represents the primary event in muscle damage with several authors believing sarcomeric deformation is the primary event (Morgan & Allen, 1999; Proske &

Morgan, 2001) and at least one author believing that 75% or more of the decline in normal physiological contractile properties of muscle come from disruption to the excitation-contraction coupling system (Warren, Ingalls, Lowe, & Armstrong, 2001). Still some research suggests that loss of function following muscle damage may be caused primarily by damage to cytoskeletal elements of the myofibril Warren et al. rounds out his excitation-contraction coupling theory by suggesting that the remaining loss of function seen for 24-72 hours post injury is caused by damage to the tension bearing elements within the muscle (Warren et al., 2001). These include the contractile proteins actin and myosin as well as the cytoskeletal proteins.

2.3.4 The Role of Excitation-Contraction Coupling in Muscle Damage Induced Loss of Function

Evidence that much of the physiological deficit caused by muscle damage stems from damage to the excitation-contraction coupling system stems from observational studies that have occurred in rodent muscle tissue. For example, it has been demonstrated that the deficit in passive tension of skeletal muscle caused by muscle damage can be partially recovered with caffeine supplementation (Balnave & Allen, 1995; Warren et al., 1993). Caffeine does not alter sarcomere length or cytoskeletal elements but it has been suggested that it may have the ability to alter excitation-contraction coupling (Balnave & Allen, 1995; Warren et al., 1993). This hypothesis has been tested experimentally. In the first of these studies passive muscle tension was nearly completely recovered by introduction of a 50 mm caffeine solution. The proposed mechanism by which caffeine alters resting muscle tension is through a Ca^{2+} dependent

pathway. Caffeine which causes release of Ca^{2+} from the sarcoplasmic reticulum and eventually leads to altered excitation contraction coupling in skeletal muscle. In the second, 10 mm caffeine was used to potentiate tension in single fibers in response to direct electrical stimulation. It was concluded that in mouse fibers changes in E-C coupling may be a major contributor to the observed fall in tension after eccentric contraction induced muscle damage (Allen, 2001).

2.3.5 The Roll of Cytoskeletal Scaffolding Damage in Muscle Damage Induced Loss of Function

Cytoskeletal scaffolding proteins including titin, nebulin, and dystrophin form a complex structure that anchors the sarcomere within the myofibril and allow for transmission of force. The sarcomeric structure exists in such a way that transmission of contractile forces laterally across the fiber as well as from fiber to fiber takes place (Banus & Zetlin, 1938; Ramsey & Street, 1940). It is therefore a logical assertion that damage to this cytoskeletal scaffolding could lead to alterations in whole-muscle contractile properties and subsequently lead to lifestyle disturbances. It has been well documented that muscle damage effects these cytoskeletal proteins (Friden & Lieber, 2001; Lieber & Fridén, 1999; Lieber, Woodburn, & Friden, 1991). Lieber and Friden have demonstrated by way of biopsies and histochemical preparations that eccentric exercise induced muscle damage disturbances in desmin can be observed as little as 5 minutes most damage. Other research has shown that cytoskeletal scaffolding damage continues long after the initial muscle damage invoking event (Allen, 2001). This has lead researchers to the following hypothesis concerning damage of cytoskeletal

proteins (Lieber & Fridén, 1999). First, as previously described, overextended sarcomeres cause a highly localized increased release of [Ca^{2+}]. Next, elevated [Ca^{2+}] causes activation of myo-cellular proteases such as calpain which hydrolyses desmin; (iii) this loss of the structural support of desmin plays a large role in debilitating the rest of the sarcomeric scaffolding. Critics of this hypothesis will quickly cite the observation that markers of muscle damage can be seen immediately following a single very large stretch (Brooks, Zerba, & Faulkner, 1995). The fact that markers of cytoskeletal element muscle damage can be seen so quickly make it unlikely to be caused by a calcium initiated proteolytic pathway (Brooks et al., 1995). It has been hypothesized that the damage to cytoskeletal scaffolding following muscle damage is overstated because of in-precise measurement techniques (Allen, 2001). It is entirely plausible that when cytoskeletal proteins become stretched (through muscle damage) the conformational change alters antibody binding chemistry. This theory gains particular traction in light of the observation that titin staining increases following muscle damage (Allen, 2001). Whether or not there is a functional reason for this or if this is simply due to an alteration in binding chemistry is unclear (Lieber, Thornell, & Friden, 1996)

2.3.6 Immediate vs Delayed IASTM in Treatment of Muscle Damage

Another valuable question the Ohio State group has set out to answer centers around the timing of soft tissue mobilization treatments. Though soft tissue mobilization is generally accepted as beneficial following muscle damage, it is less clear whether the treatments should come immediately following the muscle damage inducing exercise or if it should be delayed. In order to test this research question the previously described vivo animal model was utilized.

This allowed the researchers to induce muscle damage through an eccentric loading in a controlled and measurable manner (Crawford et al., 2014).

The researchers hypothesized that soft tissue mobilization when performed at an optimal magnitude, duration and loading frequency following an intense bout of eccentric exercise would lead to both acute (daily) and cumulative (over four-day treatment) changes in the muscle's viscoelastic properties, in particular a reduction of both the instantaneous elastic response and apparent viscosity of the tissue (Crawford et al., 2014). Researchers found that the prescribed eccentric exercise protocol increased instantaneous elastic response (AG_0) a variable commonly associated with muscle stiffness in all groups ($P < 0.05$). The same load cell/strain gauge apparatus that was used to control the pressure of the IASTM during the treatment was used to measure $AG_{0\,in}$ in vivo. This served to validate that the Ohio State groups' eccentric muscle damage protocol was effective in altering the whole muscle physiology of the TA. Recovery of AG_0 was not significant in the immediate or delayed group compared to the control group following four days of massage. However, within-day (pre/post massage) analysis revealed a significant decrease in AG_0 in both massage groups. These findings suggest that soft tissue mobilization following eccentric exercise has a greater effect on reducing muscle stiffness, estimated by AG_0, within-day rather than affecting recovery over multiple days. Further, these data suggest that soft tissue mobilization immediately following muscle damage is no more efficacious then the same treatment several hours later.

2.3.7 IASTM and Muscle/Tendon Stiffness in Humans In-Vivo

As just described, one proposed benefit of IASTM is that it decreases muscle/tendon stiffness. However, only two in-vivo human model studies have examined the effects of massage/soft tissue mobilization on passive muscle/tendon stiffness. In one study a 10-minute effleurage massage had no significant effects on passive gastrocnemius stiffness properties when compared with 10-minutes of rest (Stanley, Purdam, Bond, & McNair, 2001). Authors of this study cited that the pressure of the effleurage treatment administered by the clinician may not have been enough to elicit a physiological response.

In another study IASTM was used to explore the effect of soft tissue mobilization on passive muscle/tendon stiffness of that plantar flexors (Vardiman et al., 2015). Results from this study indicated that IASTM, when performed at standard clinical pressures, did not alter passive muscle/tendon stiffness of the plantar flexors in healthy, college aged participants. Further research is needed to investigate the effects of other soft tissue mobilization techniques.

2.3.8 Soft Tissue Mobilization and Blood Flow

Another proposed benefit of massage is an increase in blood flow. Although anecdotally authors have agreed that massage could increase blood flow, study results have been inconclusive largely because of research study design limitations. Besides having a small sample size (Bell, 1964; Dubrovsky, 1983; Hansen & Kristensen, 1973) most of these studies had no reported statistical analysis, (Bell, 1964; Dubrovsky, 1983; Hansen & Kristensen, 1973) nor did

they use a control group , (Bell, 1964; Dubrovsky, 1983; Hansen & Kristensen, 1973). These limitations make it hard to differentiate the changes in blood flow from normal variation. More importantly, the venous occlusion plethysmograph and Xenon wash-out techniques used in these studies have their own limitations.

Venous occlusion plethysmograph generally demonstrates underestimation of blood flow due to the inflation of the cuff extreme sensitivity to movement artifacts (Shoemaker, Tiidus, & Mader, 1997; P. Tiidus & Shoemaker, 1995). At times the changes of blood flow could not be expressed quantitatively because of these limitations (Hansen & Kristensen, 1973). Moreover, venous occlusion plethysmography cannot be used to measure blood flow during massage but only immediately following the treatment (P. Tiidus & Shoemaker, 1995). Further, the Xenon wash-out technique overestimated blood flow because of the local trauma from injection of the tracer (Shoemaker et al., 1997; P. Tiidus & Shoemaker, 1995; P. M. Tiidus, 1999). Pulsed Doppler ultrasound has been used to investigate muscle blood flow and has indicated that manual massage did not affect blood flow in the muscle after treatment of the muscle (Shoemaker et al., 1997; P. Tiidus & Shoemaker, 1995). However, the ultrasound used in these studies detected changes in the large artery and vein but did not detect microcirculation in muscle that could be affected by massage. Because pulsed Doppler ultrasound can only examine major blood supply it is difficult to draw any conclusion about whether or not soft tissue mobilization causes increased microcirculation the affected region.

In addition to localized changes in blood flow, it now appears soft tissue mobilization techniques may lead to alterations in systemic blood flow (Franklin, Ali, Robinson, Norkeviciute, & Phillips, 2014). Previous research shows that flow-mediated dilation (FMD), the “gold

standard" in-vivo measure of endothelial function, is impaired in healthy but sedentary young adults after an acute bout of strenuous lower extremity resistance exercise involving both eccentric and concentric muscle contractions (Phillips, Das, Wang, Pritchard, & Gutterman, 2011). Franklin et al. set out to explore if this decreased FMD could be attenuated by soft tissue mobilization and the found that it can be (Franklin et al., 2014). In that study, a single lower extremity soft tissue mobilization treatment resulted in the elevation of brachial artery FMD that lasted for 48 hours after exertion-induced muscle injury. This treatment also increased FMD in the absence of exercise (Franklin et al., 2014). These results indicate that soft tissue mobilization may have the ability to improve blood flow, particularly at the macro circulatory level.

2.4 Cellular and Sub-Cellular Effects of IASTM

2.4.1 The Role of Fibroblasts in IASTM Mechano-Transduction

Musculo-tendinous tissue that is compressed by IASTM undergo histochemical modifications that lead to a host of downstream signaling cascades. These downstream signaling cascades are apparently mediated by fibroblast proliferation but the exact mechanotransduction pathway is not fully understood. Musculo-tendinous fibroblast have a number of structural characteristics that make them unique in addition to their ability to produce molecules necessary for muscle and tendon cell structure. Musculo-tendinous fibroblasts are easily identified by their flattened, elongated shape as well as long there elongated nuclei and actin-based cytoplasmic protrusions (Kjær et al., 2009). Tendinous fibroblasts interact with the extra cellular matrix (ECM) by way of an advanced coupling

network that is often referred to as “collagen cross linking” (Kjær et al., 2009). As previously mentioned the exact mechano-transduction pathway by which soft tissue mobilization initiates molecular signaling is not fully understood but it is believed to be initiated by structural proteins located in the extra cellular matrix (ECM) of muscle.

2.4.2 Role of Fibroblast in Growth and Repair

Musculotendinous repair occurs in a three step process: inflammation, proliferation, and remodeling. During the acute inflammatory stage, blood platelets and fibrin cover the musculotendinous wound and fibroblasts and phagocytic cells translocate to the site of soft tissue injury. The fibroblasts begin to produce fibronectin. In the proliferative stage, fibroblasts increase in number and begin synthesizing Type III collagen. The remodeling or maturation stage involves a reduction in cellularity and realignment of collagen fibers. Also collagen production shifts from immature, Type III collagen, to mature, Type I collagen, and fibronectin production decreases (Davidson et al., 1997).

Perhaps the most explored aspect of the effect of IASTM on human skeletal muscle is the dose/response relationship that appears to be present. In the late nineties, research exploring the effect of various pressure loads on skeletal muscle fibroblast proliferation in rat hind-limb tissue was published out of Ball State University (Gehlsen, Ganion, & Helfst, 1999). Gehlsen et al. utilized a popular tendinopathy induction model in which unilateral tendinopathy is chemically induced allowing the other limb(s) to serve as a control group.

2.4.3 Tendinopathy and IASTM Animal Model

In order to examine the effect of IASTM on tendinopathy a common in vivo animal model was utilized. Collagenase injections were administered directly into the achilles tendon of rabbits to induce achilles tendinopathy. This research was novel not only in the sense that the relationship between the mechanical loading of skeletal muscle fibroblast proliferation had never been explored but also in the sense that there were three different pressure groups allowing researchers to explore interaction effects (Gehlsen et al., 1999). These pressure groups were designated: light IASTM (.5 N/mm^2), medium IASTM (1.0 N/mm^2) and extreme IASTM (1.5 N/mm^2). The only group that was statistically significant from the rest in terms of fibroblast activation was the Achilles compromised "extreme IASTM" group. This suggest not only that IASTM benefits appear to be dose dependent but also that unless the high pressure is administered to damaged tissue there may not be any biological effect.

2.4.4 Development of an In-Vivo Human Pressure Quantification Tooling

When studying the effects of IASTM it is vital that IASTM pressure administration volumes be reported. This is due to the fact that many of these molecular signaling responses appear to be dose dependent. However, until recently there has been no device capable of quantifying IASTM pressure administration during in-vivo human trials. This is a gap in the literature. In animal models the relationship between IASTM and tissue response has been shown to be dose dependent in regards to load administered (Gehlsen et al., 1999). Presumably the response to IASTM is dose dependent in humans as well. This dose dependency was first

demonstrated in a fibroblast proliferation model (Gehlsen et al., 1999) however it appears to be present in other variables of interest as well. For example, in addition to fibroblast response being dose dependent, it appears the relationship between mechanical loading of soft tissue, ion kinetics (McNamee, Ingber, & Schwartz, 1993), protein synthesis (Thie, Schlumberger, Rauterberg, & Robenek, 1989), gene expression (Komuro et al., 1991) and release of secondary signaling proteins (Letsou, Rosales, Maitz, Vogt, & Sumpio, 1989) are dose dependent as well.

Pilot data generated in our lab suggest that the IASTM pressure forces administered by experienced clinicians is considerably less than the pressure forces used in the animal models (Table 1). In addition to the differences in mean pressure forces applied, the animal models differ in total IASTM volume. The most commonly used IASTM volume prescription in the animal model literature is 3 proximal to distal strokes and 3 distal to proximal strokes (Gehlsen et al., 1999). Generally IASTM treatments in humans utilize substantially more volume and lower pressure. For this reason it is vital that researchers keep soft tissue mobilization dosage parameters in mind when interpreting results. Differences in outcomes could theoretically be explained solely by differences in load/volume of soft tissue soft tissue mobilization.

Table 1: Dosage Parameters: Comparison of clinical animal/human model pressure administration

Author, Year	Model Summary	Pressure Force Administered	Outcome
(Gehlsen et al., 1999)	Collagenase induced Achilles tendonitis rat model (n=30)	Light IASTM= (.5 N/mm^2) Medium IASTM= (1 N/mm^2) Extreme IASTM= (1.5 N/mm^2)	Increased fibroblast proliferation (p<.05)
(M. T. Loghmani & Warden, 2009)	Bilateral torn MCL rat model (n=51)	Estimated at (1.5 N/mm^2)	Ligament strengthening (p<.05) Ligament stiffening (p<.01)
(M. Loghmani & Warden, 2013)	Bilateral torn MCL rat model (n=30)	Estimated at (1.5 N/mm^2)	Increased tissue perfusion 24h post (p<.05)
(Davidson et al., 1997)	Collagenase induced achilles tendonitis rat model (n=20)	"Considerable pressure"	Increased fibroblast proliferation (p<.05)
(Vardiman et al., 2015)	Human Subjects, controlled trial (n=11)	N/mm^2 equivalent is estimated at 1.0	No changes in ROM or IL-6 expression (p<.05)
Ohio State Group	Rabbit tibialis anterior, eccentric loading (n= varies from study to study)	10 N	Decreased MTS (p<.05) and time to peak torque recovery (p<.05)

2.4.5 IASTM's effect on Collagen Synthesis

A common anecdotal report is that IASTM helps "realign" connective tissue. This ambiguous, anecdotal report is yet to be studied in conjunction with IASTM but there is a body of research that appears to be directly translational to IASTM treatments. The overall

contribution of collagen to whole body protein turnover is unknown, despite the fact that collagen probably contributes 3.5 kg to the lean body mass compared with 12 kg for muscle myo-cellular protein, about which much more is known (Langberg et al., 2007). Regardless of its comparative contribution to total organismal mass it is functionally vital that healthy collagen formation occurs.

Mechanical loading of skeletal muscle and tendon, whether it be compression, stretch, or IASTM elicits largely the same response (Hammer, 2008). As previously described this response begins with the activation and eventual proliferation of fibroblast cells in the extra-cellular matric (ECM). These specialized cells produce collagen, elastin, cytokines and growth factors that aid in the growth and repair process. In instances of extreme mechanical loading of muscle and tendon, as is often seen in injured tissue, the cytokine signaling is markedly pro-inflammatory and this is also seen following therapeutic mechanical loading of muscle and tendon (Hammer, 2008).

Though it is unknown if IASTM initiates collagen realignment it is known that the realignment of collagen tissue is vital for physiologically and biomechanically sound tissue (Alfredson, Thorsen, & Lorentzon, 1999). Classically, it has been thought that collagen synthesis was initiated by inflammatory cell activation and proliferation but more recent, controlled, histological data have demonstrated this is false (Alfredson et al., 1999; Langberg et al., 2007; Movin, Gad, Reinholt, & Rolf, 1997) .

For example, the sports medicine community has long considered prostaglandin E2 elevation to be a powerful initiator of collagen synthesis and therefore encouraged patients to not take prostaglandin inhibiting pharmaceuticals after the acute inflammatory stage of injury.

However, in situ models have demonstrated that prostaglandin activation and proliferation does not correlate with re-alignment (Alfredson et al., 1999). Further, it has been demonstrated that intertendinous glutamate levels correlate with collagen alignment (Alfredson et al., 1999) at a much higher level. This glutamate correlation was groundbreaking as it demonstrates that the presence glycosaminoglycan's must play a viable role in healthy collagen synthesis and re-alignment (Langberg et al., 2007). The glutamate correlation is hard to argue against because subjects with tendinopathy expressed a 4 fold increase in glutamate when compared with healthy controls (Alfredson et al., 1999).

2.4.6 Possibility of ECM Integrin Response

In order for a stimuli such as stretch or strain to result in molecular signaling, physical alterations in the cell membrane and the extra cellular matrix ECM appear to be transmitted through intermediate activating proteins known as integrin's (Carson & Wei, 2000). Integrins in turn activate several intracellular kinases that initiate mechanotransduction signals including focal adhesion kinase (FAK) and the mitogen-activated protein kinase (MAPK) family of proteins. Skeletal muscle is sensitive to several types of stretch (Hornberger, Armstrong, Koh, Burkholder, & Esser, 2005; Kumar, Chaudhry, Reid, & Boriek, 2002), and upon stretch activation, these kinase cascades activate regulatory molecules that largely control protein synthesis, glucose uptake, and immune cell recruitment and activation status (Cara, Kaur, Forster, McCafferty, & Kubes, 2001). Any physiological benefits due to soft tissue mobilization would likely be initiated through mechanical effects on skeletal muscle followed by changes to intracellular regulatory cascades (Crane et al., 2012).

