MUSCLE AND TENDON CHARACTERISTICS AFTER SIX WEEKS OF OVERLOADED STRETCH TRAINING

by

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Abstract

Stretching is used to maintain mobility, increase range of motion and rehabilitate muscles after injury. It is rarely suggested as a modality for increasing muscle size in humans, although animal studies have reported stretch induced muscle hypertrophy. The purpose of this study was to examine whether six weeks of passive stretching of the plantar flexors would stimulate muscle hypertrophy. The first hypothesis was that stretch training would induce muscle hypertrophy, increase fascicle lengths and decrease pennation angles of the medial and lateral gastrocnemius without altering the Achilles tendon, but changes in muscle architecture would be non-uniform between the gastrocnemii. The second hypothesis was stretch training would decrease maximal force and electromyography (EMG) activity of the plantar flexors through shifting the length tension relationship without changing voluntary activation or reflex activity. Eleven males stretched the non-dominant plantar flexors for six consecutive weeks by using a leg press to passively load the ball of the foot. The load was 20% of the baseline maximal voluntary contraction. EMG was monitored on-line for each session to ensure the subject was stretching and not contracting, which would be indicated by muscle activity. At week zero, three, six and one week after the completion of stretching ultrasound was used to measure muscle architectural and tendon changes. Force, EMG, twitch interpolation, reflex activity, contractile properties and body anthropometry were also measured. Both hypotheses were supported. Muscle depth increased 10.3% ($p=0.04$) by week three in the stretched leg with a 25% ($p<0.001$) increase in the length of the muscle fascicles in the muscle tendon junction, and 5.1% ($p=0.01$) increase in the fascicles of the muscle belly ($n=3600$). The pennation angle ($n=3600$) decreased ($p=0.02$) with no change in the tendon ($p=0.95$). Muscle force decreased 10.5% ($p=0.008$) at week six, with a reduction in EMG amplitude ($p<0.001$) and no change in voluntary activation (95.48% ±

0.92, $p>0.05$) or reflex activity ($p>0.05$). These data indicated six weeks of overloaded stretch training of the plantar flexors stimulated muscle hypertrophy but also caused significant reductions in force through an alteration in the length tension relationship.

Preface

The University of British Columbia's Behavioral Research Ethics Board granted ethics approval for this research on March 10, 2014. The ethics approval certificate number for the current study is H13-03377. To date, the research included in this thesis has not been published in full.

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List of Abbreviations

Ag/Cl: Silver Chloride

AT: Achilles Tendon

cm: Centimetres

CD: Contraction Duration

DOMS: Delayed Onset of Muscle Soreness

DXA or DEXA: Dual Energy X-ray Absorptiometry

EMG: Electromyography

g: Grams

H Reflex: Hoffman's Reflex

H/M Ratio: Ratio of Hoffman's Reflex to Muscle Compound Action Potential

HRT: Half Relaxation Time

Kgs: Kilograms

kHz: Kilohertz

LG: Lateral Gastrocnemius

MG: Medial Gastrocnemius

MHz: Megahertz

MHC: Myosin Heavy Chain

mm: Millimetres

MTJ: Muscle Tendon Junction

MTU: Muscle Tendon Unit

MVC: Maximal Voluntary Contraction

M Wave: Muscle Compound Action Potential

N: Newtons

PT: Peak Tension

ROM: Range of Motion

RMS: Root Mean Square

SD: Standard Deviation

SE: Standard Error

SOL: Soleus

TA: Tibialis Anterior

TPT: Time to Peak Tension

µm: Micrometre

US: Ultrasound

VA: Voluntary Activation

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Dedication

To my family and to Cayce

Chapter 1: Introduction to Thesis

Muscle hypertrophy is the result of adaptations in the nervous, muscular and metabolic systems which stimulate growth of a muscle at the cellular level. Traditionally resistance training, which utilizes repetitive shortening and lengthening muscle contractions against a load, has been the primary modality employed to effect increases in muscle size over time (Sale, 1988, 1992, Timson, 1990). When a muscle shortens through a concentric contraction the basic contractile units of the muscle, the sarcomeres, create tension by overlapping the proteins actin and myosin; during lengthening (eccentric) contractions tension is created through resisting the effect of gravity on the load and the separation of actin and myosin (Friden et al., 1984, Pensini et al., 2002). Extensive research exists around the effectiveness of resistance training for stimulating increases in muscle size, both in concentric and eccentric training, however greater increases in muscle size and strength are evident with lengthening loads (Chilibeck et al., 1998, Higbie et al., 1996, Pensini et al., 2002). Because eccentric training requires a load large enough to overcome the ability of the muscle to resist it, muscle damage can result (Friden & Lieber, 2001). Damage is vital in the remodeling process that enables a muscle to hypertrophy but excessive damage, which results from extreme stretching, can have negative effects on muscle force and the nervous system (Dolezal et al., 2000). Six days after eccentric training, damage to the myofibrils was still present (Friden & Lieber, 1992) and after a fatiguing protocol involving eccentric training maximal voluntary force was not fully recovered until seven days post training (Linnamo et al., 2000). The body eventually adapts to lengthening stimuli and each subsequent session evokes less damage and results in shorter time frames for recovery (Brockett et al., 2001). Because of this it is necessary to continually overload a muscle by increasing the load, intensity, length or volume of a training session if progressive adaptation in muscle size and

strength is the desired outcome of training. The exact load suggested to effectively employ eccentric training is equivocal in the literature but must be enough to lengthen the muscle under tension (Brockett et al., 2001, Morgan, 1990, Proske & Morgan, 2004).

1.0 Tension Generation

Lengthening a muscle to too great an extent is prevented by the non-contractile portions of the muscle, including the perimysium and the sarcomeric protein Titin. The non-contractile components also contribute to passive tension through this resistance to lengthening. Passive tension is a large portion of total tension at muscle lengths longer than resting and during stretching is the result of the muscle resisting lengthening without muscle activation. Passive tension is also a strong growth stimulator for muscles involved in lengthening exercises such as eccentric exercise and stretching (Vandenburgh et al., 1995) (Figure 1.0).