In order to explore the mechanism by which soft tissue mobilization may alter molecular signaling in muscle Crane et al. used a human subject's massage study design. Crane et al. obtained muscle biopsies from 11 young male subjects at rest, immediately after administration of massage to a randomized, single leg and after a 2.5-hour period of recovery. Crane et al. used whole-genome microarrays to screen for expressed genes induced by massage. After identifying functional categories from the array assay, they performed targeted real-time reverse transcription–polymerase chain reaction (RT-PCR), protein signaling analysis (Western Immunoblots), and metabolite quantifications to more completely characterize possible signaling pathways within skeletal muscle that are influenced by massage. Results indicated that massage activated the mechanotransduction signaling pathways FAK and extracellular signal–regulated kinase 1/2 (ERK1/2), initiated mitochondrial biogenesis signaling [nuclear peroxisome proliferator–activated receptor γ coactivator 1α (PGC-1α)], and shunted the rise in nuclear factor κB (NFκB) (p65) nuclear accumulation caused by exercise-induced muscle damage. Further, it was found that massage attenuated the production of the pro-inflammatory cytokines tumor necrosis factor–α (TNF-α) and interleukin-6 (IL-6) and reduced heat shock protein 27 (HSP27) phosphorylation, thereby mitigating cellular stress resulting from muscle damage (Crane et al., 2012).

2.4.7 Possible Role of the Alpha7Beta1 Integrin

The alpha7beta1 integrin is a mechanoreceptor that relays membrane stretch signals to the cytoplasm through focal adhesion kinase (FAK), which can then relay the signal through cSrc and ERK1/2 (Graham, Vardiman, Siedlik, Deckert, & Gallagher, 2014). Though it is logical that

the chemical and structural changes in skeletal muscle following soft tissue mobilization are mediated by this integrin no lab has found changes in this integrin's number or activation status following IASTM treatment. Additionally, the only research group to examine the effects of IASTM on integrin signaling found no significant changes in signaling in the gastrocnemius following treatment (Graham et al., 2014) .

However, it is important to note that our research study exploring the relationship between integrin count and activation status occurred in non-injured populations where the studies that cited alterations in integrin's used injury models. These data from our lab suggest that IASTM did not have any effect on the gene expression, protein expression, or phosphorylation status of the Alpha 7 Beta 1 Integrin (Graham et al., 2014). Further research should examine other integrin's and should also explore whether injury status effects integrin response.

2.5 Neural Response to Soft Tissue Mobilization

2.5.1 Effect of massage on Neuromuscular Excitability and the Hoffman Reflex

It has long been thought that massage and other similar techniques stimulate sensory receptors and decrease muscle tension by decreasing neuromuscular excitability (M Morelli, Chapman, & Sullivan, 1998; Moreno Morelli, Seaborne, & Sullivan, 1991; Moreno Morelli, Seaborne, & Sullivan, 1990; Sullivan, Williams, Seaborne, & Morelli, 1991). The body of literature exploring this phenomena is small but consistent in this finding. When neuromuscular physiologists wish to explore neuromuscular excitability they generally use the Hoffman Reflex

(H-reflex) as the outcome variable. H-reflex is a valuable assessment tool as its amplitude is analogous with stretch reflex loop behavior (Weerapong & Kolt, 2005). It has been successfully demonstrated that IASTM intensity does correlate with H-Reflex response (Goldberg, Sullivan, & Seaborne, 1992). However, one must keep in mind that the intensity and duration of treatment are vastly different between massage and IASTM.

There appears to be a link between massage and neuromuscular adaptation but it is unclear if IASTM evokes similar responses. In order to identify if testing the effect of IASTM on sensory receptor stimulation and subsequent decreases in neuromuscular excitability is a worthwhile research track one must first understand the basic biomechanics of massage. Petrissage, the type of massage used in the massage/H-reflex research features characteristic mechanical compression as well as kneading motions. This kneading motion is not easily replicated through IASTM. Further, massage treatments generally last longer and utilize lower pressure forces. None the less, massage and IASTM are similar so it is not outside the realm of possibility that they effect neuromuscular excitability in similar ways.

One study by Morelli et al. used six minutes of one handed petrissage and performed a series of H-Reflex tests to explore the effect of massage on neuromuscular excitability (Moreno Morelli et al., 1990). Researchers observed that during the massage treatment there was a decrease in H-Reflex amplitude that is characteristic of decreased excitability (Moreno Morelli et al., 1990). However, it was observed that immediately following completion of the treatment H-Reflex characteristics returned to normal, baseline values. Morelli, in another paper, described that he believed the mechanism behind the decrease in H-Reflex amplitude was likely due to a decrease in spinal reflex excitability (Moreno Morelli et al., 1991). Further, Morelli

adds that this phenomena only occurs locally (Sullivan et al., 1991), in the muscles that received treatment.

Morelli hypothesized that the inhibitory effects of neuromuscular excitability by massage could be the reason for the common anecdotal findings reported in hundreds of case studies that massage decreases resting muscle tension. However, it does not appear the reduction of H-reflex amplitude is the cause for observed post massage decreases in muscular strength as reported by Wiktorsson-Moller et. el (Wiktorsson-Moller et al., 1983). The most compelling finding that decreases in H-reflex are not the reason for strength losses post massage is that H-reflex returns to "resting" value immediately upon completion of the treatment whereas strength losses can be present for days (Weerapong & Kolt, 2005). Further research is needed to understand the mechanism by which these strength losses occur. Additionally, further research is needed to be able to determine if IASTM effects localized neuromuscular tissue in a similar way.

2.5.2 Proposed Neural Mechanism

In an effort to explore the mechanism of the decreased H-Reflex amplitude found in the Morelli et al original studies the Morelli research group designed a follow up study (M Morelli et al., 1998). The purpose of this study was to explore if cutaneous receptors contribute to the changes in the amplitude of the H-reflex during massage. In order to test this hypothesis subjects were randomly assigned to one of two groups. One was a control group and the other a cutaneous receptor "knock down" group that utilized a local anesthetic to accomplish this end (M Sabbahi & De Luca, 1981; MA Sabbahi & De Luca, 1982; Wolf & Minkwitz, 1989).

There was a marked increase in H-Reflex amplitude in both groups compared to the pre-massage tests but no group x time interaction. The fact that there was no group x time interaction suggests that the mechanism by which massage increases H-Reflex amplitude is likely independent of cutaneous sensory neuron stimulation.

2.6 The Use of Diagnostic Ultrasound to Assess Changes in Muscle Architecture

Examining muscle size, pennation angle and tissue quality in-vivo is useful when trying to determine the effectiveness of IASTM and other therapeutic modalities. These variables can be reliably explored in the hamstrings muscles using modern diagnostic ultrasound imaging (Palmer, Akehi, Thiele, Smith, & Thompson, 2015). Diagnostic ultrasound has been successfully used to document changes in skeletal muscle architecture caused by training (Ahtiainen et al., 2010), aging (Roth et al., 2001), immobilization (Abe, Kawakami, Suzuki, Gunji, & Fukunaga, 1997) and neuromuscular disease (Moreau, Teefey, & Damiano, 2009). In addition to the measurement of muscle size, thickness and pennation angle more modern measurements including muscle quality (echo intensity) are being utilized to assess the ratio of muscle to fat and fibrous content (Pillen et al., 2009). Muscle quality appears to be a variable that can change with training status (Palmer et al., 2015). For example, it has been demonstrated that 6 weeks of isokinetic resistance training can lead to alterations in muscle quality (Cadore et al., 2014). Though cross sectional area, muscle thickness, pennation angle and muscle quality as measured by echo-intensity are all trainable it remains unclear if any of these architectural variables are altered by soft tissue mobilization.

2.7 Summary:

In summary, more research is needed to explore the ability of IASTM treatments to alter the chemical and physical property of skeletal muscle. One confounding factor is how many forms of soft tissue mobilization there are. While IASTM and massage appear quite similar, it is difficult to know if application can be drawn from one form of soft tissue mobilization to the next.

Further research is needed to better understand the link between the therapeutic mechanical loading of soft tissue and the mechanism by which positive benefits occur. Better understanding of this mechanism will aid clinicians in better prescription of therapeutic modalities and could lead to quicker return to work following musculoskeletal injury. Crain et al has suggested that this mechanism is integrin initiated and ultimately carried out by production of growth and repair factors in localized fibroblast. This hypothesis is logical but needs further study to be substantiated. In addition to the further exploration of this proposed mechanism researchers should explore interactions between therapeutic mechanical loading of muscle and other therapeutic modalities as well as common, over the counter analgesics.

The effect of IASTM on neuromuscular physiology is largely understudied as well. The body of research that has demonstrated massages ability to alter H-reflex is perhaps the most compelling at suggesting IASTM potentially does affect the neuromuscular function of skeletal muscle. The fact that massage has repeatedly been shown to transiently increase H-Reflex amplitude during treatment suggests massage (and potentially other forms of soft tissue mobilization) plays a role in the alteration of basal neuro-muscular activity. However, Morelli et al proved its own cutaneous sensory fiber activation theory wrong in their subsequent follow

up study. Future research should explore the mechanism by which massage effects H-Reflex and whether or not other forms of soft tissue mobilization effect H-Reflex in the same manner.

Chapter 3: Methods

3.1 Study 1- Instrument-assisted soft tissue mobilization: effects of on the properties of human plantar flexors

3.1.1 Subjects

11 healthy men (mean±SD age=23±3 years; stature=181±7 cm; mass=83±11 kg) volunteered for this investigation. Each participant was screened for current or ongoing neuromuscular diseases, musculoskeletal injuries, or skin disorders specific to the plantar flexors. Participants reported that they had neither recently taken nor were currently using non-steroidal anti-inflammatory drugs (NSAID), aspirin, or other anti-thrombotic over-the-counter or prescription medications. Participants were instructed not to participate in exercise 24 h prior to their first scheduled visit to the laboratory or throughout the 4 subsequent days during data collection. This study was approved by the University Institutional Review Board for Human Subjects, and all participants completed a written informed consent form and a Health & Exercise Status Questionnaire. This study also meets the ethical standards established by the International Journal of Sports Medicine (Harriss & Atkinson, 2011).

3.1.2 Study Design

A repeated measures design was used to examine the acute effects of the IASTM on plantar flexors musculotendinous stiffness (MTS), passive range of motion (PROM), maximal voluntary contraction peak torque (MVPT), perception of functional ability questionnaire (PFAQ) responses, intramuscular levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-

α) cytokines. Participants visited the laboratory 5 times. The first day included familiarization with the protocol, screening for inclusion and exclusion criteria and signing the IRB approved informed consent document. The second visit was within 7 days of the initial visit and was the first of four concurrent days of data collection. The first day of data collection included random assignment of the IASTM treatment leg (TL), muscle biopsy 1 (MB1) from the control leg (CL), pre-IASTM isokinetic assessment of the TL and CL, the IASTM treatment protocol to the TL and the post-IASTM isokinetic assessment of the TL and CL. The post-IASTM assessments occurred immediately after the treatment. The subsequent three visits included muscle biopsy of the TL only and isokinetic assessment of the TL and CL. All experimental trials were performed at the same time of day (±15 min).

3.1.3 Muscle Tendon Stiffness

Musculotendinous stiffness of the plantar flexors was quantified. MTS is the ability of the combined musculotendinous unit to prevent a change in length when force is applied (Pearson & McMahon, 2012). MTS was calculated for each 1° increment in the passive angle-torque relationships from the neutral ankle position of 0° (i. e., 90° between the foot and leg) to the end of the range of motion. The ROM for each measure was determined using the position signal from the isokinetic dynamometer. The final MTS value that was calculated for the joint angles commonly achieved during both the pre- and post-treatment trials was analyzed. For example, if MTS values were obtained for a subject at 10°, 11° and 12° during the pre-treatment assessment, and MTS values were calculated at 10°, 11°, 12° and 13° for the post-

treatment assessment, then the values at 12° were used for analysis, because this joint angle was common to both the pre- and post-treatment assessments.

MTS was measured using a fourth-order polynomial regression model that was fitted to the passive angle-torque curves for each subject (Nordez, Cornu, & McNair, 2006). The fourth-order polynomial model was chosen over other models (i. e., second-order polynomial and Sten-Knudson) based on the comparative recommendation of Nordez (Nordez et al., 2006) and because the fourth-order polynomial is classically used in the literature to assess in vivo MTS (Magnusson, Simonsen, Aagaard, Sørensen, & Kjaer, 1996; Magnusson, Simonsen, Aagaard, & Kjaer, 1996; Riemann, DeMont, Ryu, & Lephart, 2001). MTS quantifies the joint angle-specific stiffness of the musculotendinous unit based on the passive angle-torque relationship. No gravity correction was performed, as based on the methods of Salsich et al, (Salsich, Mueller, & Sahrmann, 2000) who indicated that the foot constituted approximately 1.4% of the body's mass and suggested that this mass can be considered negligible (T. Herda et al., 2009). Values utilized in analysis were slope values from three common joint angles across all subjects (T. Herda et al., 2009).

3.1.4 Passive Range of Motion

The passive range of motion (PROM) of the plantar flexors was determined for each participant during the pre- and post-treatment assessments using the isokinetic dynamometer programmed in passive mode. PROM is the measure of the terminal end of motion in a joint facilitated by passively moving the limb. This is typically determined by the patient's subjective indication of when the limbs movement becomes painful (T. J. Herda, Cramer, Ryan, McHugh, &

Stout, 2008). Maximum PROM was determined for each individual during the trial as the point of discomfort, but not pain, as verbally acknowledged by the subject during a passive stretch of the plantar flexors while the leg was in terminal knee extension. The dynamometer lever arm passively dorsiflexed the foot at an angular velocity of 5°/s until the end range of motion. PROM was calculated as the range of motion attained from 0° (neutral) to the maximum tolerable point of passive dorsiflexion. No gravity correction was performed based on the methods of Muir (Muir, Chesworth, & Vandervoort, 1999), who indicated that the foot constituted approximately 1.4% of the body's mass (Winter, 2009) and suggested that this mass can be considered negligible.

3.1.5 Maximal Voluntary Contraction Peak Torque

To determine maximal voluntary contraction peak torque (MVPT), each participant performed two 5-s isometric MVCs of the plantar flexors at a neutral ankle joint angle (0°=90° between the foot and leg), while the knee joint was in terminal knee extension. The MVPT is the force produced at a specific angle in a patient's range of motion. A 2-min rest was allowed between trials. The MVPT for each trial was determined as the highest consecutive 0.25 s epoch. The same 0.25 s epoch were selected for the EMG signals to calculate the time domain estimates during the MVC trials. The mean PT value from the 2 MVC trials was used as the representative score for further analyses. The participants were instructed to give a maximum effort for each trial and strong verbal encouragement was provided by the investigators.

3.1.6 Surface EMG Collection

EMG was collected to ensure all PROM assessments were passive according to Gajdosik et al. (2005). Bipolar, active surface EMG electrodes were placed on the medial gastrocnemius (MG) and soleus (SOL) muscles. The electrode configuration (TSD150B, Biopac Systems Inc.; Santa Barbara, California, USA) had a fixed center-to-center interelectrode distance of 20 mm, built-in differential amplifier with a gain of 350 (nominal), input impedance of 100 MΩ, and common mode rejection ratio of 95 dB (nominal). For the SOL, the electrodes were placed along the longitudinal axis of the tibia at 66% of the distance between the medial condyle of the femur and the medial malleolus. The electrodes for the MG were placed on the most prominent bulge of the muscle per the recommendations of (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000). A single pre-gelled, disposable electrode (Ag-Ag Cl, Quinton Quick Prep, Quinton Instruments Co., Bothell, Washington, USA) was placed on the spinous process of the seventh cervical vertebrae to serve as a reference electrode. To reduce interelectrode impedance and increase the signal-to-noise ratio, local areas of the skin were shaved, lightly abraded, and cleaned with isopropyl alcohol prior to placement of the electrodes.

3.1.7 Signal Processing

The EMG and torque signals were recorded simultaneously with a Biopac data acquisition system (MP150WSW, Biopac Systems, Inc. Santa Barbara, California, USA) during each MVPT and PROM assessment. The for torque (Nm) data, signals from the dynamometer and EMG (μV) signals were sampled at 2 kHz and recorded from the SOL and MG. All signals were stored on a personal computer (Dell Inspiron 8200, Dell, Inc., Round Rock, Texas, USA), and processing was completed off-line using custom written software (LabVIEW v 7.1, National Instruments, Austin, Texas, USA). The EMG signals were digitally filtered (zero-lag fourth-order Butterworth filter) with a pass band of 10–500. The torque signal was low-pass filtered with a 10 Hz cutoff (zero-phase fourth-order Butterworth filter). All subsequent analyses were performed on the filtered signals.

3.1.8 PFAQ Questionnaire

The Perception of Functional Ability Questionnaire (PFAQ) was developed by panel of physicians, athletic trainers, and patients. Six critical domains were identified for the assessment of functional ability during a functional task: physical health, flexibility, muscular strength, pain, restriction of sport, skill, and activity of daily living (ADL) performance. To assess the six domains, an 8-question questionnaire with associated visual analogue scale from 0–10 was developed. The PFAQ was evaluated for test-retest reliability using 60 college-aged students following procedures described by Levine (Levine et al., 1993). Internal consistency was assessed for all items collectively using Chronbach's alpha (=0.856), with a score of 0.8

being considered good and 0.9 excellent. Each participant completed the PFAQ prior to the muscle biopsy and isokinetic testing on each of the testing days.

3.1.9 IASTM Treatment Dosage

The IASTM protocol was administered by a certified athletic trainer with over 13 years of experience. On day 1, subjects underwent the IASTM protocol on the plantar flexors of the randomly assigned TL. The IASTM was a 7–8 min, soft-tissue mobilization protocol using one convex shaped and one concave shaped stainless steel instrument designed for IASTM. The plantar flexors of the TL were divided into 4 treatment sections. Each section received 3 sets of 7 strokes in both proximal and distal directions. A bubble level was applied to both instruments to provide the clinician with a consistent treatment angle of 45°. Flexiforce-Economical Load and Force pressure sensors (ELF™) (Tekscan, South Boston, MA) were applied to the instrument's treatment surface to ensure standardized treatment pressures throughout the protocol. Measures of peak and mean pressure for each of the 4 treatment quadrants were quantified using LabVIEW (LabVIEW v 7.1, National Instruments, Austin, Texas, USA).

3.1.10 Muscle Biopsies

On each day following the collection of ROM, and Passive Torque data, percutaneous muscle biopsies (~100 mg) (Bergstrom, 1962) were taken from the gastrocnemius. The baseline biopsy was taken from the control leg while the other biopsies (-immediately post, -24 post, 48 post and 72 post) were taken from the treatment leg. This methodology was used to avoid

biopsy induced inflammation from confounding the immediately post treatment biopsy values. All biopsies were obtained from the mid-belly region of the muscle, and each biopsy was 2–3 cm proximal from the previous site and within the region treated by IASTM. Each subject received standard antiseptic application to each biopsy site followed by an injection of 3 cc of local anesthetic (2% lidocaine) to each biopsy site. The subject then rested for 5 min to ensure that the area was sufficiently anesthetized. An incision approximately 0.5 cm wide and 1 cm deep was then made using a scalpel (#11 Blade) approximately 6–8 cm from the joint line of the knee. All samples were then placed in liquid nitrogen and stored at −80°C until analysis.

3.1.11 Muscle Processing

Approximately 20 mg of each muscle sample was homogenized in extraction buffer (Biosource; Carlsbad, CA) using a glass-on-glass tissue grinder. Homogenized samples were centrifuged at 4°C at 3 000 rpm for 4 min. For determining total protein, supernatant was separated from the pellet and the sample was diluted (1:1 000) in preparation for analysis using a bicinchoninic acid (BCA) protein assay (Pierce; Rockford, IL). All samples were measured in triplicate using a Synergy microplate reader (BioTek, Winooski, VT) at 450 nm.

3.1.12 Western Immuno-Blotting

Muscle samples were diluted with 5x buffer (IL-6) or 1x buffer (TNF-α) and heated for 3 min at 100°C. 80 μg of protein was loaded for each sample and placed on a 5% stacking and

10% separating gel at 0.05 mA for 1 h. Proteins were transferred to hydrophobic polyvinylidene difluoride (PVDF) membranes at 0.20 mA for 2 h. Membranes were blocked for 1 h in a Tris-buffered saline with 5% nonfat dry milk on a rocker at room temperature. Membranes were then incubated at 4°C on a plate rocker overnight in a 1:1 000 IL-6 (Cell Signaling Technology, Inc., Beverly, MA) or TNF-α antibody (Cell Signaling Technology, Inc., Beverly, MA) which was normalized to tubulin (Cell Signaling Technology, Inc., Beverly, MA) in TBST and 1% nonfat dry milk solution. Following the overnight incubation, membranes were rinsed 3 times for 5 min in TBST. Membranes were incubated in horseradish peroxidase conjugated secondary antibody for an hour and once again rinsed 3 times for 5 min in TBST. Membranes were then incubated in chemiluminescence. IL-6 and TNF-α (Santa Cruz Biotechnology, Santa Cruz, CA) protein bands were then visualized and quantified using densitometry (AlphaView® FluorChemHD2 v.3.4.0.0, Protein Simple, Santa Clara, CA).

3.1.13 Statistical Analyses

3 separate 2×5 repeated measures ANOVAs [Leg (CL vs. TL)×Time (pre-IASTM, post-IASTM, day 2, 3, and 4)] were used to analyze MTS, PROM, and MVPT data. Three separate one-way repeated measures ANOVAs were used to analyze PFAQ, IL-6 and TNF-α. When appropriate, follow-up analyses were performed using paired samples t-tests and with Bonferroni's corrections. Mauchly's sphericity test was also run for each of the previously described ANOVA models and sphericity could be assumed in all ANOVA models.

3.2 Study 2- The effects of different IASTM pressure volumes on the passive properties of skeletal muscle

3.2.1 Subjects

Forty-three healthy college aged subjects (mean±SD age=23±3 years; height=181±7 cm; mass=83±11 kg) volunteered for this investigation. Each participant was screened for current or ongoing neuromuscular diseases, musculoskeletal injuries, or skin disorders specific to the plantar flexors. Participants reported that they had neither recently taken nor were currently using non-steroidal anti-inflammatory drugs (NSAID), aspirin, or other anti-thrombotic over-the-counter or prescription medications. Participants were instructed not to participate in exercise 24 h prior to their first scheduled visit to the laboratory or throughout the 4 subsequent days during data collection. This study was approved by the University Institutional Review Board for Human Subjects, and all participants completed a written informed consent form and a Health & Exercise Status Questionnaire. This study also meets the ethical standards established by the International Journal of Sports Medicine (Harriss & Atkinson, 2011).

3.2.2 Study Design

A four group (control, sham, reduced IASTM pressure, clinical IASTM pressure) repeated measures design was used to examine the effects of IASTM pressure force dosages on calf muscle musculotendinous stiffness (MTS), passive range of motion (PROM), maximal voluntary contraction peak torque (MVPT) and perception of functional ability questionnaire (PFAQ) responses. Once participants were recruited and an IRB approved informed consent document

was obtained participants were asked to come in for a total of three testing visits. On the first visit an emollient sensitivity trial was performed. The purpose of this was to examine if participants were allergic to the topical emollient that would be used during the IASTM treatment. Upon completion of this test and a health history questionnaire, participants were asked to fill out the PFAQ questionnaire and went through MVC and PROM testing. These values served as baseline values. Upon collection of these baseline values participants were given treatment based on the group they were assigned to. When the assigned treatment was completed subjects were once again tested using the same battery of tests. This "immediately post" test marked the end of visit one. Visit two and three were scheduled -24 and -48 hours post treatment, respectively. These visits consisted of having participants fill out the PFAQ questionnaire and go through the previously described MVC and PROM testing.

3.2.3 Muscle Tendon Stiffness

MTS data collections were carried out using the methodology previously described in section 3.1.3.

3.2.4 Passive Range of Motion

Passive ROM data collections were carried out using the methodology described in section 3.1.4.

3.2.5 Maximal Voluntary Contraction Peak Torque

MVC-PT data collections were carried out using the methodology described in 3.1.5.

3.2.6 Surface EMG Collection

sEMG data collections were carried out using the methodology described in 3.1.6.

3.2.7 Signal Processing

sEMG raw signals were collected and post processed using LabVIEW (LabVIEW v 7.1, National Instruments, Austin, Texas, USA). This was carried out using the methodology described in 3.1.7.

3.2.8 PFAQ Questionnaire

The PFAQ was administered at the beginning of each visit and twice on visit one in accordance the methodology outlined in section 3.1.8.

3.2.9 IASTM Treatment Dosage

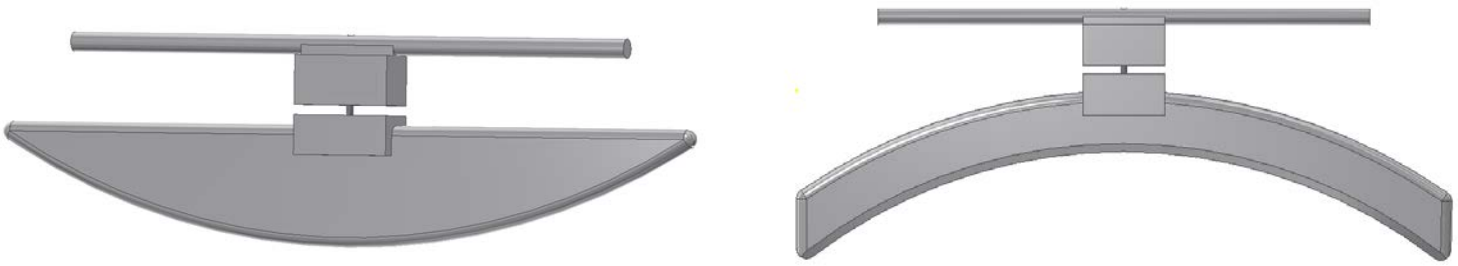
The IASTM treatments were the same as previously described in section 3.1.9 with one exception. Due to the nature of the research question there were two different groups

receiving IASTM treatment. The “clinical pressure” group received its treatment in exact accordance with the previously described method. The sub-clinical group received the same total volume of treatment at a lower pressure intensity. The mean and peak pressure for the scanner tool were 1.1± .54 N/mm^2 and 2.1± .93 N/mm^2 respectively. The mean and peak pressure for the concave tool were 1.0± .32 N/mm^2 and 2.2± .84 N/mm^2 respectively. These values were determined by taking the mean of individuals mean values and the mean of the peak pressure values respectively.

3.2.10 Pressure force quantification

Pressure forces were quantified using a modified load-cell apparatus that was machined in house (Figure 1). Voltage signals were collected using a custom written signal acquisition program (LabVIEW v 7.1, National Instruments, Austin, Texas, USA). Post processing and quantification were also carried out using LabVIEW.

Figure 1: Custom load-cell device attached to each tool



3.2.11 Statistical Analyses

Three separate 2x3×2 repeated measures ANOVAs [leg(treatment vs control)xgroupxtime] were used to analyze MTS, PROM and MVPT data. The immediately, -24hr and -48hr post time points were collapsed into one "post" time point. This was done to more clearly describe the data and to simplify the statistical model for ease of interpretation for clinicians. A 3x2 (groupxtime) mixed factorial ANOVA was used to examine changes in PFAQ response. When appropriate, follow-up analyses were performed using paired samples t-tests with Bonferroni's correction for type-1 error. Mauchly's sphericity test was also run for each of the previously described ANOVA models and sphericity could be assumed in all ANOVA models.

3.3 The Effects of IASTM Treatment on Short Hamstring Syndrome in College Aged Adults

3.3.1 Study Design

To examine the abilities of IASTM to alter skeletal muscle architecture a repeated measures study design was utilized. Participants were asked to come in for two visits with a one week wash-out between visits. Subjects received -pre testing, -post testing as well as a treatment (IASTM or Sham) on each visit.

3.3.2 Subject Recruitment

The purpose of this investigation was to examine the effects of IASTM on the flexibility and hamstrings muscle architecture (biceps femoris) in clinically-identified hamstring-restricted college-aged adults. In order to accomplish this, we recruited 17 college-aged females (age= were recruited with clinically-identified restricted leg flexors were recruited (age= 21.12 ± 0.78 years; height= 165.41 ± 7.66 cm; weight= 61.34 ±18.55 kg). However, there were certain exclusion criteria present. Namely, any subjects who had experienced traumatic injury to the lower extremity within the previous 3 months prior to participation. Additionally, anyone who has difficulty laying on their back for an extended period of time (> 1 hour) were excluded. Participant were asked to visit the lab on two occasions, for a total of three hours over the course of two weeks with a minimum of one week between testing (testing day 1 and 2). Once subjects were recruited they were classified into one of two groups.

Subjects were situated on a cushioned table to assess hamstring flexibility. To determine whether a subject was considered range of motion restricted or not a straight leg passive

stretch assessment was utilized. During this straight leg raise assessment the subjects knee and ankle were immobilized (90° flexion at the ankle and 0° flexion at the knee). After subjects were fitted with proper immobilizers the investigator slowly elevated subject's foot toward their head until they reached the point of mild discomfort prior to pain

3.3.3 PFAQ and Survey of Treatment Quality Questionnaires

The PFAQ was administered prior to the beginning of all other testing on each visit. The PFAQ is more fully described in section 3.1.8. The survey of treatment quality was developed for this examination and is a four-item survey designed to qualitatively measure then perception of mood following IASTM. The survey of treatment quality was administered immediately following both the sham and the IASTM treatments.

3.3.4 Range of Motion Assessment for Subject classification

Subjects were situated on a cushioned table to assess hamstring flexibility. To determine whether a subject was considered range of motion restricted or not a straight leg passive stretch assessment was utilized. During this straight leg raise assessment the subjects knee and ankle were immobilized (90° flexion at the ankle and 0° flexion at the knee). After subjects were fitted with proper immobilizers the investigator slowly elevated subject's foot toward their head until they reached the point of mild discomfort prior to pain. Additionally, a second range of motion assessment was completed during each visit both –pre and –post treatment or sham. During this assessment, the subject's hips and knees were placed at 90 degrees respectively. Again, the investigator elevated the subjects foot upward toward their head until subjects

indicated they had reached the maximal amount of stretch without discomfort. During this range of motion assessments both the maximal ROM and ROM at which subjects began to feel a stretch were recorded.

3.3.5 IASTM and Sham Treatment Dosage

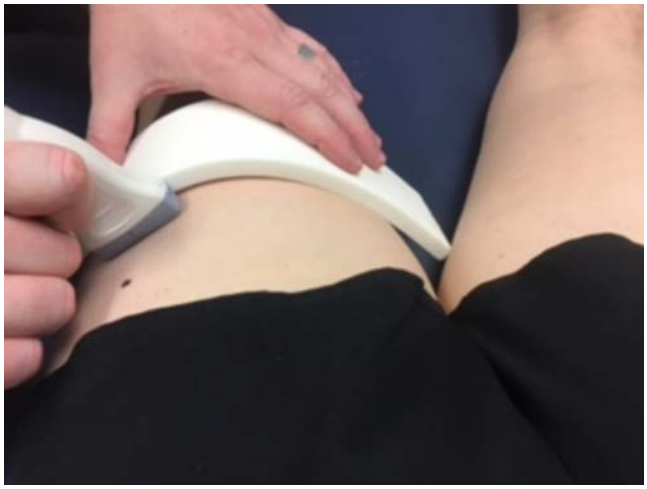
The IASTM treatments were the same as previously described in section 3.1.9 aside from the treatment being applied to the hamstrings muscles rather than the gastrocnemius complex. The sham treatment was carried out using a therapeutic ultrasound machine (Chattanooga Medical Supply, Chattanooga, TN USA). The sham treatment lasted five minutes to match the IASTM treatment time. The ultrasound machine was not turned on during the sham treatment.

3.3.6 Diagnostic Ultrasound Parameters

For diagnostic ultrasound assessments the subjects were placed in a prone position and the knee was extended and relaxed (Potier, Alexander, & Seynnes, 2009). Subjects were instructed to lie prone for five minutes prior to image collection to allow for intramuscular fluid shifts to occur. Ultrasound data were collected using the NextGen LOGIQ e ultrasound console (GE Healthcare UK, Ltd., Chalfont, Buckinghamshire, UK) with a multi-frequency linear array transducer (Model 12L-RS; 5-13 MHz; 38.4 mm field-of-view). The ultrasound probe was placed at the midpoint of the femur from the greater trochanter to the lateral epicondyle of the femur for the BF muscle (Umegaki et al., 2015) and its position was marked on the skin to ensure uniformity between pre and post ultrasound measurements (Potier et al., 2009) (Figure 2).

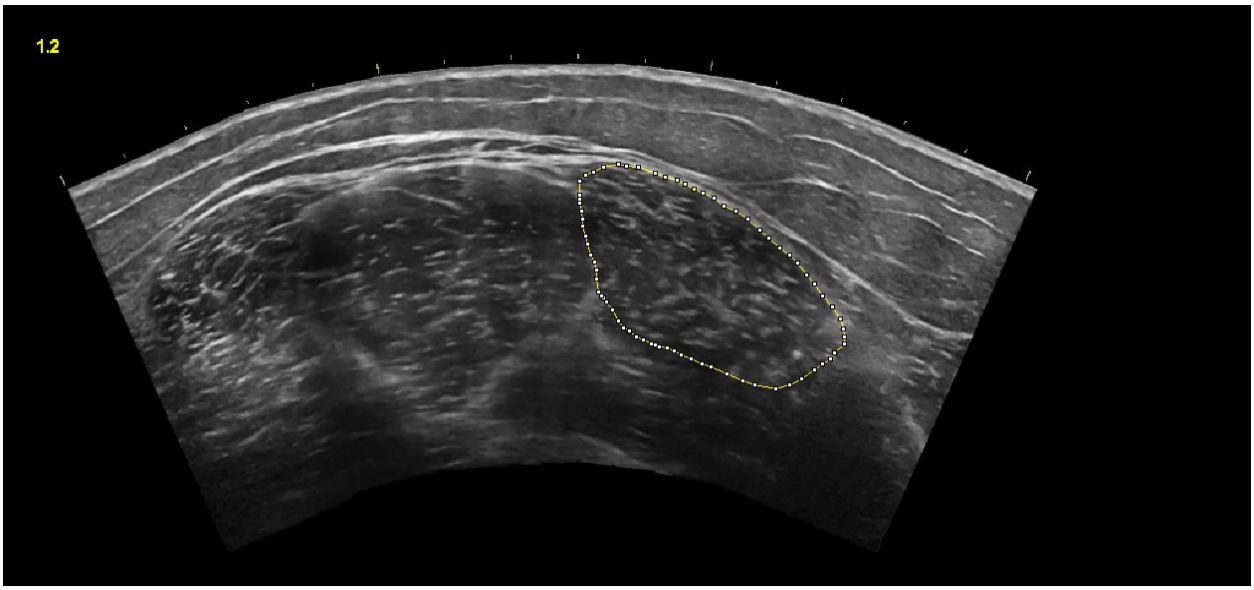
Once the probe was properly placed two images were taken and stored digitally for analysis of pennation angle (Potier et al., 2009).

Figure 2: Panoramic Scan of Hamstrings Muscle



Upon collection of B-mode ultrasound images we began collecting panoramic ultrasound signal in order to determine biceps femoris cross-sectional area and muscle quality (echo intensity). The muscle belly of the biceps femoris was carefully outlined in ImageJ (ImageJ, National Institute of Health, Bethesda, Maryland, USA). The mean echo intensity of this region was next calculated with a standard histogram function (Pillen et al., 2009). The Image J histogram function isolates each pixel and assigns an echo intensity value between 0 (black) and 255 (white) as is consistent with 8-bit greyscale post-processing (Pillen et al., 2009). The same outline was used for determination of biceps femoris cross-sectional area. During the analysis of muscle quality and muscle CSA the fascia was not included.

Figure 3: Outline of Biceps Femoris used for CSA and Echo Intensity determination

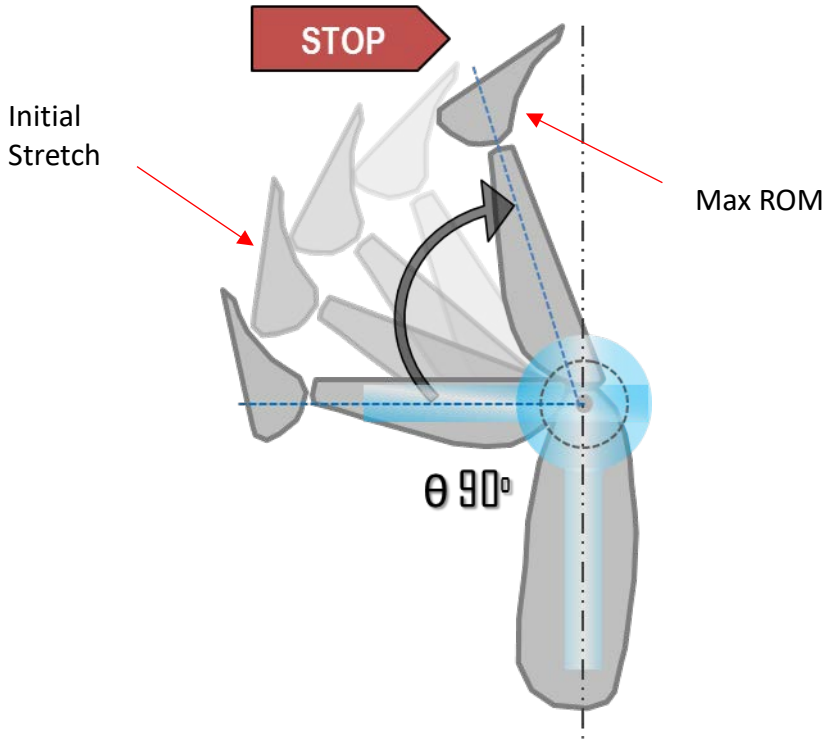


Pennation angle was defined as the positive angle between the superficial aponeurosis and the muscle fascicle (Gajdosik, Rieck, Sullivan, & Wightman, 1993; Potier et al., 2009; Woodley & Mercer, 2005).