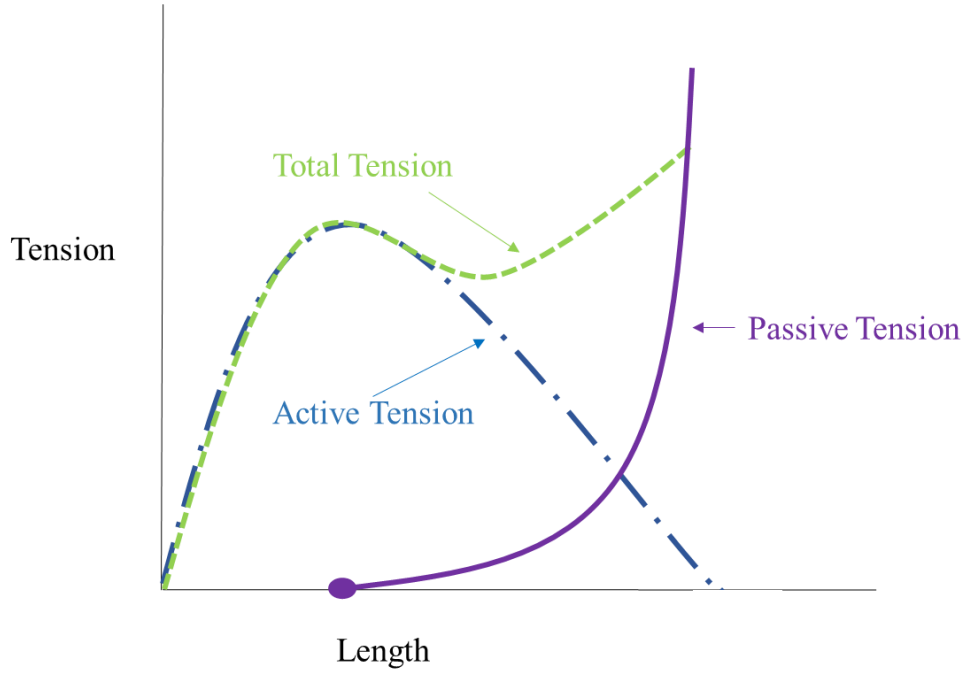

Figure 1.0: Schematic of the contribution of active, passive and total tension to the length tension relationship for whole muscle

Eccentric exercise is dependent on active tension created by breaking the bond between actin and myosin to produce force against a load (Friden, 1984); however, Holly et al. (1980) demonstrated that neuromuscular mediated active tension generation is not necessary to induce muscle growth following stretch in an animal model. During hypertrophy, augmentation is driven by overloading a muscle which results in deformation of sarcomeres and characteristic pattern of muscle damage (Warhol et al., 1985). There is evidence that stretching actin and myosin cross-links and deformation of connective tissue can evoke damage to the component parts of a muscle, such as in eccentric exercise (Gajdosik, 2001, Mafi et al., 2001). Further, stretching endosarcomeric and exosarcomeric structures responsible for passive tension generation can stimulate muscle growth and inflammatory cell migration. Inflammation, muscle soreness and myofibrillar lesions are common following resistance training; however, these are thought to initiate the processes that result in the concomitant increase in muscle strength and muscle fibre cross section (Friden et al., 1984). Central nuclei and satellite cell infiltration are pivotal components of the regenerative response (Rosenblatt et al., 2004, Schultz & McCormick, 1994) and these adaptations appear to be integral for the repair and growth of damaged muscle fibres and might be essential for stretch induced hypertrophy.

Animal studies have demonstrated the ability of stretch training, in which the muscle undergoes passive lengthening against a load much like the active lengthening during eccentric training, to evoke hypertrophy in the stretched muscles (Antonio & Gonyea, 1993, 1994, Goldspink, 1999). These studies have also demonstrated lengthening of muscle fascicles and neuromuscular adaptation to stretch training, such as increased electromyography (EMG) amplitude, are comparable to traditional eccentric resistance training (Antonio & Gonyea, 1993, Goldberg et al., 1975, Goldspink et al., 1999, Hayes et al., 2012, Holly et al., 1980, Sola et al.,

1973). Recent work in human studies have adopted a stretch training model reflective of traditional eccentric resistance training to examine the ability of repeated bouts of stretching to stimulate changes at the level of the nervous, muscular and metabolic systems (Blazevich et al., 2012, 2014, Guissard & Duchateau, 2004, Hayes et al., 2012, Kubo et al., 2001, Nakamura et al., 2012). However, the results of these studies remain equivocal in terms of whether the training adaptations are centrally or peripherally mediated and to date no study in humans has examined the effect of repeated bouts of intermittent overloaded stretching on skeletal muscle hypertrophy.

1.1 The Principle of Progressive Overload

Resistance training is based upon the principle of overload (Hellebrandt & Houtz, 1956) which incorporates progressive increases in the resistance or load applied to the muscle. This principle can also include increasing the sets or repetitions performed during a resistance training session. Chronic loading leads to an increase in the strength, size and neural function of the muscle tendon unit (MTU) but these gains can plateau if variation in exercise is not introduced. After the initial adaptation to the demands of a training program further adaptation, such as continued muscle growth, is unlikely if the exercise load is not increased and exercise is not combined with planned periods of rest to allow for recovery, which is key to hypertrophy (Kraemer & Fleck, 2007). Rest allows the nervous, muscular and metabolic systems to recover, and prevents fatigue so a muscle can continue to work against a load which induces growth (McNeil et al., 2004). During recovery increased protein production and incorporation to the muscle fibres at the sites of damage stimulates growth through upregulation of muscle metabolism (Dolezal et al., 2000). Amino acid supplementation has yet to be applied in stretch training however in resistance training amino acid supplementation is popular and has been reported to further stimulate metabolism and increase protein synthesis (Drummond et al., 2009).

Supplementation has also been reported to increase muscle mass and strength during resistance training beyond training alone (Cermak et al., 2012) suggesting that in addition to overload, the application of amino acid supplementation during stretch training could be important in stimulating hypertrophy. As a muscle gains strength, less of the muscle is recruited to perform the same task against the same load. This adaptation occurs at both the muscular and neural level as these systems become more efficient over the course of training. In order to continue to recruit the same percentage of a muscle's mass during training the intensity of the training must increase and this progression in load, repetitions or sets is known as the principle of overload.

The application of the principle of overload often centres on resistance training where specific values of load are set in order to enhance strength and augment size of the muscle. Chronic stretching in animal studies has successfully applied this principle as the weighting far surpassed that which the animal could lift (Barnett et al., 1980, Holly et al., 1980, Sola et al., 1973); however, in human studies stretches were typically administered using body weight and did not focus on the principle of overload (Blazevich et al., 2014, Hayes et al., 2012, Nakamura et al., 2012). This provides a unique opportunity to study the principle of overload in humans and examine whether this training principle when applied to stretch training stimulates hypertrophy.

1.2 Muscle and Tendon Structure

Using ultrasound, the response of tendon and muscle fascicles to stretch training can be measured *in vivo*. Tendons are easily visualized with ultrasound due to the reflective or 'hyper-echoic' nature of the collagen fibres and represent the non-contractile portion of the MTU. The Achilles tendon (AT) is the largest tendon in the body and attaches distally to the calcaneus at a position which minimizes the change in the tendon moment arm as the foot moves from dorsi to

plantar flexion (Benjamin et al., 2008). Along its length, the AT adopts three distinct shapes: round and thick near the calcaneus, thick and flat in the middle and thin and flat near the muscle tendon junction (MTJ) (Kubo et al., 2001). Tendons have inherent stiffness, lengthen during passive stretch (Herbert et al., 2002, Morse et al., 2008) and have viscoelastic properties which are vulnerable to stretch training (Kubo et al., 2001). The viscosity of a tendon is the ability to resist changing shape in relation to imposed lengthening, shortening or twisting, while the elasticity is the tendon's ability to return to its original form once its shape has been manipulated. Human research studies have suggested that stretch training primarily affects the viscosity of the tendon, rather than the elasticity with no change in the stiffness (Kubo et al., 2001). This indicates that after stretch training, the tendon is more likely to change shape, or deform, in response to loading, as opposed to increase size, and because elasticity is unaffected after stretching, the tendon will return to its original length. This deviates from resistance training where a significant increase in tendon stiffness and cross section has been reported after nine weeks of resistance training (Seynnes et al., 2007). However, without a change in tendon stiffness, even with increased muscle size, it is likely that in stretch training the tendon will remain unchanged in length and cross section. To measure these changes accurately the three tendon 'sections' must be considered and so multiple cross sectional measurements are necessary when examining AT adaptations to stretch training.

Like the tendon, the perimysium and connective tissue surrounding the muscle and muscle fascicles are also hyper-echoic under ultrasound and easily visualized. As a muscle undergoes chronic hypertrophic adaptation in relation to resistance training the cross section increases, fascicles shorten, the pennation angle increases and these adaptations cause the fascicles to align in parallel to optimize force (Maganaris et al., 1998). Recent work in humans

suggested no alteration to the muscle fascicles in the muscle belly after stretch training when measured with ultrasound (Blazevich et al., 2014, Nakamura et al., 2012). However, in animals there is strong evidence that chronic stretch causes damage and fascicle lengthening at the MTJ (Dix & Eisenburg, 1990). During static stretch the slow type fibres are more likely to be affected as the movement is slower and sustained when compared to rapid movements which target fast twitch fibres. However, there is greater potential for hypertrophy in the fast twitch fibres. Since the MTJ is dense with fast twitch fibres (Dix & Eisenburg, 1990) it is likely those fibres are more susceptible to stretch induced lengthening through the addition of sarcomeres in series and possibly stretch induced hypertrophy. There is evidence that muscle mass is related to the length of the muscle fibres and as the fibres lengthen the muscle belly becomes larger to accommodate the increase in tissue (Gajdosik, 2001). Thus, stretch induced hypertrophy is likely to affect both the MTJ and the muscle belly.

The gastrocnemius is a mixed fibre-type muscle involved in plantar flexion and is part of the triceps surae muscle group in the lower leg. Since slow twitch and fast twitch muscle fibres are present in the gastrocnemius (Coggan et al., 1991), the muscle is proficient at producing both sustained and fast force. The gastrocnemius is comprised of the lateral gastrocnemius (LG) and medial gastrocnemius (MG), each with characteristics specific to the function of the muscle. The LG and MG are pennate muscles, meaning the fascicles attach obliquely to the tendon and where they meet the aponeurosis a pennation angle is formed (Maganaris et al., 1998). Muscle pennation depends on the connection between the perimysium and the intramuscular parts of the tendon, rather than on the direct connection between the tendon and muscle fibre itself (Benjamin et al., 2008). The LG generally exhibits smaller pennation angles and longer muscle fascicles, while the MG tends to have larger cross section, pennation angles, and shorter muscle

fascicles (Kawakami et al., 1995). Both muscles terminate in the Achilles tendon (AT) and together they make up the MTU; however, the MG aligns more linearly with the tendon, whereas the LG is more oblique and it's fascicles adopt a twisted conformation (Figure 1.1).