3.3.7 Dynamic Ultrasound Measurements

In addition to the previously described prone images a series of dynamic ultrasound measurements were taken. All dynamic measurements were taken in B-mode to quantifying pennation angle. Measurements were taken with the subject's hip and knee at ninety degrees as well as the ROM where participants first perceived stretch of the hamstrings and the maximal ROM prior to pain.

Figure 4:Visual depiction of the ROM where three dynamic B-mode measurements were taken



3.3.8 Statistics

To analyze the panoramic ultrasound (CSA and Echo Intensity) two separate 2x2x2 (groupxTreatmentxTime) ANOVA were utilized. Similarly, to analyze pennation angle at the three previously described joint angles three separate 2x2x2 (GroupxTreatmentxTime) ANOVA were utilized. Lastly, to statistically analyze the range of motions at the first perception of stretch as well as the maximal allowable stretch two additional 2x2x2 (GroupxTreatmentxTime) ANOVA were utilized. PFAQ data were analyzed using one way ANOVA. The Survey of Treatment Quality was analyzed using paired samples T-tests. Independent statistical analysis revealed sphericity could be assumed for all previously described 3-way ANOVA models.

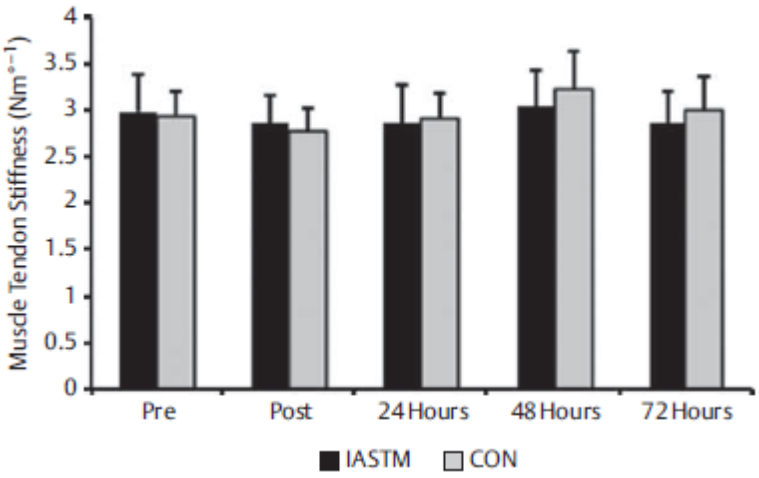
Chapter 4: Results

4.1 Study 1- Instrument-assisted soft tissue mobilization: effects of on the properties of human plantar flexors

4.1.1 MTS

For MTS, there were no significant two-way interactions (time×treatment, p=0.92) and no significant main effects for time (p=0.63) or treatment (p=0.89) (Figure 2).

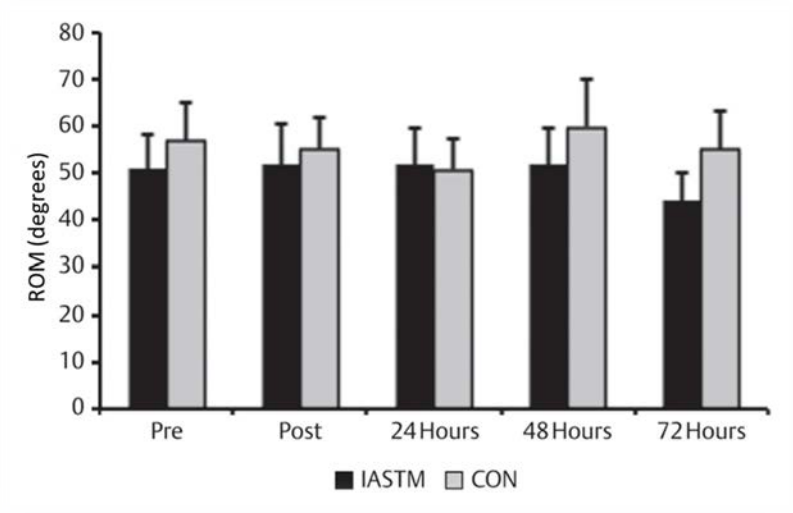
Figure 5: MTS was unaltered by treatment and time



4.1.2 Passive range of motion

For PROM, there were no significant two-way interactions (time×treatment, P=0.78) and no significant main effects for time (p=0.11) or treatment (p=0.64) (Figure 3).

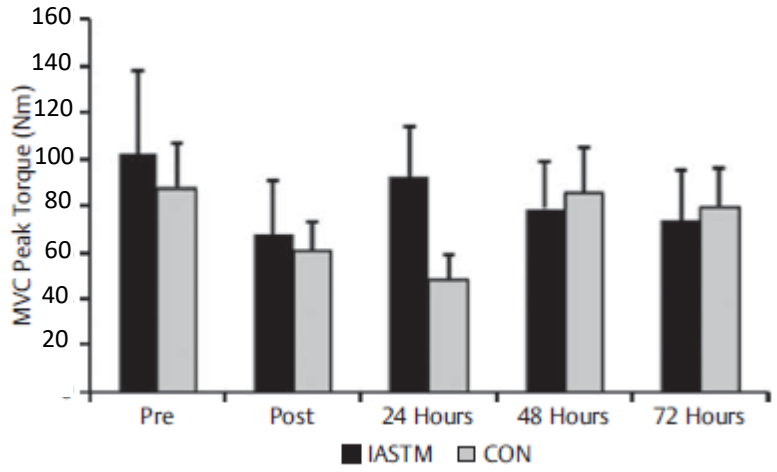
Figure 6: Passive ROM was unaltered by treatment and time



4.1.3 MVC-PT

For MVPT, there were no significant two-way interactions (time×treatment, P=0.25) and no significant main effects for time (p=0.6) or treatment (p=0.45) (Figure 4).

Figure 7: *Maximal voluntary contraction peak torque was unaltered by treatment and time*



4.1.4 Myokine Expression

For IL-6, there were no significant differences (p=0.82) at any point in time following IASTM. For TNF- α, there were no significant differences (p=0.68) at any point in time following IASTM treatment (Figure 5).

Figure 8: Protein expression values were quantified indirectly by imaging luminescence after the myokines of interest were tagged with chemical luminescent.

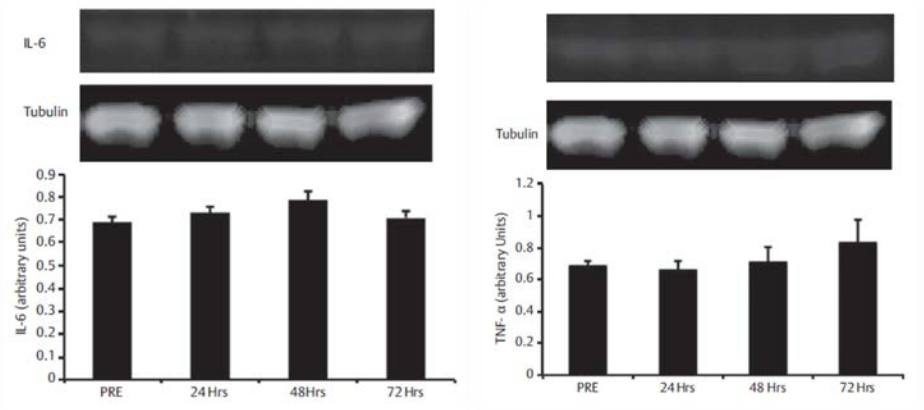


Figure 9: Individual TNF-α expression values following IASTM treatment

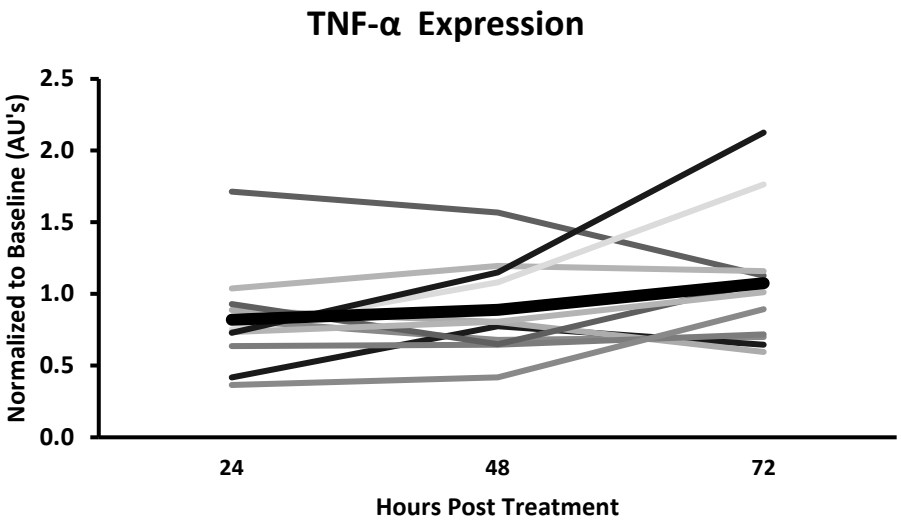
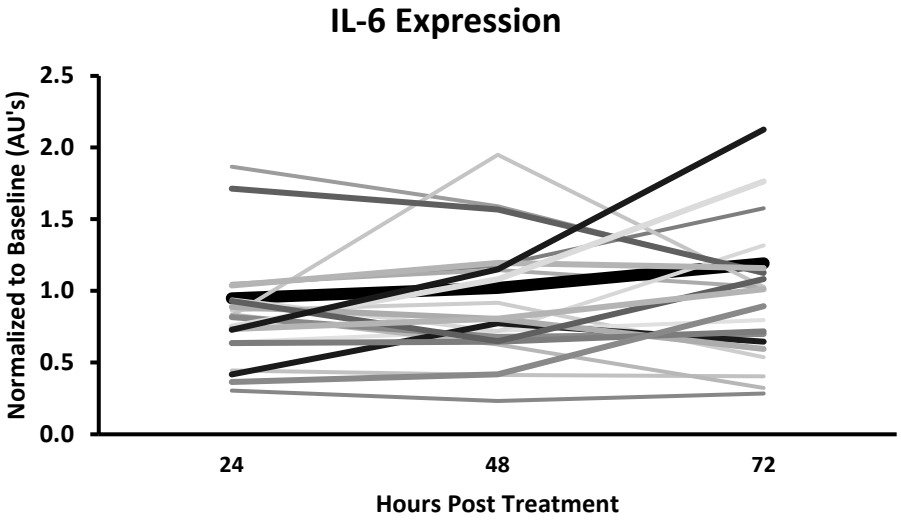


Figure 10: *Individual IL-6 expression values following IASTM treatment*



4.2 Study 2- The effects of different IASTM pressure volumes on the passive properties of skeletal muscle

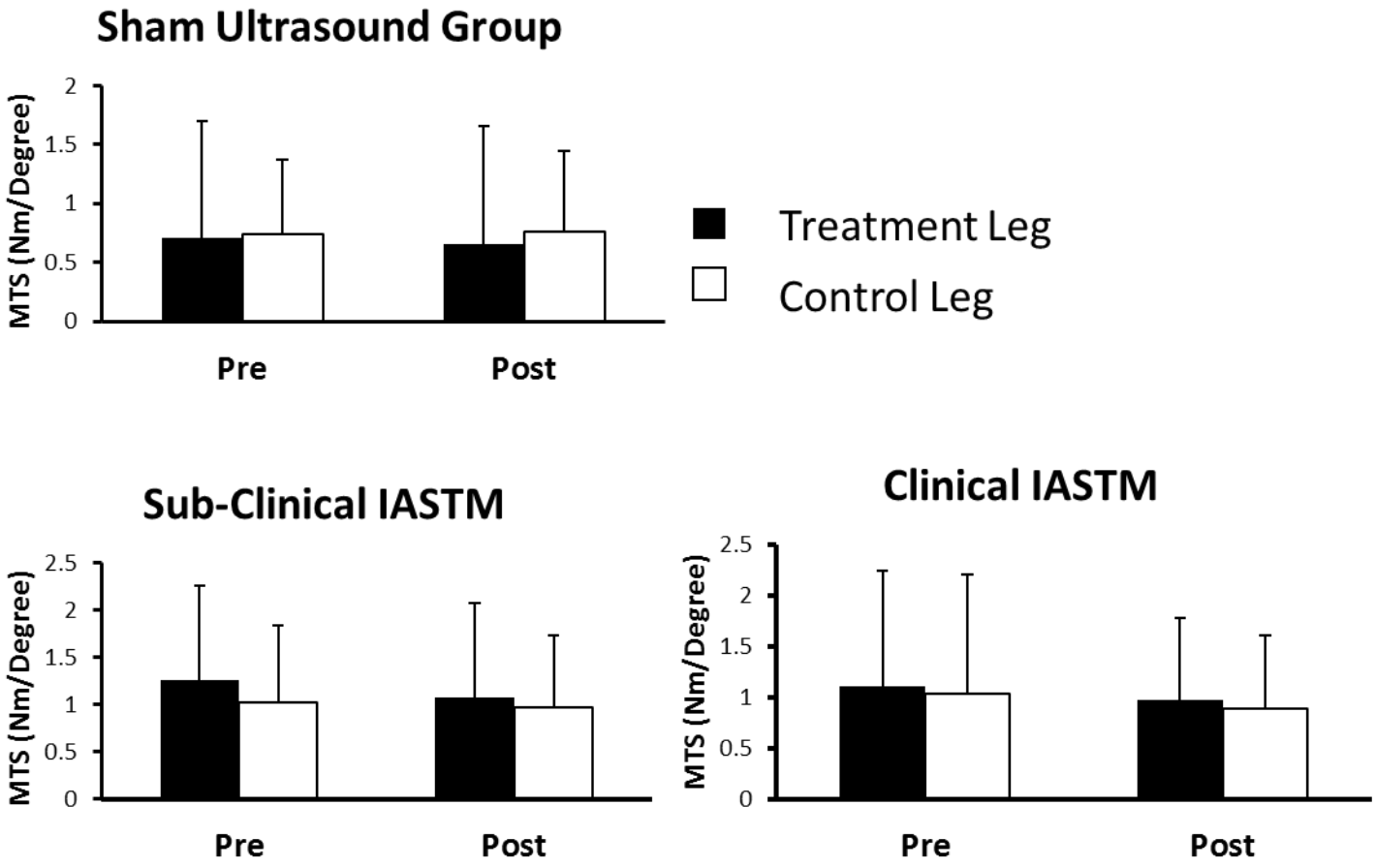
4.2.1 Muscle-Tendon Stiffness

As previously described, a 2x3×2 mixed factorial, repeated measures ANOVA [leg(treatment vs control)xgroupxtime] was used to analyze MTS results. ANOVA revealed no significant interactions or main effects (Table 2).

Table 2: MTS ROM-ANOVA results

Term	P value	Partial Eta Squared
GroupxTimexLeg interaction	p= 0.621	= 0.032
TimexLeg interaction	p= 0.637	= 0.008
LegxGroup interaction	p= 0.985	=0.001
TimexGroup interaction	p= 0.254	=0.090
Time main effect	p= 0.605	=0.009
Leg main effect	p= 0.320	=0.034
Group main effect	p= 0.350	=0.070

Figure 11: Graphs displaying mean MTS values across time (-pre and -post). Statistical analysis revealed no interactions or main effects for MTS data.



4.2.2 Passive Torque

As previously described, a 2x3×2 mixed factorial, repeated measures ANOVA [leg(treatment vs control)xgroupxtime] was used to analyze passive torque results. ANOVA revealed no significant interactions or main effects (Table 3).

Table 3: Passive Torque ANOVA results

Term	P value	Partial Eta Squared
GroupxTimexLeg interaction	p= 0.217	= 0.029
TimexLeg interaction	p= 0.394	= 0.025
LegxGroup interaction	p= 0.511	=0.045
TimexGroup interaction	p= 0.240	=0.094
Time main effect	p= 0.120	=0.081
Leg main effect	p= 0.355	=0.030
Group main effect	p= 0.771	=0.018