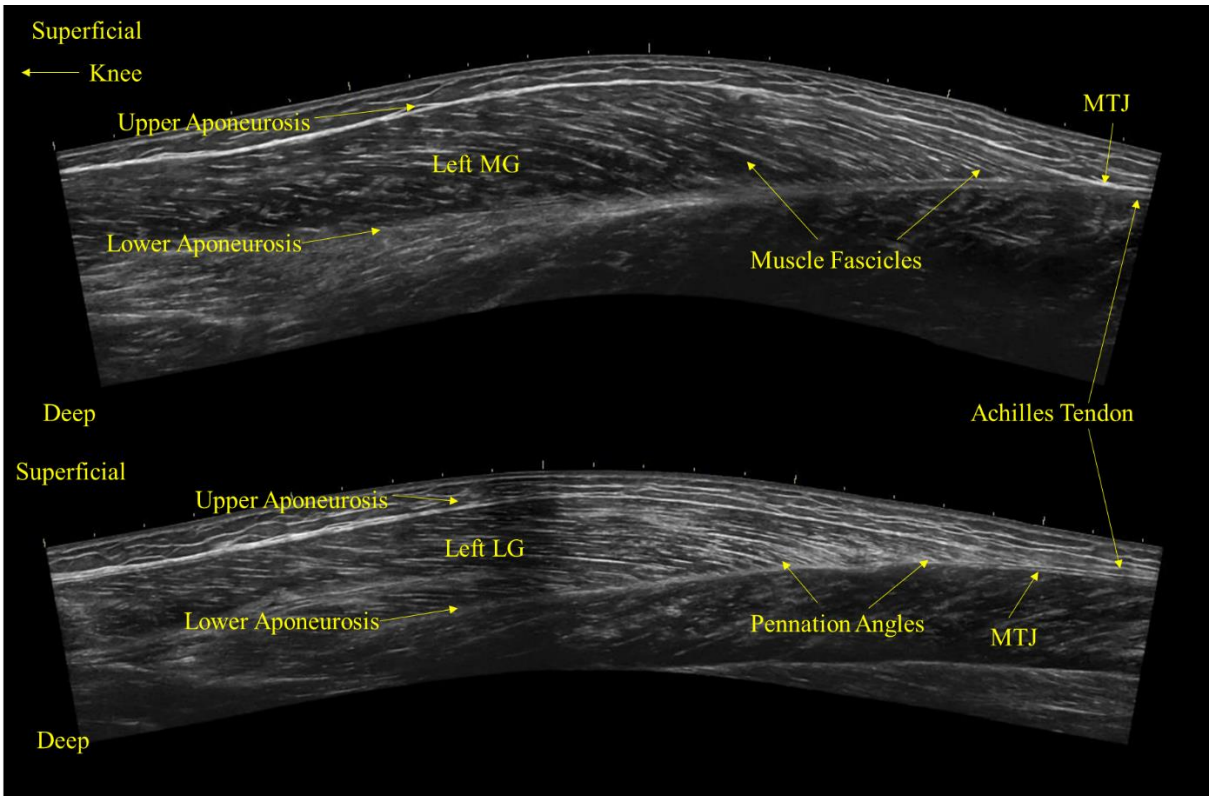

Figure 1.1: Gastrocnemii attachment to the Achilles tendon. The architecture and arrangement of the medial and lateral heads of the gastrocnemius are visible with the oblique attachment of the lateral gastrocnemius evident. MTJ is the muscle tendon junction, LG is lateral gastrocnemius, MG is medial gastrocnemius. Images were collected with a LOGIQview scan from the MTJ to the popliteal crease with a ML6-15 probe (4.5-15.0 MHz linear array, 13 x 58 mm foot print, 30 frames per second, 50 mm field of view, 8 cm depth of field LOGIQview, GE©)

1.3 Length Tension Relationship

Muscle force is dependent upon length of the sarcomere, muscle fascicles and MTU. Lengthening or shortening of muscle fascicles can compromise the position of actin and myosin overlap and alter force output from the sarcomere. The length tension relationship is well established in accordance to resistance training (Leonard & Herzog, 2010) and shows the muscle

functions most effectively to produce force at lengths that fall within the plateau region. With lengthening, the myosin heads cannot contact the actin as effectively and therefore force produced by the sarcomere decreases. If the sarcomere becomes excessively shortened the actin and myosin overlap too much and little force can be generated. The optimal sarcomere length for force production is ~2.26μm - 2.43μm (Leonard & Herzog, 2010) and this makes up the plateau region of the length tension relationship for sarcomeres. Resistance training can alter this relationship through shifting the relationship to the ideal portion of the plateau, while increasing passive tension and the addition of sarcomeres in parallel, which would increase force (Leonard & Herzog, 2010). In animal studies, increased length of muscle fascicles have been reported after stretch training (Dix & Eisenburg, 1990) which would shift the relationship to the descending limb and reduce force at the level of the sarcomere. This shift, coupled to a reported decrease in passive tension and increase in muscle lengthening after stretch training (Blazevich et al., 2014) would serve to further reduce the force created from the MTU as the reliance on passive tension would be compromised. Studies involving stretch training in humans have yet to report a shifting of the length tension relationship or alterations to muscle force caused by adaptations in muscle architecture including sarcomere and muscle fascicle lengths.

The length tension relationship can be passively manipulated by changing the joint angle. In the gastrocnemius AT MTU, as the ankle is rotated to plantar flexion, the fascicles are passively shortened, and in dorsi flexion the fascicles are passively lengthened. Therefore, testing muscle force at different ankle angles would provide insight into the effect of stretching on the length tension relationship of the muscle fascicles and sarcomeres.

1.4 Dual Energy X-ray Absorptiometry

Based on resistance training, stretch training in humans could affect more than just the MTU. There is documentation of increases in lean mass of the stretched muscle as well as whole body following stretch training in animal models (De Jager & Herzog, 2005, Dix & Eisenburg, 1990). Because of the potential to induce whole body adaptation it is important to monitor changes throughout the body to a novel training intervention like stretch training. Dual energy X-ray absorptiometry (DXA or DEXA) is a fast and precise technique of body anthropometrical measures including bone mineral density and muscle mass (Fuller et al., 1992). In animals, after stretching the muscle is typically excised from the body to measure wet weight and length, but in humans these measurements can be performed *in vivo* by use of the DXA. The DXA is a non-invasive tool that is sensitive to delineate training related changes in muscle size and mass as well as bone mineral density and works through scanning the entire body with a fan beam which delivers a low dose of X-rays. These rays have two distinct energy peaks: one is absorbed by soft tissue, the other by bone. Using a mathematical formula, bone can be separated from soft tissue, making the DXA the gold standard for bone compositional analysis (Visser et al., 1999) and it has been validated against the gold standard for measuring tissue mass, which is the multi-slice computerized tomography (CT) scan (Levine et al., 2000). The estimates of muscle mass are from a model suggested in Fuller et al., (1992) which takes into account assumptions of skin weight and non-muscle tissue to report a measure of lean mass.

1.5 Muscle Activity and Reflexes

During and following stretch training nociceptor (type IV afferent) and mechanoreceptor (type III afferent) feedback may dampen reflex responses. In animal studies, after approximately 90 seconds of stretching afferent responses were abolished or depressed (Hutton & Nelson,

1986) decreasing conscious and unconscious inhibition to further stretching (Magnusson, 1996) and allowing increased stretch intensity. In humans, adaptations from stretch training can only be considered resultant to the stretch when little to no muscle activity is present during stretching. If inhibition to stretching to the full extent is decreased at 90 seconds then, adhering to the principle of overload, in order to reach the intensity needed to affect continual muscular and neural changes the time of the stretch must be extended beyond 90 seconds. In humans there is no standardized time for stretches to be held, and this, in part, may contribute to the equivocal nature of the muscular and neural outcomes of stretch training (Blazevich et al., 2012, 2014, Guissard & Duchateau, 2004, Hayes et al., 2012, Kubo et al., 2001, Nakamura et al., 2012). The Hoffman's (H) reflex amplitude also decreases during prolonged stretch. Because this reflex pathway is associated with the efficacy of homonymous Ia afferents to activate spinal motor neurons the larger amplitude suggests greater recruitment of motor neurons (Blazevich et al., 2012). After stretch training, there are reports of reduced H reflex amplitude (Guissard & Duchateau, 2004) but this finding is debated by more recent literature where no change in reflex activity was observed (Hayes et al., 2012, Blazevich et al., 2014). Neural changes induced by stretching remain equivocal and although no studies have reported that these alterations led to muscle hypertrophy in humans, stretch training increased range of motion (ROM) (Alter, 1996, Stanley & McNair, 1996, Wienmann & Hahn, 1997, Young & Elliot, 2001) and decreased passive stiffness of the MTU (Blazevich et al., 2014). Both of these adaptations have been cited to have neural as well as mechanical origins (Guissard & Duchateau, 2004, Magnusson et al., 1996).

Few studies in humans have examined central adaptations to stretch training (Blazevich et al., 2014, Guissard & Duchateau, 2004, Hayes et al., 2012), although results from resistance

training strongly suggests peripheral and central adaptation are tightly intertwined (Sale, 1988, 1992). In animal and human studies muscle activity after stretch training was unchanged (Hayes et al., 2012, Holly et al., 1980, Guissard & Duchateau, 2004, Magnusson et al., 1996) which differed from observations of resistance training where EMG amplitude increased with muscle strength and size (Sale, 1992). An increase in EMG amplitude is indicative of a greater number of active motor units and increased motor unit firing rates both of which suggest synchronization of motor units, and thus greater force potential (Bigland-Ritchie, 1979). EMG along with voluntary activation (VA) are indicative of the drive from the central nervous system to maximally activate a muscle during voluntary contraction. The technique of twitch interpolation is administered to assess VA and incorporates addition of a super imposed electrical stimulation during maximal voluntary contraction (MVC). If the twitch elicits additional force to the MVC, the activation of the muscle is less than 100% and this is interpreted as a decrease in drive to the muscle from the central nervous system. In practice, if VA is less than ~95% decreases observed in force at MVC could be due to a lesser voluntary drive to the muscle (Jakobi & Rice, 2001). If either EMG or VA decrease, voluntary force decreases providing a link to the contribution of the nervous system to stretch induced adaptations in strength.

Based on the well-established results of resistance training (Sale, 1988) and the reported neural and mechanical adaptations in animals to stretch training when the principle of overload is used it is probable that stretch training in human muscle will induce muscle hypertrophy. However, whether the adaptation in muscle fascicle lengths and pennation angles in the LG and MG will be non-uniform across each muscle due to the architectural arrangement of each head with the tendon remains to be established.

1.6 Research Question

Two distinct questions were addressed in order to determine whether stretch training of the plantar flexors differentially affects the LG and MG muscle architecture, induces hypertrophy, and impacts neural activity and force production at various muscle lengths.

Question one evaluated whether six weeks of stretch training of the plantar flexors induced muscle hypertrophy of the gastrocnemius and altered muscle fascicle length and pennation angle non-uniformly across the LG and MG. Furthermore, whether these changes in size and architecture resulted in increased plantar flexion force and left the tendon unaffected was considered. It was hypothesized that stretch training would be a sufficient stimuli to increase muscle depth and preserve force without altering the tendon length or depth. It was also postulated that the muscle fascicles would lengthen and pennation angles would decrease at both the MTJ and muscle belly but these changes would be disproportionate across the LG and MG.

Question two examined the effect of stretch training on the neural, muscle architectural, and force characteristics of the plantar flexors at five different lengths of the muscle tendon unit imposed by changing the ankle angle. It was hypothesized that stretch training was a feasible modality to shift the length tension relationship of the gastrocnemius AT MTU to the descending limb of the curve and increase reliance on passive tension for force generation. This would result in reduced voluntary force related to a reduction in passive stiffness without changing voluntary activation or reflex activity. It was further hypothesized that the rates of the twitch contractile properties would be faster and there would be increased muscle size and length of muscle fascicles as the muscle responded to chronic overload stretching.

Chapter 2: Question 1

2.0 Background

Repetitive stress, such as that encountered through resistance training when one lifts and lowers a weight multiple times, alters muscle architecture including the length of muscle fascicles and magnitude of pennation angles (Churchward-Venne et al., 2012, Sale, 1988, 1992). If the load is large enough, resistance training will also stimulate increases in muscle cross section and greater force transmission through the tendon to the bone to effect movement. Muscle and tendon responses to resistance training are well defined at the level of the MTU (Abe, 2000, Kraemer et al., 2001) but adaptations to stretch training are not as well understood.

Recently, studies have considered whether stretch training impacts muscle architecture and the tendon similarly to resistance training (Blazevich et al., 2012, 2014, Guissard & Duchateau, 2004, Hayes et al., 2012, Nakamura et al., 2012, Seynnes et al., 2007). Stretching causes lengthening of muscle and this increases passive tension by stressing the non-contractile series and parallel elastic components of the sarcomere and MTU. This rise in passive tension through the non-contractile elements stimulates the muscle fascicles to become longer and in animal studies muscle hypertrophy was reported but this adaptation was not observed in human muscle following stretch training (Barnett et al., 1980, Holly et al., 1980, Sola et al., 1973).

Hypertrophy is gained through a compensatory response to increased load applied during resistance training which initiates a cascade of cellular signalling events and incorporation of protein into various structures within the muscle fascicles. In animal studies chronic stretch lengthened the muscle fascicles through the addition of sarcomeres in series, specifically at the MTJ (Dix & Eisenburg, 1990, Williams & Goldspink, 1971), and also increased the cross section of muscle fascicles (Goldberg et al., 1975). In these studies, the muscles of the animals were

overloaded through chronic stretch under increasing tension, suggesting that the intensity of the stretch influences the magnitude of muscle fascicles lengthening as well as the augmentation in size. The role of intensity in resistance training is well known as the principle of overload but this factor has not been evaluated in human stretch training studies.

Pennation angle is an important determinant of force production and with an increase in muscle cross section the pennation angle also increases (Kawakami et al., 1995, Fukunaga et al., 1997). Larger pennation angles increase the attachment area between muscle and tendon, bringing the tendon into contact with a greater portion of contractile material and this in turn transmits more force to the bone (Aagaard et al., 2001, Alexander & Vernon, 1975, Kraemer et al., 2001). In resistance training, the tendon gains stiffness and muscle size increases, stimulating greater force through pennation angle and tendon adapting in tandem. However, in stretch training the viscosity of the tendon decreased while the elasticity was unchanged (Kubo et al., 2001, Seynnes, 2007). These findings suggest stretch training is likely to affect the muscle architecture more than the tendon. To date, no study has reported changes in pennation angles in response to stretch training (Blazevich et al., 2014, Guissard & Duchateau, 2004) even though the link between smaller pennation angle and longer muscle fascicles is well documented (Fukunaga et al., 1997).