Figure 12: Individual responses in passive torque at maximal range of motion

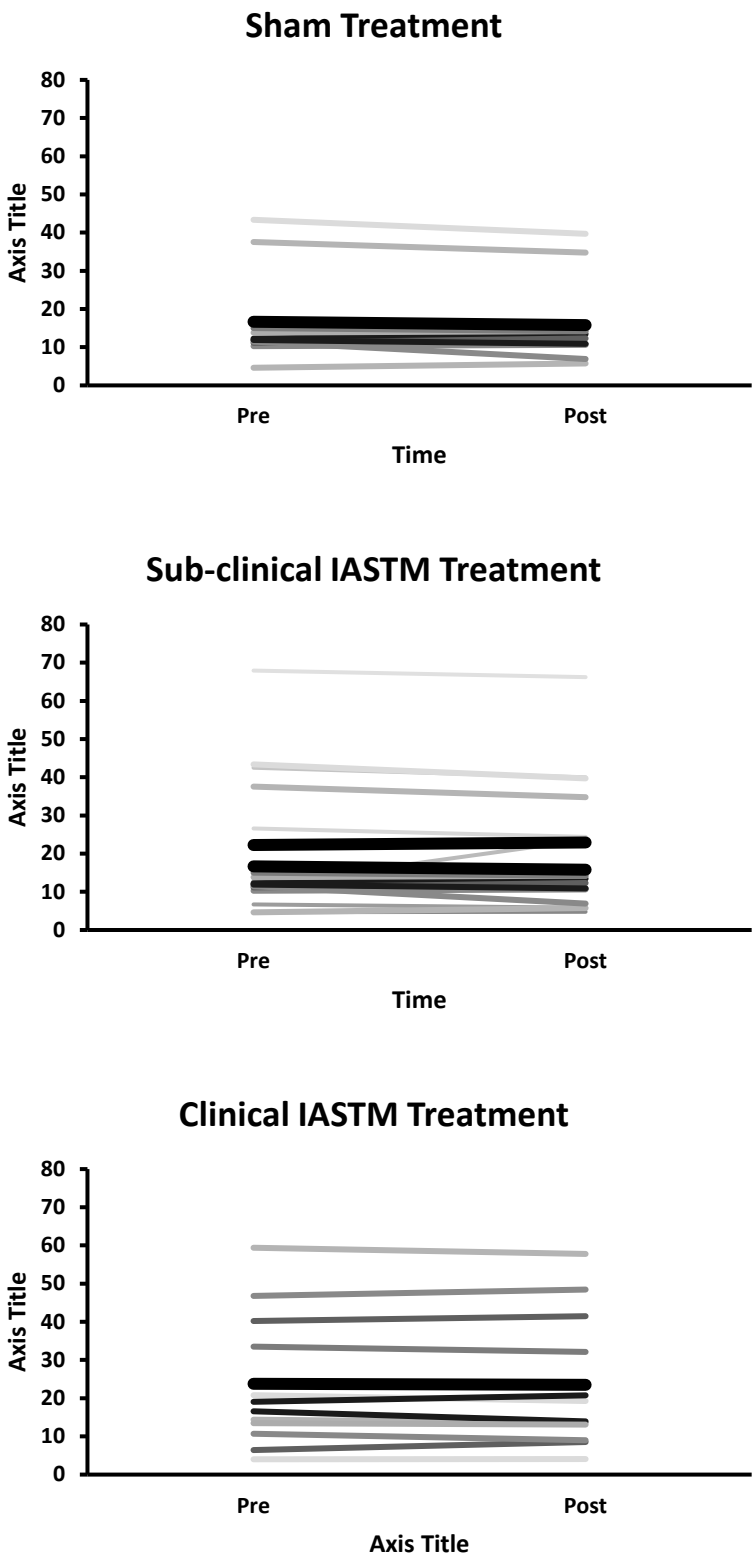
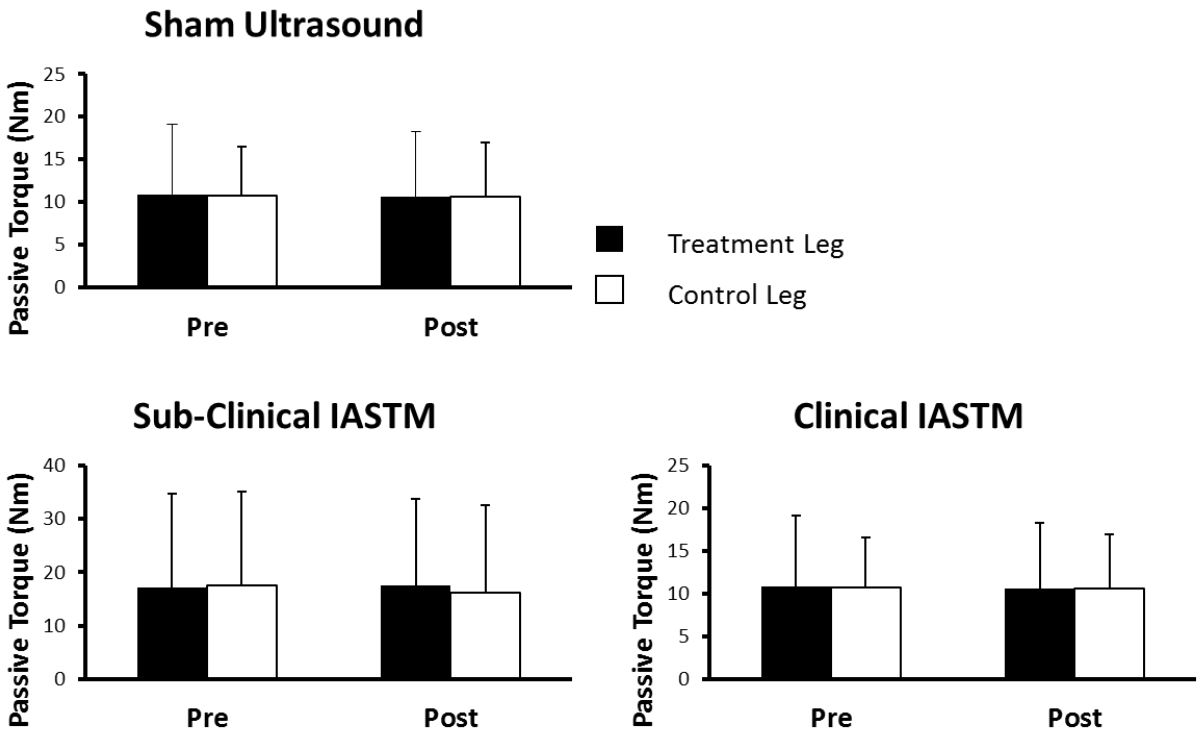


Figure 13: Graphs displaying mean Passive Torque values across time (-pre and -post). Statistical analysis revealed no interactions or main effects for Passive Torque data.



4.2.3 Range of Motion

As previously described, a 2x3×2 mixed factorial, repeated measures ANOVA [leg(treatment vs control)xgroupxtime] was used to analyze ROM results. ANOVA revealed no significant interactions or main effects (Table 4).

Table 4: Passive Torque ANOVA results

Term	P value	Partial Eta Squared
GroupxTimexLeg interaction	p= 0.504	= 0.046
TimexLeg interaction	p= 0.952	= 0.000
LegxGroup interaction	p= 0.450	=0.054
TimexGroup interaction	p= 0.102	=0.146
Time main effect	p= 0.277	=0.041
Leg main effect	p= 0.693	=0.005
Group main effect	p= 0.356	=0.069

Figure 14: Graphs displaying mean maximal ROM values across time (-pre and -post). Statistical analysis revealed no interactions or main effects for ROM data

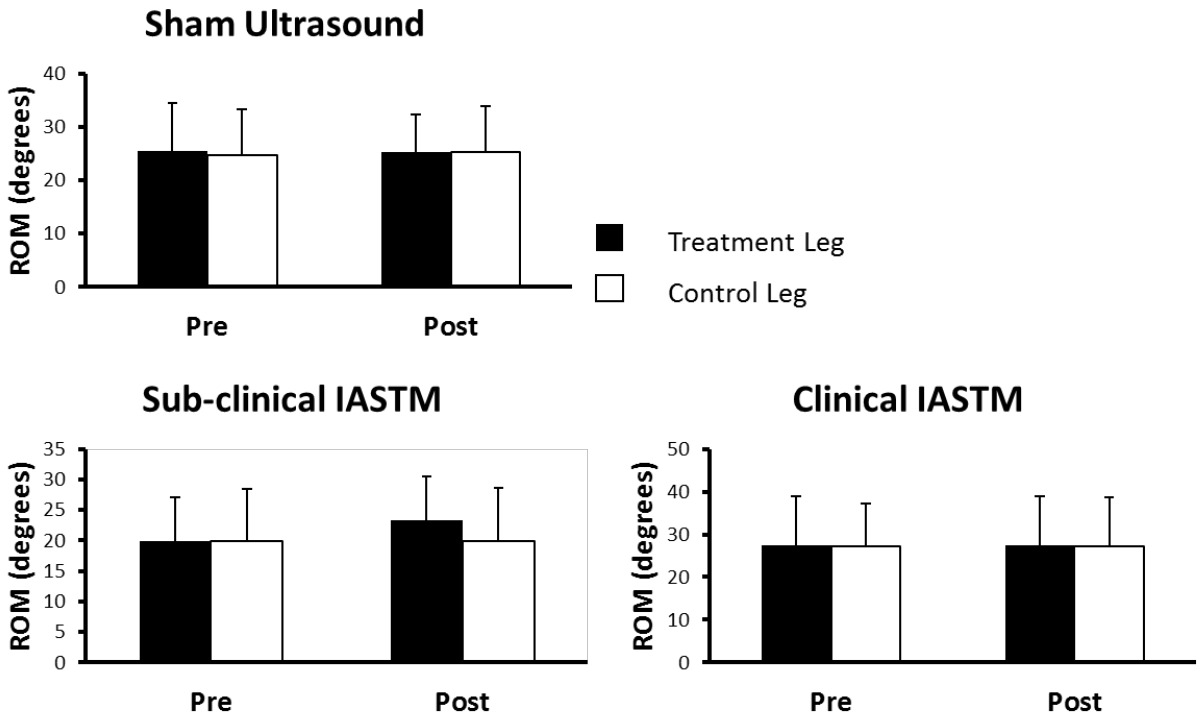


Table 5: Perception of Functional Ability Questionnaire collapsed across group

Question	p Value	Partial Eta Squared
Overall Health	0.154	0.038
Overall Flexibility	0.120	0.042
Overall Strength	0.205	0.033
Calf Flexibility	0.201	0.036
Calf Strength	0.233	0.031
Calf Pain	0.099	0.055
Calf Functional Ability	0.126	0.044
Calf Ability to Carry out Activities of Daily Life	0.169	0.036

4.3 The Effects of IASTM Treatment on Short Hamstring Syndrome in College Aged Adults

4.3.1 Hamstrings flexibility

There were no significant interactions or main effects concerning the range of motion at which participants reported sensation of initial stretch. Nor was there a between subject's main effect for group (p=0.234).

Figure 15: Hamstrings range of motion associated with initial stretch perception collapsed across treatment

	Restricted		Non Restricted	
	Pre	**Post**	**Pre**	**Post**
Mean	129.38	127.00	154.00	155.11
SD	12.02	14.22	14.04	14.02

Table 6: ANOVA in hamstrings range of motion associated with initial stretch perception

Term	P value	Partial Eta Squared
TreatmentxTimexGroup	p=0.389	=0.05
TreatmentxTime	p= 0.389	=0.105
TimexGroup	p= 0.207	=0.641
TreatmentxGroup	p= 0.184	=0.114
Treatment	p= 0.989	=0.000
Time	p= 0.556	=0.024

There was however a significant between subject's main effect for group (p= 0.002).

Figure 16: *Spaghetti plot displaying individual stretch perception responses to sham treatment*

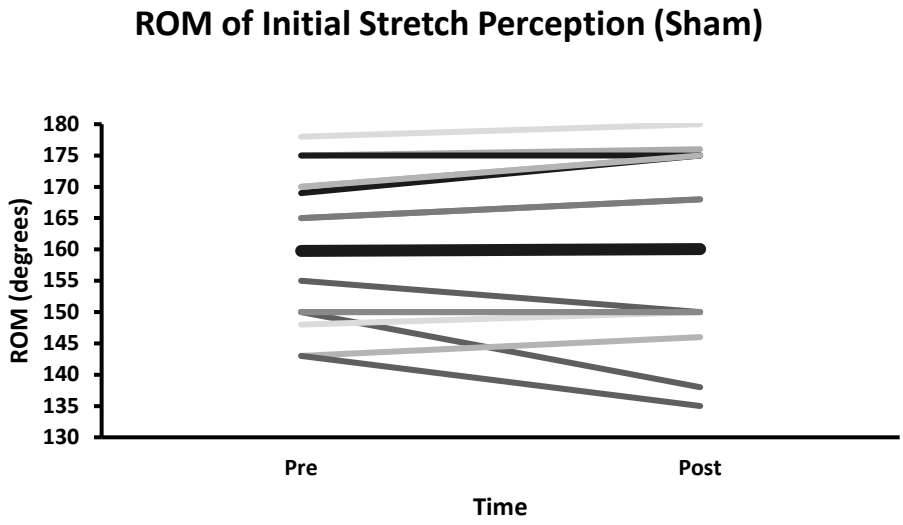
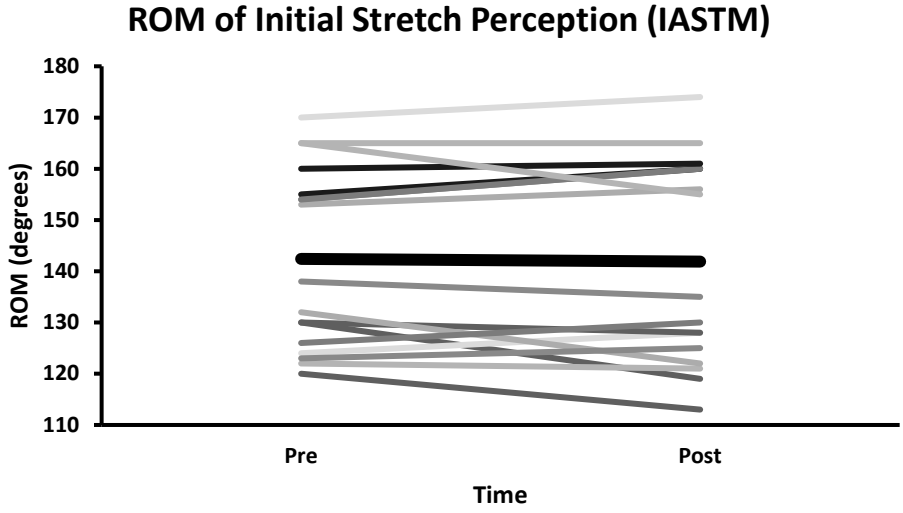


Figure 17: *Spaghetti plot displaying individual stretch perception responses to IASTM treatment*



Similarly, there were no significant interactions or main effects for maximal hamstrings stretch range of motion. Further, there was no between-subjects main effect for group ($p=0.166$).

Figure 18: Hamstrings range of motion associated with maximal stretch perception collapsed across treatment

	Restricted		Non Restricted	
	Pre	**Post**	**Pre**	**Post**
Mean	150.50	148.38	168.00	169.89
SD	8.69	9.91	8.69	7.79

Table 7: *ANOVA in hamstrings range of motion associated with maximal passive stretch*

Term	P value	Partial Eta Squared
TreatmentxTimexGroup	p=0.127	=0.148
TreatmentxTime	p= 0.556	=0.024
TimexGroup	p= 0.294	=0.073
TreatmentxGroup	p= 0.391	=0.050
Treatment	p= 0.446	=0.039
Time	p= 0.446	=0.039

There was however a significant between subject's main effect for group (p=-.00002).

Figure 19: Spaghetti plot displaying individual responses in maximal ROM to IASTM treatments

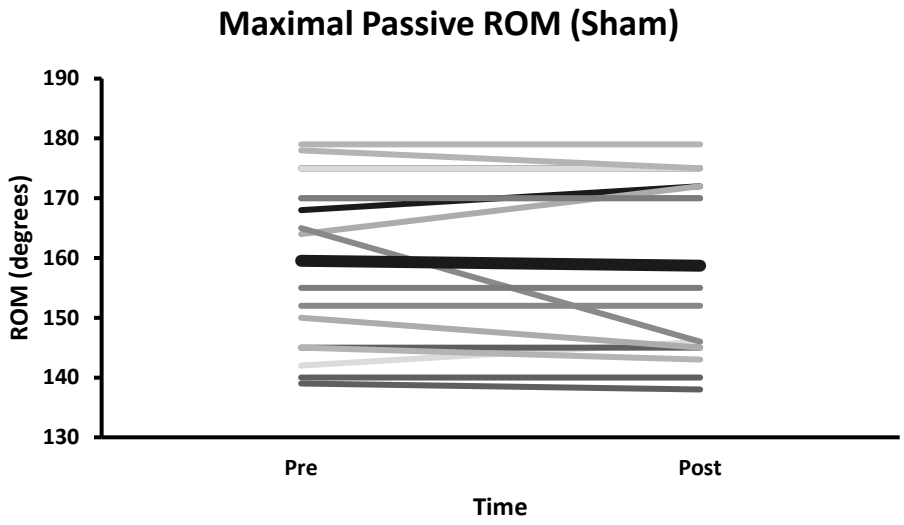
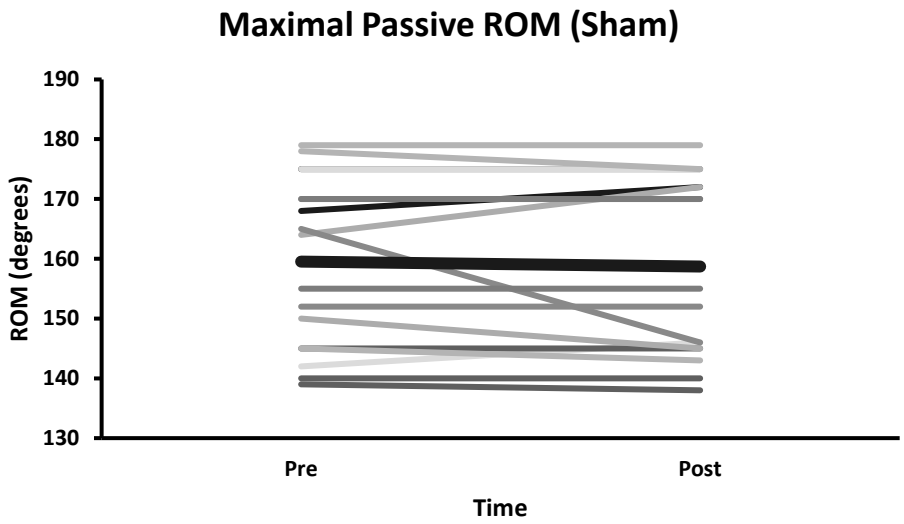


Figure 20: Spaghetti plot displaying individual responses in maximal ROM to Sham treatments



4.3.2 Biceps fermoris muscle quality (echo intensity)

There were no significant interactions or main effects in the muscle quality of the biceps femoris as measured by ultrasound echo intensity.

Figure 11: Biceps Femoris echo intensity (muscle quality) values collapsed across group

Figure 21: Biceps Femoris echo intensity (muscle quality) values collapsed across group

	IASTM		Sham	
	Pre	**Post**	**Pre**	**Post**
Mean	59.33624	55.72171	58.53512	58.94624
SD	10.01471	15.36098	10.5303	10.52788

Table 8: ANOVA in muscle quality of the Biceps Fermoris

Term	P value	Partial Eta Squared
TreatmentxTimexGroup	p=0.443	=0.040
TreatmentxTime	p= 0.395	=0.049
TimexGroup	p= 0.385	=0.051
TreatmentxGroup	p= 0.755	=0.007
Treatment	p= 0.616	=0.017
Time	p= 0.491	=0.032

Similarly, there was no between-subjects main effect for group (p=0.160).

Figure 22: Spaghetti plot displaying individual responses in echo intensity following IASTM treatment

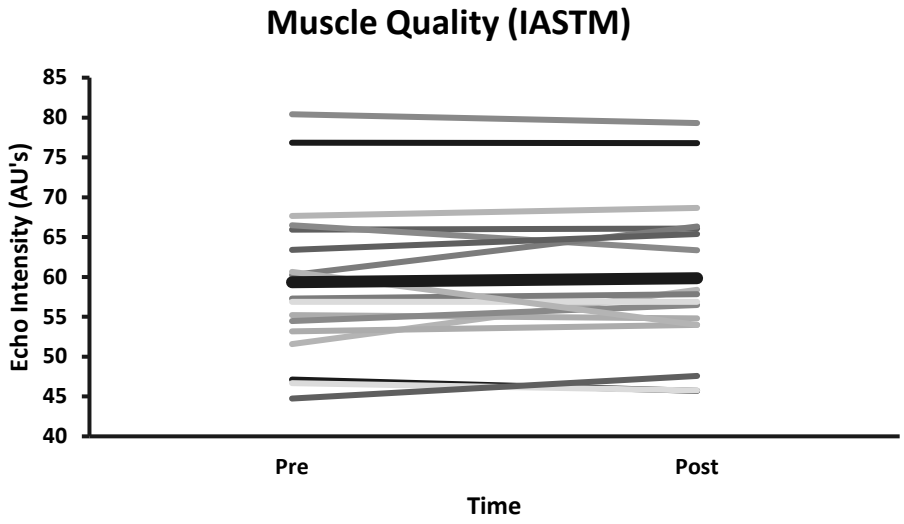
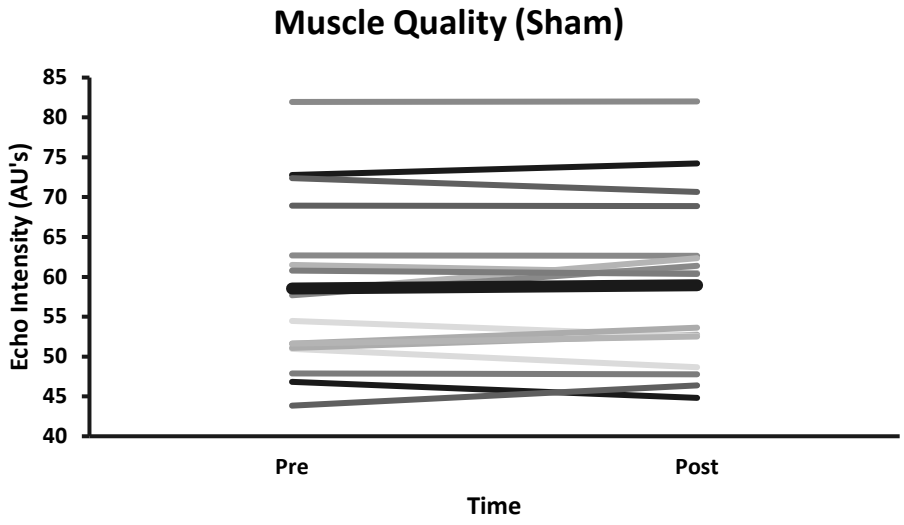


Figure 23: Spaghetti plot displaying individual responses in echo intensity following sham treatment



4.3.3 Biceps fermoris cross-sectional area

There were no significant interactions or main effects for cross-sectional area as measured using ultrasound.