Muscles contract as a unit with their associated tendon; however, when a MTU is composed of a bipennate muscle such as the gastrocnemius, the individual heads may respond independently to the stress of training and therefore the outcome of stretch training might be non-uniform across the muscles. The LG and MG both attach to the AT and collectively transmit plantar flexion force to the ankle joint but the lateral head has an oblique attachment while the medial head's is linear. During movement this causes the MG to experience greater force as the

LG muscle fascicles dissipate a portion of the force through the angle at which they attach to the tendon (Edama et al., 2014). The unequal force transmission through the gastrocnemii creates greater pennation angles and shorter muscle fascicles resulting in larger size of the MG compared with the LG (Fukunaga et al., 1997, Luthi et al., 1986). In resistance training these differences in muscle architecture result in greater mass and force in the medial head (Kubo et al., 2001); however, in stretch training force was not altered (Blazevich et al., 2014, Guissard & Duchateau, 2004, Nakamura et al., 2012). The individual response of the LG and MG to stretch training has not been reported and so it is unknown if the architecture of these two muscles are non-uniformly affected by stretch training like they are in resistance training.

To date, no study in humans has evaluated the architectural adaptation of the LG and MG separately or reported muscle hypertrophy in response to stretch training. Thus, the aim of this study was to determine whether chronic stretch training of the plantar flexors caused non-uniform adaptation in fascicle length and pennation angle between the LG and MG, and resulted in hypertrophy of the gastrocnemius. It was hypothesized that chronic stretch training applying the principle of overload would increase muscle depth while lengthening fascicles and decreasing pennation angles but these responses would be non-uniform between the LG and MG.

2.1 Method

Subjects

Twenty two healthy males aged 19-30 years were recruited for this study. Twelve took part in the stretching intervention. One subject did not complete the stretching protocol so data are based on 11 subjects in the experimental group and 10 control subjects (Table 2.0).

Table 2.0: Subject characteristics. cm=centimetres, kgs=kilograms. Values are mean ± SD

	Number	**Height**	**Weight**	**Age**
Training Group	11	179.45 cm ± 8.33	76.77 kgs ± 15.15	22 years ± 2
Control Group	10	179.03 cm ± 10.93	83.93 kgs ± 15.98	22 years ± 4

The subjects in both the training and control group were moderately active as defined by the Canadian Physical Activity Guidelines of at least 150 minutes of moderate to vigorous activity per week (Canadian Society for Exercise Physiology, 2015). Activities in the training group included sports such as basketball and badminton, resistance training, hockey, track and field, ballet and longboarding. In the control group, subjects participated in soccer, hockey, basketball, badminton, volleyball, swimming, resistance training and cross country running. The self-reported non-dominant leg (left for all subjects) as defined by the leg the subject would prefer to kick a soccer ball with (Hoffman, 1995) underwent the stretching intervention and the dominant leg (right) served as an internal control. Subjects were instructed to maintain the same exercise and diet regimes for the duration of the protocol with the addition of the stretching intervention, and the post stretch protein drink. The exclusion criteria for this study included individuals who had a lower body injury, were under the age of 19 or over the age of 30, had neurological or musculoskeletal disorders, or were unable to read or speak English fluently. At the start and end of the protocol, physical activity and diet was reported. Informed written consent was obtained prior to participation in this study. All procedures were approved by the University of British Columbia Behavioral Research Ethics Board (H13-03377) and conformed to the Declaration of Helsinki.

Experimental Set up

Assessments

Over the duration of seven weeks four full experimental assessments were recorded at week 0 (baseline), week 3, week 6 and one week after the completion of the stretching protocol, at week 7. During these assessments muscle depth, fascicle lengths, pennation angles and tendon length and depth were measured with ultrasound (GE LOGIQ E9, Connecticut USA) with a ML6-15 probe (4.5-15.0 MHz linear array, 13 x 58 mm foot print, 30 frames per second, 50 mm field of view, 8 cm depth of field LOGIQview, GE©) and MTJ position with a RSP6-16 3D/4D probe (5.6-18.4 MHz linear array, 38.4 x 44.5 mm footprint, volume 37.4 mm x 29º field of view), muscle force with a dynamometer (Biodex Medical Systems, New York USA) and anthropometric measures with dual energy x-ray absorptiometry scans (DXA, Hologic, Inc. Bedford Ma, USA). A partial assessment was performed at day 5 of stretching immediately after the daily stretching session. During this visit only ultrasound measures of the LG, MG and AT were collected.

To begin the assessment ultrasound measures were collected with the subject laying prone with the soles of the feet restricted against the base of the bed so the ankle angle was 90º as measured by a goniometer (JAMAR® Easy Read™, Patterson Medical, Mississauga, Ontario, Canada). Using ultrasound with a RSP6-16D 3D/4D probe the MTJ was identified. This probe allowed for the true position of the MTJ to be determined in the 3D setting through simultaneous capture of longitudinal, cross sectional and frontal position. The ML6-15 probe, which was also used in this study, does not have the 3D/4D capabilities of the RSP6-16D making both probes necessary. The MTJ was defined as the position at which the muscle receded to tendon in all three of these views. Aquasonic clear ultrasound transmission gel (Parker Laboratories, Inc.,

New Jersey USA) provided acoustic contact between the probe and skin. At the skin the MTJ position was marked using an echo-absorptive marker. Similarly, the insertion of the AT at the calcaneus was identified using the same method and also marked. The distance between these two points was determined at the skin surface using a measuring tape.

A longitudinal scan of the MG and LG was performed using the ML6-15 probe in LOGIQview setting. This probe was used as the RSP6-16D does not have a LOGIQview scan setting. Each head of the gastrocnemius was scanned separately from the MTJ to the insertion on the femur over the widest part of the muscle along the medial aspect. A longitudinal scan of the AT was similarly performed from the insertion of the AT on the calcaneus and continued superior along the medial aspect of the tendon to the defined MTJ. The AT cross section on both legs was measured at three locations which represented the round, middle and flat portions of the tendon: i) 3 cm superior to the horizontal projection of the medial malleolus to the AT (defined as inferior); ii) 3 cm superior to the first point (middle); and iii) 3 cm superior to the second point (superior) using the ML6-15 probe in the standard still image setting. In an identical manner ultrasound measures and scans were repeated for the opposite leg.

Subsequent to the ultrasound measures, maximal voluntary contraction (MVC) and voluntary activation (VA) were executed. Positioning straps were secured across the chest and shoulders and the foot was held to the foot plate with custom designed ratchet straps. The medial malleolus was aligned with the centre of rotation of the Biodex, and the ankle angle was set to 90°. Force was produced on the Biodex dynamometer (Biodex Medical Systems, New York USA) sampled at 496Hz, converted from analogue to digital format (Power 1401, Cambridge Electronic Design, Cambridge UK) and displayed in Spike 2 software (Cambridge Electronic Design, Cambridge UK).

Twitch interpolation technique was employed to assess VA during MVC. Supra-maximal stimuli were established through progressive increases of the stimulation intensity (200μs pulse width, 400 volts) until a plateau of the peak twitch amplitude in the force tracing occurred. Once maximal amplitude was achieved the intensity was increased a further 10% to achieve supra-maximal level. Three stimulations were applied prior to, during and immediately post MVC with an inter-stimulation time of one second. All measures were repeated for the opposite leg.

A DXA scan was performed with the body positioned supine, arms rested at the side and palms down (Body Composition and Analysis Centre, Boston, MA). During the DXA scan, the subject laid with hands pressed flat to the surface of the bed, and fingers spread. The heels were separated and the toes inverted so the hallux of each foot touched. To ensure consistent body positioning for all sessions the distance between the heels was measured using a measuring tape and the body was aligned and positioned vertically and measured for all scans.

30 Stretching Sessions

Stretching occurred five times per week for six consecutive weeks. Stretching was organized into blocks of five stretches with two days of rest between each block of five. The leg press sled was loaded with 20% MVC for each session (TuffStuff PPL-960 45°Leg Press, TuffStuff Fitness Equipment, Chino, CA) which was progressively increased each week to adhere to overload. To ensure stretch rather than contraction muscle activity was measured with EMG and subjects were reminded to relax. When muscle activity exceeded 2% of the MVC (3 stretching sessions out of 330) as marked on the EMG recording with horizontal lines, the subject had ten seconds to relax the muscle. If they could not the stretching session was stopped, ten minutes of rest was given, and the stretch attempt was repeated.

EMG and ankle angle were measured during the stretch training sessions with a portable surface EMG device (Biometrics DataLOG P3X8, Gwent, UK). The skin of the vastus lateralis, biceps femoris, MG, LG, SOL and TA were exfoliated with 70% isopropyl alcohol swabs and low friction cleansing pads. Biometrics SX230 electrodes (Gwent, UK) were placed mid-belly of the two major upper leg muscles (vastus lateralis, biceps femoris) and four lower leg muscles (MG, LG, SOL, TA) with an inter-electrode distance of 20 mm. Electrodes were adhered with Hypafix™ (BSN Medical Ltd., Laval, Canada). High conductivity electrode cream (Signa Creme, New Jersey, USA) was used with the reference electrode (R200, Biometrics), which was positioned on the lateral malleolus of the fibula. The cables from the electrodes were placed into the data logger (9.5 x 15.8 x 3.3 cm; 380 g; DataLOG P3X8, Gwent, UK) and the portable EMG unit was placed next to the subject. An electronic goniometer was attached over the ankle joint so the inferior portion aligned with the midline of the foot, and the superior portion aligned with the midline of the shank, and the cables were placed into the data logger (Figure 2.0).

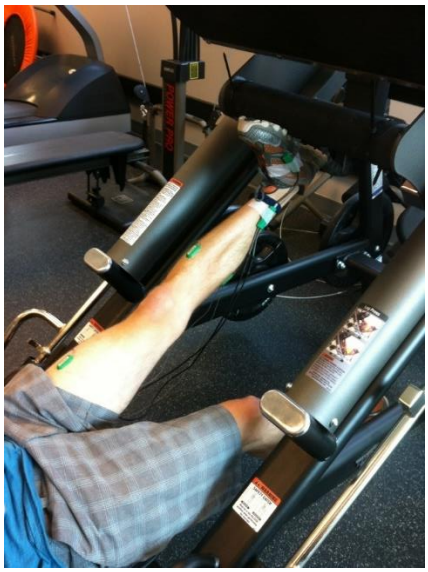

Figure 2.0: Experimental set up during stretching sessions. Visible is the non-dominant leg undergoing stretch and surface electromyography electrodes.

EMG signals were sampled at 1,000 Hz, amplified (100x), band-pass filtered (20 Hz-10 kHz), and displayed in real time on a computer screen visible to the subject. Two horizontal lines defining ± 2% MVC were visible on the screen and the subject was informed what the data displayed and if the EMG activity exceeded the lines the stretch was not passive and they needed to relax. Immediately after the completion of the stretch training, electrodes were removed and the calf circumference was measured around the widest part of the muscle using a tape measure at the skin.

Experimental Procedures

Prior to commencing training on day one baseline measures of self-reported caloric consumption and physical activity were collected for the three days preceding the assessment. Isometric plantar flexion MVC was executed as hard and as fast as possible and this effort was held for five seconds before relaxing. The twitch interpolation technique was administered to ascertain voluntary activation. The opposite leg was tested in an identical manner.

At the onset of each training session a Likert scale of 0-10 was administered where 0 represented no pain and 10 represented excruciating pain. Warm up was conducted prior to each stretch training session on a mini rebounder (Tempo Fitness, Cottage Grove, WI). Subjects maintained a low intensity bounce for three minutes while focusing on maximal plantar flexion at each push off. Then subjects performed 15 body weight calf raises on a custom built step (61 cm x 15 cm x 20 cm) which enabled the heels to drop toward the floor (dorsi flexion) thus stretching the plantar flexors, before raising to full plantar flexion. Subjects were instructed to reach full dorsi and plantar flexion for each of the 15 repetitions. Electrodes were attached to the non-dominant leg of the subject and the subject sat on the leg press machine so the hips were flexed to 90° as set by the angle of the back rest. The balls of the feet were placed on the calf

raise portion of the leg press machine. Using both legs the subject pressed the weight up so the legs were extended and the sled was raised at a 45° angle against gravity. On "go" the subject removed the dominant leg from the leg press, the three minute timer was started, and the subjects were encouraged to relax their plantar flexors so the ankle of the non-dominant leg could rotate to dorsi flexion, thus stretching the calf and AT. Muscle activity was monitored for the duration of the stretch and visual feedback was provided to encourage the subject to stretch and not contract the muscles. The stretch was held for three minutes and at one minute the ankle angle was recorded from the goniometer. At three minutes, the subject replaced the dominant foot on the leg press, and both legs guided the weight back to the resting locks. The subject stood and calf circumference was measured. Within 10 minutes of completing the stretch, the subject drank a protein and water mixture consisting of eight ounces of water and 0.25g protein powder per kilogram of body weight (Natural Whey Isolate, 25 g protein per scoop, Kaizen Canada, Calgary, AB).