Figure 24: Biceps Femoris cross-sectional area values collapsed across group

	IASTM		Sham	
	Pre	**Post**	**Pre**	**Post**
Mean	7.685118	7.473941	7.358588	7.252471
SD	1.731234	1.620817	1.671481	1.607253

Table 9: ANOVA in Biceps Femoris Cross-sectional area

Term	P value	Partial Eta Squared
TreatmentxTimexGroup	p=0.227	=0.096
TreatmentxTime	p= 0.564	=0.023
TimexGroup	p= 0.582	=0.281
TreatmentxGroup	p= 0.795	=0.005
Treatment	p= 0.080	=0.191
Time	p= 0.132	=0.145

Further, there was no between-subject main effect for the group (p=.996).

Figure 25: Individual changes in cross sectional area following IASTM treatment

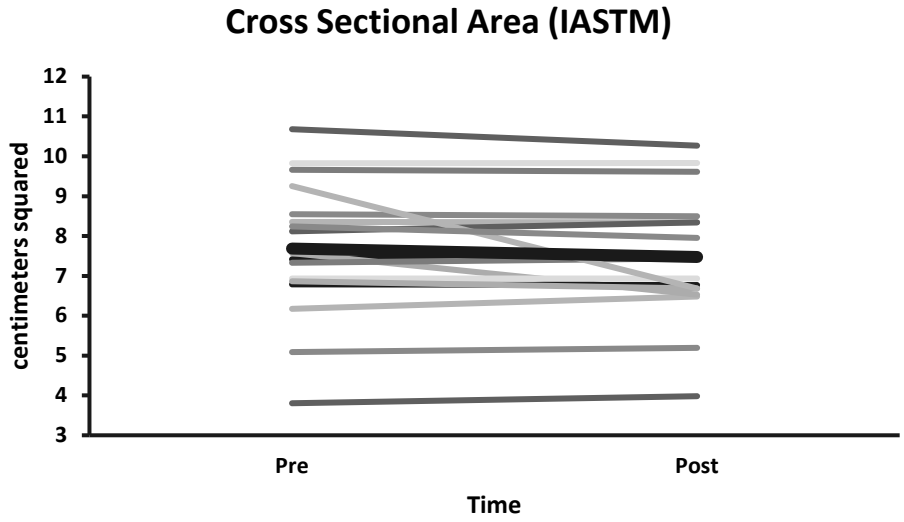
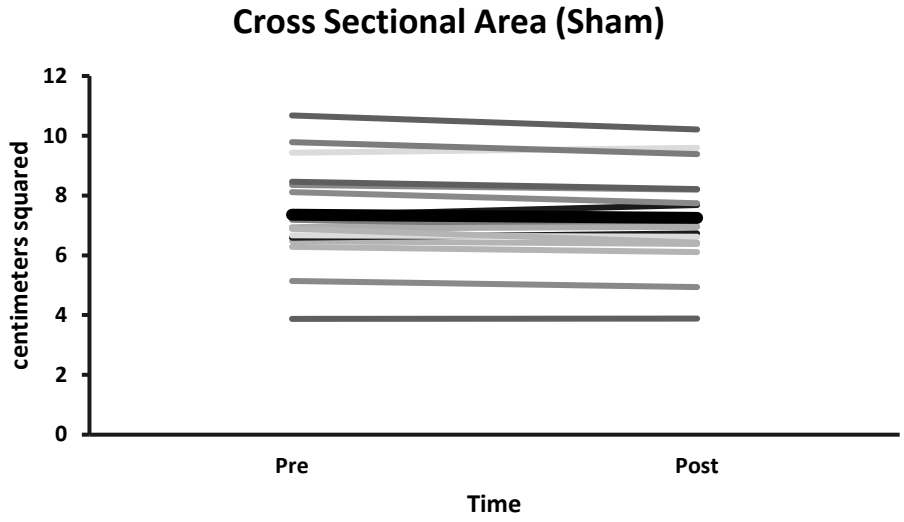


Figure 26: Individual changes in cross sectional area following Sham treatment



4.3.4 Biceps fermoris pennation angle

There were no significant interactions or main effects in the pennation angle data barring a main effect for joint angle (p=.0001). It is important to note that the TreatmentxGroup interaction approached significance (p=.057). This indicates IASTM may be more beneficial for subjects who are suffering from range of motion restriction.

Figure 27: Delta scores of pennation angle from the ninety degree position

	IASTM Treatment				**Sham Treatment**			
	Medium Stretch		**Max Stretch**		**Medium Stretch**		**Max Stretch**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Mean	-0.43	-1.74	-2.22	-2.70	-0.99	-1.59	-2.78	-2.24
SD	2.17	1.59	1.63	2.11	0.93	1.46	1.64	2.18

Figure 28: Spaghetti plot displaying individual responses in pennation angle in the un-stretched position following IASTM

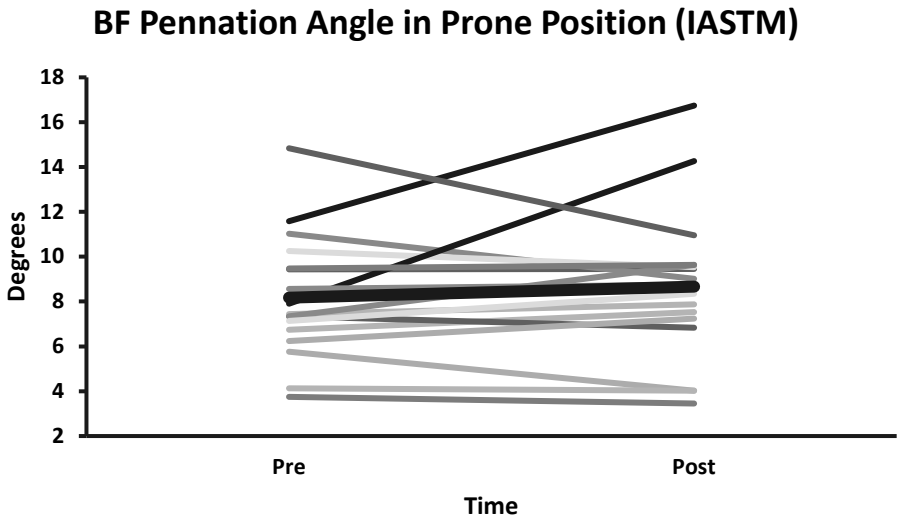


Figure 29: Spaghetti plot displaying individual responses in pennation angle in the un-stretched position following IASTM

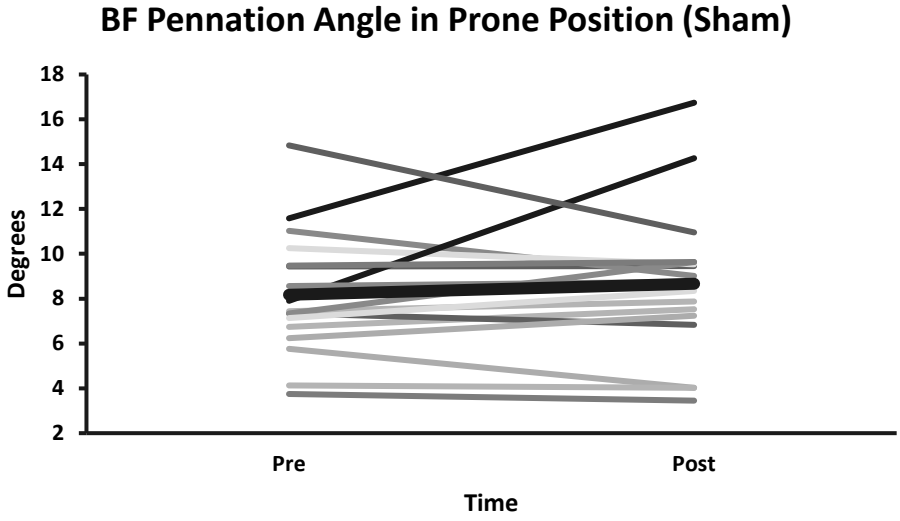


Table 10: ANOVA in Biceps Fermoris pennation angle

Term	P value	Partial Eta Squared
TreatmentxAnglexGroup	p= 0.945	=.004
TreatmentxTimexGroup	p= 0.528	=.027
TreatmentxAngle	p= 0.905	=.007
TreatmentxTime	p= 0.450	=.038
AnglexGroup	p= 0.988	=.001
TimexGroup	p= 0.344	=.060
TreatmentxGroup	**p= 0.057**	**=.220**
Angle*	**p= 0.0001***	**=.684***
Time	p= 0.944	=.0001
Treatment	p= 0.101	=.169

Figure 30: *Spaghetti plot displaying individual responses in pennation angle at the 90 degree position following IASTM treatment*

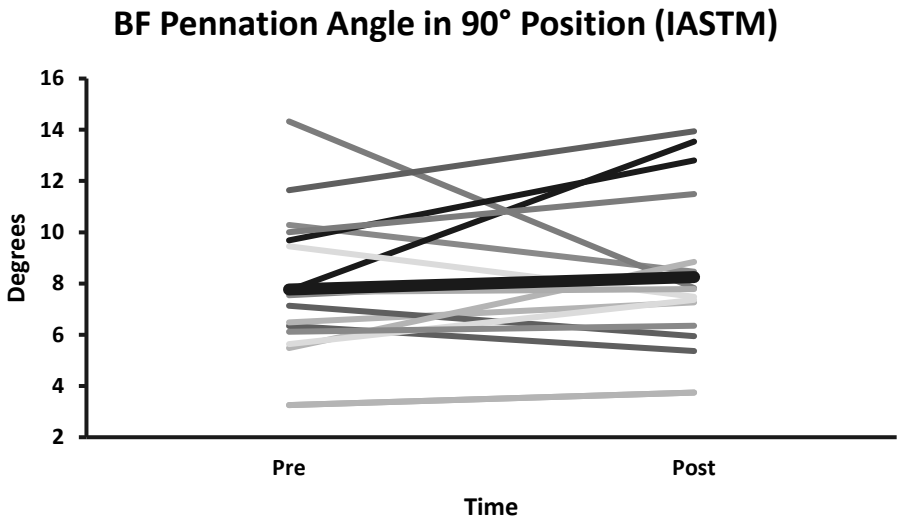


Figure 31: Spaghetti plot displaying individual responses in pennation angle at the 90 degree position following sham treatment

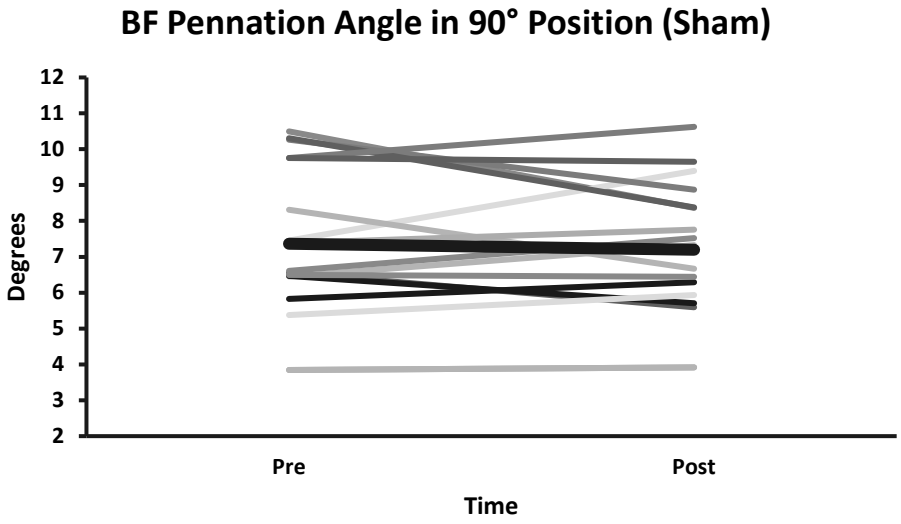


Figure 32: Spaghetti plot displaying individual responses in pennation angle at the mid-stretch position following IASTM treatment

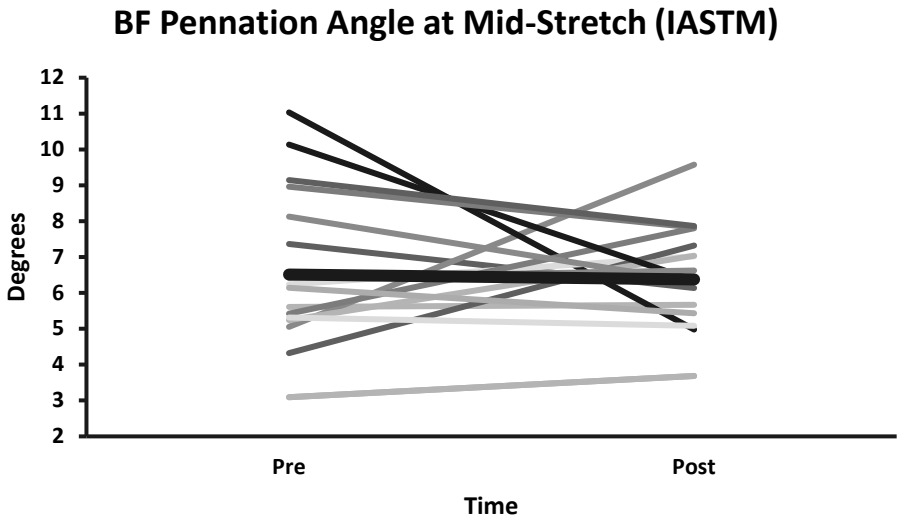


Figure 33: Spaghetti plot displaying individual responses in pennation angle at the mid-stretch position following sham treatment

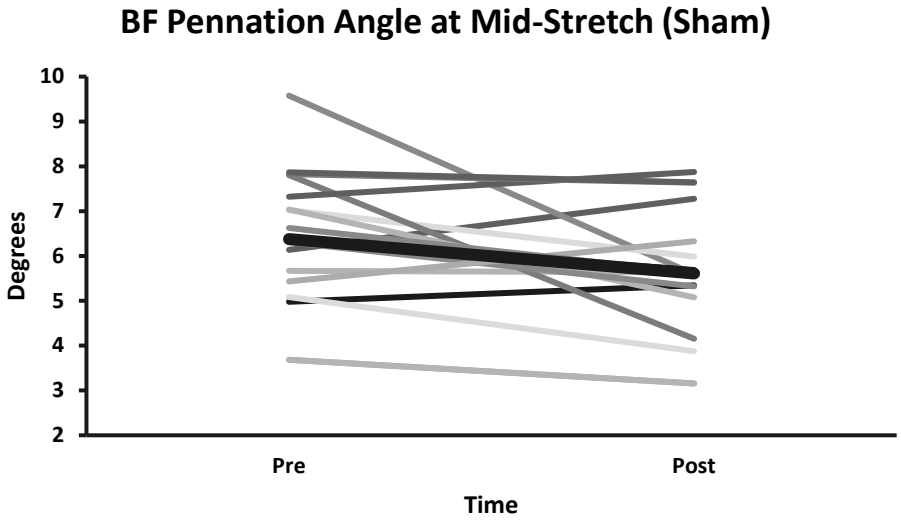


Figure 34: Spaghetti plot displaying individual responses in pennation angle at the maximal stretch position following IASTM treatment

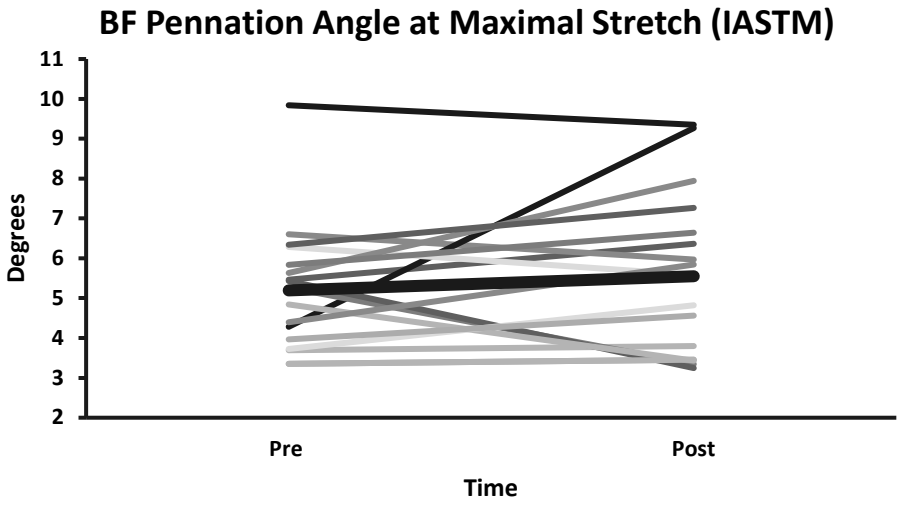
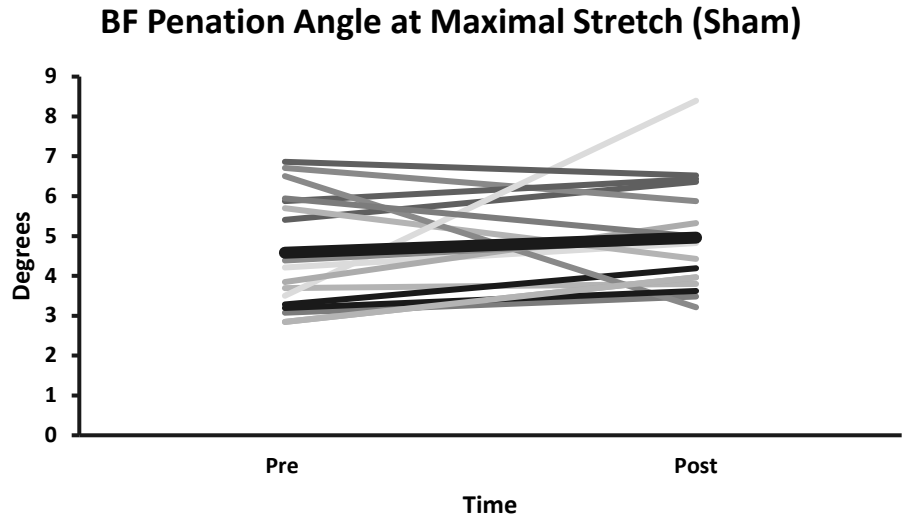


Figure 35: Spaghetti plot displaying individual responses in pennation angle at the mid-stretch position following IASTM treatment



4.3.5 Qualitative Assessments of Treatment Efficacy

There were no statistically significant interactions or main effects associated with the perception of functional ability data.

Table 11: PFAQ ANOVA Results

Question	p Value	Partial Eta Squared
Overall Health	0.470	0.028
Overall Flexibility	0.768	0.020
Overall Strength	0.087	0.108
Calf Flexibility	0.057	0.129
Calf Strength	0.128	0.094
Calf Pain	0.501	0.040
Calf Functional Ability	0.109	0.100
Calf Ability to Carry out Activities of Daily Life	0.106	0.129

Further, paired samples T-tests revealed IASTM didn't evoke a sense of healing when compared with the sham treatment.

Figure 36: Survey of Treatment Quality Results

	Question 1			Question 2		
	Treatment	**Sham**	**p value**	**Treatment**	**Sham**	**p value**
Mean	3.53	3.18	0.172	3.59	3.18	0.055
SD	0.51	0.81		0.51	0.88	

Chapter 5: Discussion

5.1 Discussion Intro

Results from this multi-study examination of the effects of IASTM have suggested that IASTM may not be the most efficacious treatment available for degenerate soft-tissue. Our three investigations found no changes in MTS, PROM, MVC-PT, myokine expression, perception of functional ability as measured by the PFAQ, muscle quality (echo intensity), pennation angle or hip ROM. However, there are considerations that should be made prior to passing judgement on IASTM as a treatment for soft tissue injury. These considerations include the role of IASTM as a small part of a larger treatment plan, the type of patient that IASTM is indicated for and the dosage parameters and frequency of treatments.

5.2 IASTM as part of a larger treatment plan

A recent review article examined the way IASTM should be used as a part of a larger treatment plan (Kim, Sung, & Lee, 2017). Using prior published peer-reviewed articles, they made the following recommendations (Table 12).

Table 12: IASTM as part of a larger treatment plan

Treatment	Objective	Protocol	Reference
Warm-up	↑ Blood Flow ↑ Muscle Temp ↑ Plasticity	10-15 light exercise or 3-5 min with hot pack/ultrasound	(Black, 2010; Hammer, 2008)
IASTM	↓ Scar Tissue ↑ Collagen Synthesis ↑ Collagen Alignment	40-120 seconds	(Laudner, Compton, McLoda, & Walters, 2014)
Stretching	Correct the shortened tissues and prevent re-injury	3 repetitions lasting 30 seconds each	No peer reviewed evidence suggesting interaction with IASTM
Strengthening Exercise	Strengthen the treated tissue and prevent re-injury	High repetition with low load	(Hammer, 2008)
Cryotherapy	Reduce pain, control residual inflammation, and prevent secondary cell hypoxia	10-20 minutes	No peer reviewed evidence suggesting interaction with IASTM

This paper points out a paradox within the soft-tissue mobilization research body (Kim et al., 2017). In order to have more control over confounding variables researchers generally study one treatment at a time. While this is logical from a study design perspective it is detached from the reality that clinicians do not use IASTM as a stand-alone treatment. It is possible that other treatments (light exercise, stretching, and heat/cryotherapy) serve as a moderator of the IASTM treatment and that if IASTM were used in conjunction with other treatments, over time in an indicated population that measurable differences the the prior mentioned dependent variables would be present. There is a clear disconnect between the way IASTM is used clinically verses how it is studied in laboratories. As the basic science, mechanisms behind IASTM become clearer through controlled laboratory examination

(particularly in animal models) researchers will be able to begin exploring the effects of IASTM as part of a larger subject treatment plan.

5.2 The need for IASTM research in indicated populations

One possible reason our findings for MTS, PROM, MVC-PT, myokine expression, perception of functional ability as measured by the PFAQ, muscle quality (echo intensity), pennation angle or hip ROM were statistically insignificant could be due to the healthy populations that were used (Kim et al., 2017). With the exception of our third investigation, which used subjects who were classified as “hamstrings restricted” (Halbertsma & Goeken, 1994), all participants in these research investigations were healthy, college-aged students. Generally, the investigations, which found clear benefit from IASTM (Davidson et al., 1997; Gehlsen et al., 1999) were carried out in animal models utilizing injured rodents including collagenase induced achilles tendinopathy. It makes sense from both a physiological and statistical point of view that treating an injured population with clear physiological deficits in the variables of interest would be more likely to yield statistically significant, positive outcomes. Our study designs in the first two investigations used IASTM as more of a training tool then a rehabilitation tool. Our data from these investigations indicate that IASTM has little efficacy as a training tool to increase ROM or cause detectable increases in strength measures in asymptomatic populations.

The third study utilized one group of subjects who were suffering from short hamstrings syndrome as determined by unilateral straight leg raise. Therefore, these subjects did have a positive indication for receiving the IASTM treatment. Of these restricted subjects, none

reported any symptoms that are often associated with shortened hamstrings and/or other posterior kinetic chain restriction (low back pain, sciatica etc.). These participants were likely asymptomatic because they were young and active. Irrespective of symptoms, we would expect that the biomechanical and possible genetic predispositions that lead to these subjects having chronically shortened hamstrings had also caused alterations in pennation angle. This hypothesis that baseline pennation angle in the hamstrings restriction group would differ from that of the healthy population proved to be a faulty hypothesis. In addition to there being no differences in baseline pennation angle between the two groups there was no group interaction for any of the ultrasound data in study three.

As previously mentioned, prior to our third investigation we suspected that the primary reason we were finding no physiological changes in skeletal muscle performance following IASTM was due to the fact that we were carrying out our investigations in healthy, asymptomatic populations. The fact that we saw no detectable changes in pennation angle following IASTM within our hamstrings restricted population causes us to wonder if our lab is using sensitive enough measurements to detect change. Further, these results suggest that future research should include symptomatic subjects who have recently suffered acute injury or those who are suffering from chronic pathologies (i.e. tendonitis, tendinosis).

The fact that there were no changes in hamstrings ROM following IASTM was surprising as it has been demonstrated that massage can alter hamstrings ROM even in healthy, asymptomatic populations who are not suffering from short-hamstrings syndrome (Diana Hopper et al., 2005; D Hopper et al., 2005). It is possible that the use of manual goniometry rather than an isokinetic dynamometer added variance to these ROM values. Manual

goniometry was used, as it was more cost effective than having a custom lever arm built and much more efficient from a time per visit perspective. Due to time restraints associated collaborators this method seemed most appropriate.

5.3 Time course of proposed benefits of IASTM

One research question that has stumped both researchers (Crawford et al., 2014) and clinicians alike is the question of when to use IASTM following damage to skeletal muscle. This is a complex question as many variables including, training status, type of damage and stage of healing come into play. The data at hand does not speak to this research question, as it was not carried out following any training or injury event. However, prior research has suggested that following eccentric loading-induced muscle damage starting IASTM treatments immediately following the damaging event or -24 hours post were both equally efficacious in returning muscle/tendon stiffness to pre-damage values (Crawford et al., 2014).

5.3.2 IASTM Treatment during Acute Injury Phase

A common debate amongst clinicians is if IASTM treatments are appropriate during the acute injury phase of healing in damaged/pathologic tissue. It has been anecdotally reported that many clinicians avoid IASTM during the acute injury phase as it may cause further inflammation and damage. Conversely, it has also been anecdotally reported that clinicians use IASTM tools to help facilitate lymphatic reabsorption of inflammatory secretions by use of superficial strokes towards the heart. Data generated during investigation one demonstrated

that IASTM does not initiate inflammation signaling in healthy, asymptomatic tissue (Vardiman et al., 2015). However, in the presence of positive indications for IASTM evidence has demonstrated that soft tissue mobilization may have the ability to initiate inflammation at the cellular level (Davidson et al., 1997; Gehlsen et al., 1999; Hammer, 2008). This ultimately leads to increased fibronectin and fibroblast proliferation and aids in healing of degenerate tissue (Hammer, 2008).

In addition to IASTM potentially causing localized cellular inflammation, it appears IASTM and other forms of soft tissue mobilization more than likely elicit localized and possibly systemic increases in blood flow (Franklin et al., 2014). If IASTM is efficacious it is likely through an inflammation-mediated pathway. With this in mind it seems prudent to not further exacerbate inflammation in already inflamed tissue. Especially considering IASTM has been shown to be efficacious in respect to fibroblast proliferation irrespective of time administered post injury (Crawford et al., 2014).

Though it is likely unwise to elicit further inflammatory response using IASTM immediately following acute muskulo-skeletal injury, there is a growing body of literature suggesting that it may be similarly unwise to use therapeutic modalities and compression to reduce inflammation (Fredericson, Moore, Guillet, & Beaulieu, 2005; Kim et al., 2017; Molloy, Wang, & Murrell, 2003). Restriction of the localized inflammatory response has been suggested to “disorganize and weaken” the soft tissue structure (Fredericson et al., 2005). However, the more compelling argument not to combat inflammation following an acute injury stems from the reports that localized inflammation leads to secretion of growth factors, which aid in the

facilitation of healing through fibroblast proliferation and subsequent collagen synthesis. (Molloy et al., 2003).

5.4 Dosage Recommendations

As previously stated, one variable that makes exploring the efficacy of IASTM difficult is the fact that there are no commercially available devices that quantify load and volume administered during IASTM treatments. However, using consistent, uniformed pressure is not a problem unique to researchers. Though no research has compared and contrasted forces administered between clinicians during IASTM it is likely highly variable. The purpose behind investigation two was to help clarify the effects of different pressure forces on muscle performance in human subjects. Literature review revealed that there is a dose/response main effect for pressure administered for fibro-blast proliferation and likely down-stream collagen synthesis (Gehlsen et al., 1999) (Table 1). Exploring this pressure dose/response relationship was the primary reason for carrying out investigation two.

Table 1: Dosage Parameters: Comparison of clinical animal/human model pressure administration

Author, Year	Model Summary	Pressure Force Administered	Outcome
(Gehlsen et al., 1999)	Collagenase induced Achilles tendonitis rat model (n=30)	Light IASTM= (.5 N/mm^2) Medium IASTM= (1 N/mm^2) Extreme IASTM= (1.5 N/mm^2)	Increased fibroblast proliferation (p<.05)
(M. T. Loghmani & Warden, 2009)	Bilateral torn MCL rat model (n=51)	Estimated at (1.5 N/mm^2)	Ligament strengthening (p<.05) Ligament stiffening (p<.01)
(M. Loghmani & Warden, 2013)	Bilateral torn MCL rat model (n=30)	Estimated at (1.5 N/mm^2)	Increased tissue perfusion 24h post (p<.05)
(Davidson et al., 1997)	Collagenase induced achilles tendonitis rat model (n=20)	"Considerable pressure"	Increased fibroblast proliferation (p<.05)
(Vardiman et al., 2015)	Human Subjects, controlled trial (n=11)	N/mm^2 equivalent is estimated at 1.0	No changes in ROM or IL-6 expression (p<.05)
Ohio State Group	Rabbit tibialis anterior, eccentric loading (n= varies from study to study)	10 N	Decreased MTS (p<.05) and time to peak torque recovery (p<.05)

Our second research investigation explored dose response using a "medium pressure" group (1.03 N/mm^2) (Gehlsen et al., 1999) and a "very light" group. Neither of our groups demonstrated statistically significant differences from the control or sham group so these data are not helpful in making dosage parameter recommendations. Based on literature review, the

most desirable load during an IASTM treatment is 1.0 N/mm^2 performed over the course of three minutes with strokes running both proximal to distal as well as distal to proximal (Gehlsen et al., 1999). Further, It would appear that in instances of tendinopathy the tendon should be scraped primarily as opposed to the muscle belly (Gehlsen et al., 1999).

As previously stated most clinicians do not have the equipment or expertise available to quantify and control for pressure administered during an IASTM treatment. During our second investigation we administered treatments that corresponded with the ideal pressure force of 1.0 N/mm^2(Gehlsen et al., 1999). Anecdotally, we observed no bruising during subsequent IASTM follow-up visits, which suggests that the most efficacious IASTM treatments will more than likely not elicit bruising.

5.5 Future Research Recommendations

Future research investigations should continue to explore the effects of dosage parameters, treatment frequency and how IASTM responses are moderated when used as one part of a holistic rehabilitation plan. Further, future research should use controlled, clinical trials featuring symptomatic populations. Of particular interest should be patients suffering from acute muscle tears and strains, those with chronic tendinopathies including tendinitis and tendinosis and those recovering from post-surgical tendon repair. Though these populations have been explored in rodents using collagenase injections to induce tendinopathy (Davidson et al., 1997; Gehlsen et al., 1999) there are no controlled, clinical trials who have investigated these populations in humans.

One issue that presents its self when conducting in-vivo experiments in injured and pathologically degenerate muscle tissue in humans is the difficulty of correctly quantifying the

severity of an injury for between-subjects comparison. For this reason, the most practical study design may be the use of age and sex matched post-surgical tendon repair subjects.

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Appendices

Documents used in all studies

Health History Questionnaire

APPLIED PHYSIOLOGY LABORATORY
UNIVERSITY OF KANSAS

MEDICAL HISTORY FORM

NAME:_____ DATE:_____

AGE:_____ HEIGHT:_____ WEIGHT: _____

A. Have you ever experienced any of the following conditions or procedures?

1. Myocardial Infarction	YES	NO
2. Angiography	YES	NO
3. Coronary Surgery	YES	NO
4. Chest Discomfort	YES	NO
5. Hypertension (high blood pressure)	YES	NO
6. Hypotension (low blood pressure) Systolic ≤ 100mmHg or Diastolic ≤ 60mmHg	YES	NO
7. Shortness of breath upon light exertion	YES	NO
8. Dizziness upon light exertion	YES	NO
9. Pulmonary disease	YES	NO
10. Heart palpitation	YES	NO
11. Heart murmur	YES	NO
12. Diabetes If “YES”, Type I or Type II	YES	NO

13. Extremity discomfort YES NO

14. Claudication (circulation problems cause leg pain) YES NO

15. Peptic Ulcers YES NO

16. Metal implants (including pins) YES NO

Does anyone in your family have a history of cardiovascular disease? YES

NO If "YES", who? _____

_____

B. Do you smoke? YES NO

C. Are you currently using any anti-asthmatic medications? YES NO

D. Are you currently using any anti-hypertensive medications? YES NO

E. Are you currently taking any anti-inflammatory medications? YES NO

F. Are you currently taking any blood thinners (i.e.: Coumadin, aspirin) YES NO

G. Are you currently taking any other kind of medication? YES NO

If "YES", please list below:

_____

_____

_____

_____

_____

H. Are you allergic to idodine (Betadine, tincture of Iodine)? YES NO

I. Have you ever been treated for a heat related illness (heat exhaustion, heat stroke)? YES NO

J. What is your current Cholesterol level? (If known) _____

K. What is your current Blood-Pressure? (must be measured by APL staff) _____

L. Do you currently or have you been recently diagnosed with any of the following conditions?

Unhealed Fractures	YES	NO
Thrombophlebitis (blood clots)	YES	NO
Recent Surgery	YES	NO
Recent Uncontrolled Bruising	YES	NO
Osteomyelitis (acute or chronic bone infection)	YES	NO
Myositis Ossificans (hardened scarring in muscle tissue of the thigh)	YES	NO

M. Do you currently or have you been recently diagnosed with any of the following conditions?

Varicose Veins	YES	NO
Burn Scars	YES	NO
Rheumatoid Arthritis	YES	NO
Acute Inflammatory Conditions	YES	NO
Pregnancy	YES	NO
Osteoporosis	YES	NO

N. Have you ever experienced a skin irritation from common hand lotions or soaps? YES NO

Perception of Functional Abilities Questionnaire

Default Question Block

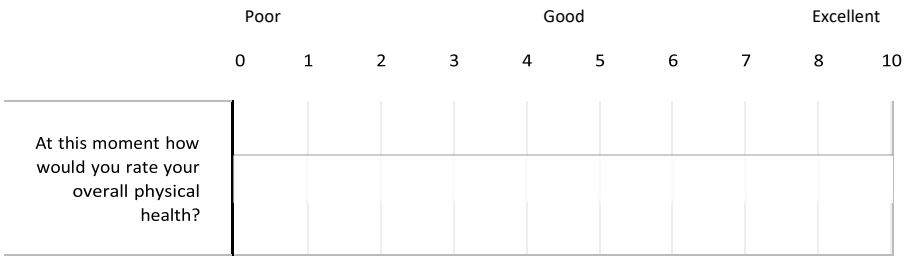
ID number

In the following questionnaire, you will be asked to rate your overall physical health, muscular flexibility, and muscular strength. You will also be asked to rate these relative to a affected body part. You will also be asked to rate your pain and ability to perform sport specific activities or activities of daily living.

Please indicate if this is the first or second time you have completed this survey

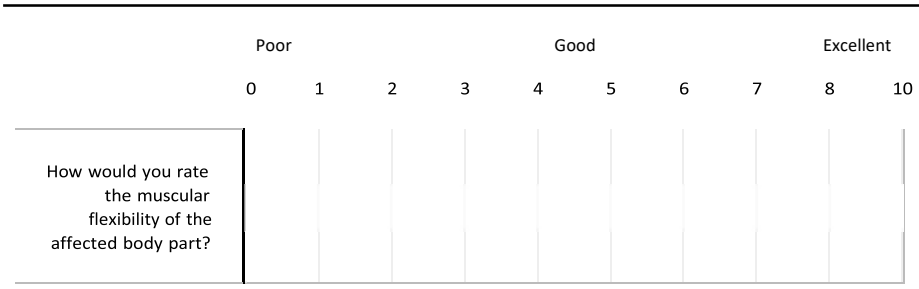
- First (Pre)
- Second (Post)

Please rate the following by circling the most appropriate number



Please rate the following by circling the most appropriate number

Please rate the following by circling the most appropriate number



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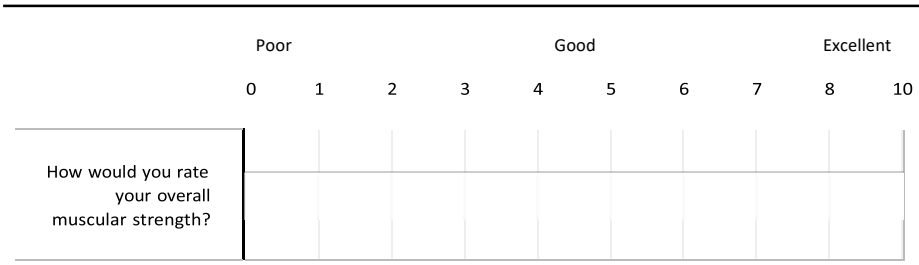

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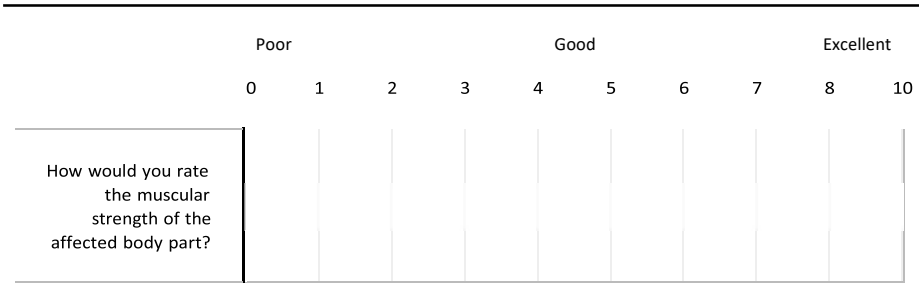
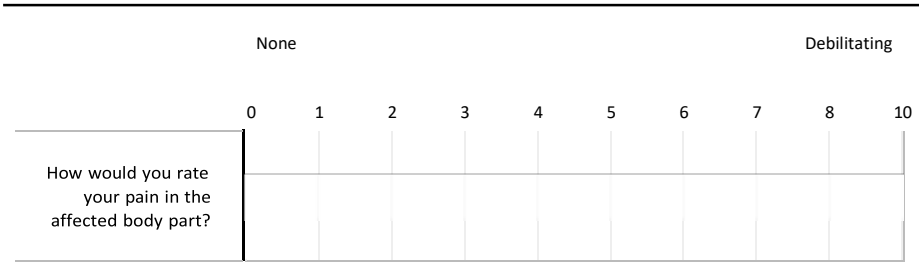


Please rate the following (note the different scale form the one used previously) by circling the most appropriate number



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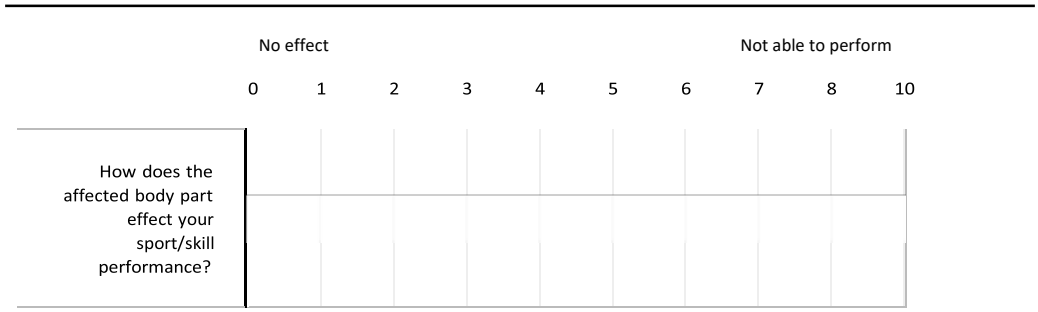
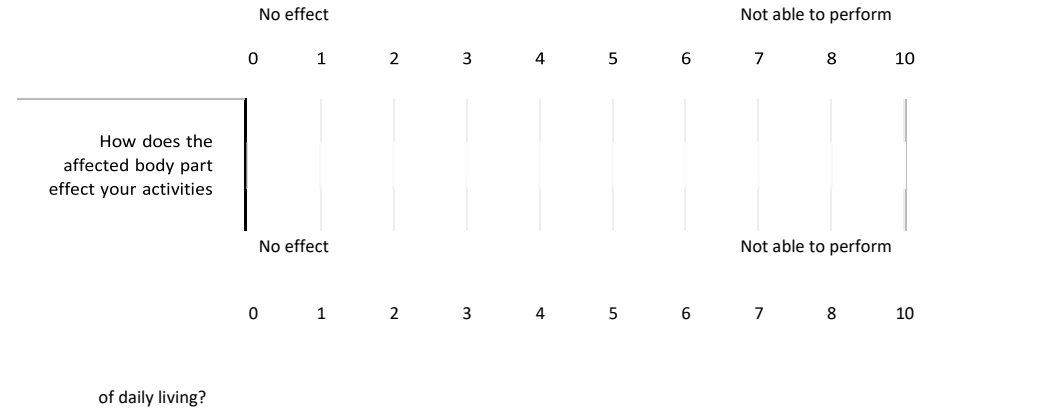


Please rate the following (note the different scale form the one used previously) by circling the most appropriate number



PAR-Q

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	N	
☐	☐	**1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart con- dition?**
☐	☐	**7. Do you know of any other reason why you should not do physical activity?**

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional.

Ask whether you should change your physical activity plan.

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to par

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT _____ WITNESS _____

or GUARDIAN (for par ticipants under the age of majority)

Study 3 Specific Document

Perception of Treatment Survey

Survey Item	Scale				
	Strongly Disagree	Disagree	Neither Agree or Disagree	Agree	Strongly Agree
1. My leg that received the IASTM treatment feels better than it did before treatment.	1	2	3	4	5
2. My leg that received the therapeutic ultrasound treatment feels better than it did before treatment.	1	2	3	4	5
3. My leg that received the IASTM treatment feels more flexible following treatment than it did before treatment.	1	2	3	4	5
4. My leg that received the therapeutic ultrasound treatment feels more flexible following treatment than it did before treatment.	1	2	3	4	5

Data Used in Statistical Analysis

Investigation one: Western Immuno-blots

Fold Changes in TNF.a Normalized to Tubulin

Subject	TNF-a Pre	TNF-a 24	TNF-a 48	TNF-a 72
1	1	1.714	1.568	1.127
2	1	1.038	1.195	1.160
3	1	0.818	0.680	0.699
4	1	0.418	0.775	0.646
5	1	0.751	1.080	1.764
6	1	0.888	0.804	0.595
7	1	0.636	0.645	0.718
8	1	0.930	0.649	1.083
9	1	0.734	0.806	1.012
10	1	0.365	0.418	0.894
11	1	0.729	1.151	2.126

Fold Changes in IL-6 Normalized to Tubulin

Subject	IL-6 Pre	IL-6 24	IL-6 48	IL-6 72
1	1	0.866	0.917	0.538
2	1	0.305	0.232	0.286
3	1	0.445	0.415	0.404
4	1	1.867	1.591	1.118
5	1	1.038	1.177	1.577
6	1	1.050	1.146	1.027
7	1	0.642	0.722	0.797
8	1	0.949	1.021	1.193
9	1	0.940	0.719	1.318
10	1	0.828	1.950	1.028
11	1	0.836	0.626	0.323

Investigation two:

	Treatment Leg						Control Leg					
	MTS		Passive Torque		ROM		MTS		Passive Torque		ROM	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
2-1-D	2.821178	1.576427	11.89271	10.87963	49.10145	41.20717	2.2461	1.952967	54.06972	54.94787	43.13156	43.13883
2-3-D	2.482985	2.710641	37.55169	34.77573	30.25586	30.26538	2.437162	2.278546	37.55169	34.77573	37.02732	37.18489
2-4-N	0.521657	0.550558	10.27658	10.61874	16.3242	16.33521	0.38963	0.45394	10.13496	11.88465	18.32156	18.33219
2-5-D	0.318931	0.510028	12.26015	13.44345	27.05474	27.07239	0.89984	0.760686	21.0784	20.4409	30.02038	30.15443
2-6-N	1.252213	0.946128	43.36631	39.69874	25.72845	25.81309	0.478985	0.090383	52.34943	56.83242	22.98598	22.8492
2-7-D	0.527627	0.55644	13.82284	14.22142	24.04275	24.04291	0.574925	0.695137	15.31428	16.52106	24.03493	24.02042
2-8-N	0.584166	0.523964	15.01284	14.18222	25.07176	25.03949	0.523411	0.398391	16.46153	11.35407	25.89914	26.06176
2-9-D	0.384128	0.479928	11.13783	12.30659	20.94518	20.97701	0.316593	0.281383	7.354236	6.196119	14.84818	14.82063
2-10-N	0.138991	0.194408	4.621034	5.746051	16.88695	16.88341	0.16872	0.175215	5.164603	5.680721	16.9334	16.8775
2-11-D	0.135072	0.196283	11.46654	6.913528	26.05698	26.11458	0.149356	0.254678	11.67334	7.743747	22.97754	23.03486
2-12-N	0.576363	0.518801	11.89271	10.87963	17.86399	17.85983	0.496658	0.46208	11.67557	11.11484	20.93023	20.94333
Mean	**0.885756**	**0.796691**	**16.66375**	**15.78779**	**25.39385**	**24.69186**	**0.789216**	**0.709401**	**22.07525**	**21.59019**	**25.19184**	**25.21982**
SD	**0.9259**	**0.739539**	**12.11782**	**10.99732**	**9.066664**	**7.103465**	**0.795384**	**0.727542**	**17.66214**	**18.83122**	**8.587142**	**8.629082**

	Treatment Leg						Control Leg					
	MTS		Passive Torque		ROM		MTS		Passive Torque		ROM	
4-1-N	2.532903	2.75939	40.2171	41.47736	43.95313	39.25364	2.490712	2.537012	35.18299	34.47552	36.1359	39.25364
4-2-D	0.292787	0.277818	14.37606	13.56938	46.11847	48.91441	0.203563	0.236079	11.86797	12.55537	49.24552	48.91441
4-3-N	3.891787	3.316543	46.78885	48.45302	38.29715	38.00273	2.926806	3.084741	32.67581	37.29745	36.18153	38.00273
4-4-D	0.381389	0.502002	16.57375	13.93262	31.19185	31.16316	0.396759	0.566452	13.10265	20.08976	32.21701	31.16316
4-5-N	0.881546	0.753906	20.79225	19.26939	30.094	30.08564	0.882796	1.109558	18.95623	20.87284	24.98509	30.08564
4-6-D	0.7474	0.736257	13.46596	13.09174	18.83505	18.89016	0.937232	0.856476	15.45212	15.18813	19.80871	18.89016
4-7-N	1.434177	1.624385	33.50951	32.12365	28.90189	28.9389	2.013333	1.602279	31.20865	25.86843	29.99496	28.9389
4-8-D	0.182532	0.468782	6.422307	8.52671	14.8421	14.88445	0.297049	0.421355	9.378553	11.07497	19.97546	14.88445
4-9-N	6.002048	2.864125	59.4033	57.78249	26.12808	26.1998	4.916738	4.559201	68.21339	69.49049	30.08478	26.1998
4-10-D	0.944059	0.690133	10.69307	8.995461	13.87588	12.87384	0.9334	0.846048	12.63228	11.54248	15.93084	12.87384
4-11-N	0.858539	1.008717	19.05977	20.75127	26.23519	26.15534	0.562882	0.61882	12.54443	13.01177	21.10681	26.15534
4-12-D	0.182965	0.263076	4.009044	4.067839	11.85888	11.8583	0.045443	0.199654	5.79186	6.189264	15.02268	11.8583
Mean	**1.527678**	**1.272095**	**23.77591**	**23.50341**	**27.52764**	**27.26836**	**1.383893**	**1.386473**	**22.25058**	**23.13804**	**27.55744**	**27.26836**
SD	**1.781202**	**1.0979**	**17.33275**	**17.4033**	**11.38297**	**11.37753**	**1.450728**	**1.344869**	**17.42677**	**17.43671**	**10.04731**	**11.37753**

	Treatment Leg						Control Leg					
	MTS		Passive Torque		ROM		MTS		Passive Torque		ROM	
3-1-D	0.171512	0.213558	4.657364	4.764066	14.86452	14.83746	0.302736	0.325026	9.646121	10.82118	25.0637	14.83746
3-2-N	0.374946	0.436189	12.94816	13.72957	27.0666	27.05714	0.302736	0.325026	9.646121	10.82118	25.0637	27.05714
3-3-D	0.174083	0.129398	6.694918	5.827707	23.16009	23.1659	0.239663	0.234606	6.788771	6.625407	17.98572	23.1659
3-4-N	1.064236	0.919075	16.65584	15.91354	18.95675	18.98182	1.379207	1.197092	21.64088	20.71909	23.14895	18.98182
3-5-D	1.415144	1.457078	10.71205	11.88601	8.740042	8.611056	2.112794	1.765946	11.50996	11.45904	8.744107	8.611056
3-6-N	3.263294	2.202749	67.93216	66.2166	33.275	33.30527	3.08016	3.807906	72.55964	67.68129	38.21157	33.30527
3-7-D	0.553747	3.025446	11.60595	23.59997	18.96918	18.98459	0.527055	0.680671	11.41829	13.20111	18.97537	18.98459
3-8-N	3.738518	1.767131	26.58878	24.38284	16.6228	16.465	4.108902	1.803206	43.64477	34.82336	36.29186	16.465
3-9-D	1.533463	1.454649	42.48809	39.9586	17.93482	17.9651	2.571299	3.229047	56.88151	44.26156	16.06305	17.9651
Mean	**1.365438**	**1.289475**	**22.2537**	**22.91988**	**19.95442**	**19.93037**	**1.62495**	**1.485392**	**27.08178**	**24.49036**	**23.28311**	**19.93037**
SD	**1.315271**	**0.968237**	**20.70919**	**19.50833**	**7.133646**	**7.175282**	**1.418737**	**1.303945**	**24.41268**	**20.51665**	**9.411272**	**7.175282**

Investigation three: ROM, muscle quality, CSA and pennation angle data

Pre IASTM

		Stretches				Pennation Angle			
Subject	Group	Initial Stretch	M-PROM	Quality	BF-CSA	relaxed	90	Mid	Max
2	1	130	150	65.932	3.804	9.436	6.345	6.114	5.463
3	2	165	170	51.576	6.173	6.741	6.482	5.668	3.693
4	1	154	165	66.482	5.088	11.025	10.285	9.474	6.603
5	2	155	169	47.126	7.417	11.583	7.728	6.171	4.281
6	1	124	148	46.675	9.826	10.254	9.452	7.562	6.284
7	2	153	175	55.213	7.57	5.762	3.254	6.123	3.352
9	2	154	165	57.287	7.329	3.748	14.326	7.829	5.265
10	1	130	155	44.743	10.68	7.342	7.132	6.983	5.432
11	1	122	143	67.652	8.354	7.44	5.481	5.634	4.842
12	2	138	160	54.467	8.547	7.34	7.542	6.843	5.632
13	2	160	175	76.841	6.787	7.904	9.684	8.836	9.84
15	2	170	178	56.876	6.934	7.135	5.635	4.962	3.725
16	1	132	150	53.172	6.865	6.238	7.665	5.892	3.965
17	2	126	150	60.235	9.662	2	10.003	7.436	5.836
18	1	120	143	63.396	8.117	3	11.642	7.865	6.34
19	2	165	170	60.634	9.253	4	3.254	6.123	3.352
20	1	123	150	80.409	8.241	5	6.117	6.442	4.395

Post IASTM

		Stretches		Longitudinal		Pennation Angle			
Subject	Group	Stretches	M-PROM	Quality	BF-CSA	relaxed	90	Mid	Max
2	1	119	138	66.071	3.981	9.453	5.364	7.364	6.364
3	2	165	175	58.385	6.482	7.529	7.263	5.609	3.798
4	1	160	168	63.346	5.194	9.015	8.461	5.054	5.969
5	2	160	175	45.712	7.517	16.741	13.539	11.032	9.267
6	1	128	150	45.759	9.832	9.532	7.481	6.235	5.584
7	2	156	176	54.8	6.529	4.023	3.743	3.092	3.455
9	2	160	168	57.834	7.439	3.451	7.824	5.421	3.345
10	1	128	150	47.58	10.267	6.832	5.945	4.321	3.245
11	1	121	146	68.659	8.343	7.874	8.843	5.241	3.432
12	2	135	160	56.477	8.496	9.635	8.324	8.127	7.943
13	2	161	175	6.787	6.77	14.265	12.804	10.135	9.351
15	2	174	180	56.876	6.934	8.346	7.362	5.314	4.821
16	1	122	150	53.983	6.683	7.238	7.784	6.143	4.562
17	2	130	150	66.325	9.612	9.635	11.492	8.961	6.642
18	1	113	135	65.386	8.342	10.955	13.941	9.145	7.265
19	2	155	175	53.983	6.683	4.023	3.743	3.092	3.455
20	1	125	150	79.306	7.953	8.752	6.351	6.423	5.843

Pre Sham

		Stretches		Longitudinal			Pennation Angle		
Subject	**Group**	**Initial Stretch**	**M-PROM**	**Quality**	**BF-CSA**	**relaxed**	**90**	**Mid**	**Max**
2	1	119	139	68.932	3.874	9.806	6.555	6.136	5.406
3	2	170	179	61.496	6.475	6.343	6.453	5.668	3.693
4	1	160	170	62.687	5.142	11.196	10.496	9.575	6.708
5	2	152	168	46.826	7.328	9.475	5.826	4.978	3.283
6	1	124	142	50.963	9.431	9.784	7.453	7.021	4.21
7	2	142	164	51.082	6.937	3.836	3.844	3.684	2.845
9	2	160	170	47.881	7.189	4.392	10.265	7.803	3.076
10	1	128	145	43.841	10.68	6.561	10.305	7.321	5.876
11	1	128	145	58.105	6.284	6.861	8.311	7.035	5.698
12	2	145	165	57.687	8.354	6.818	6.608	6.308	6.503
13	2	161	175	72.778	6.589	6.525	6.466	6.349	3.195
15	2	150	175	54.468	6.669	6.376	5.376	5.083	3.501
16	1	122	150	51.62	6.891	7.453	7.384	5.432	3.846
17	2	130	155	60.785	9.786	10.757	9.754	7.824	5.943
18	1	113	140	72.384	8.462	11.924	9.754	7.865	6.862
19	2	150	178	51.62	6.891	3.836	3.844	3.684	2.845
20	1	140	152	81.942	8.114	6.354	6.495	6.624	4.386

Post Sham

		Stretches		Longitudinal			Pennation Angle		
Subject	**Group**	**Initial Stretch**	**M-PROM**	**Quality**	**BF-CSA**	**relaxed**	**90**	**Mid**	**Max**
2	1	119	138	68.879	3.884	9.591	5.593	7.275	6.36
3	2	170	179	60.492	6.381	7.925	7.326	5.634	3.798
4	1	165	170	62.641	4.938	9.207	8.364	5.592	5.875
5	2	148	172	44.816	7.683	9.869	6.29	5.343	4.191
6	1	134	146	48.661	9.596	11.872	9.395	5.986	4.824
7	2	148	172	52.745	7.248	3.641	3.916	3.156	3.964
9	2	165	170	47.771	6.963	4.151	8.871	4.153	3.482
10	1	128	145	46.382	10.211	8.576	8.376	7.873	6.432
11	1	122	143	62.341	6.109	7.636	6.667	5.076	4.426
12	2	121	146	61.385	8.197	9.321	7.521	5.321	3.212
13	2	167	175	74.229	6.734	7.635	5.705	5.686	3.621
15	2	170	175	52.586	6.623	10.897	5.934	3.875	8.395
16	1	128	145	53.621	6.954	9.235	7.757	6.327	5.324
17	2	140	155	60.364	9.386	10.345	10.623	7.635	4.986
18	1	120	140	70.653	8.216	11.364	9.649	7.641	6.518
19	2	150	175	52.514	6.427	3.641	3.916	3.156	3.964
20	1	140	152	82.006	7.742	6.348	6.442	5.624	4.935