Data Analysis

Offline assessment of muscle and tendon architecture (OsiriX Dicom Viewer imaging software 5.5, Switzerland) was undertaken after calibration of the software to the measurements inherent to the B1500 (GE LOGIQ E9, Connecticut USA, ML6-15 probe) ultrasound. Calibration was performed by using a pre-set one centimetre distance on the dicom image to calibrate the straight line distance of the OsiriX software, and then an intra-observer measurement reliability of 0.01cm and 0.1° was established by measuring various lengths and angles of the same muscle fascicles on both the B1500 ultrasound and OsiriX. Researchers were blinded to the subject, condition and assessment number during analysis.

Muscle depth was the perpendicular distance between the parallel upper and lower aponeuroses across the widest part of the muscle and was measured for both the LG and MG. Muscle fascicle length was measured from the lower to the upper aponeurosis with a spline trace where any deviations from linear could be accounted for and included within the trace pattern. Where the muscle fascicle met the lower aponeurosis the pennation angle was measured using the inherent angle function. The AT length was measured using a spline trace from the insertion on the calcaneus to the recession of the tendon into the MG along the deep edge of the tendon. Tendon depth was measured in the same manner as the muscle depth (Figure 2.1).

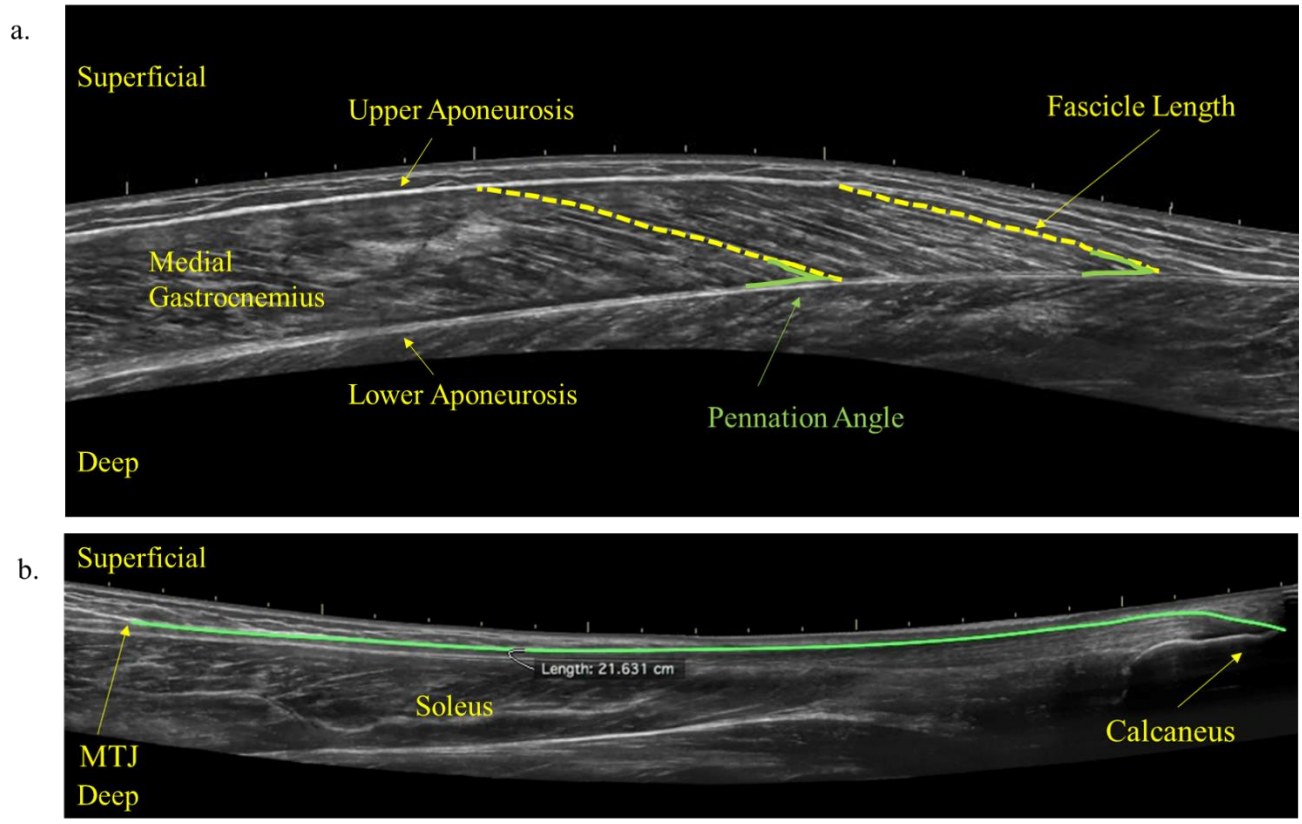

Figure 2.1: Representation of ultrasound images and measurements of the muscle fascicles and pennation angles (a) and the Achilles tendon (b). MTJ is the muscle tendon junction. Muscle image (a) was collected with a LOGIQview scan from the MTJ to the popliteal crease. Tendon image (b) was collected from the tendon insertion on the calcaneus to the MTJ. Both images were captured with a ML6-15 probe (4.5-15.0 MHz linear array, 13 x 58 mm foot print, 30 frames per second, 50 mm field of view, 8 cm depth of field LOGIQview, GE©)

MVC and VA were analyzed off line using custom scripts in Spike 2 software (Cambridge Electronics Design, Cambridge, UK). MVC was measured as the highest force achieved during the contraction and VA was assessed according to Jakobi & Rice (2001) and based on the equation: voluntary activation (%) = (1- (interpolated twitch/resting twitch))*100.

To obtain measures of the individual calf anthropometry after the completion of a full body DXA scan, the calf of each leg was isolated with segmental boxes that allowed for compartmental body analysis. Each calf was measured from the centre of the knee joint (across the superior end of the tibia and inferior end of the femur) to the ankle joint (at the meeting of the inferior portion of the tibia and the superior portion of the talus). The box was wide enough to accommodate both muscle and subcutaneous tissue of the calf. This allowed for analysis of the lean muscle mass, bone density and fat mass of the calf, isolated from the whole body.

The ankle angle during stretch was measured with an electronic goniometer (DataLOG P3X8, Gwent, UK) based upon the difference between midline of the foot and the midline of the shank. Full plantar flexion was considered 180°, where 0° was full dorsi flexion. As the ankle became more dorsi flexed (and thus flexibility was gained), the ankle angle approached zero. For the stretch training sessions self-reported perception of pain was measured as the distance between the point of origin on the left and the marked position on the Likert Scale measured in millimetres.

All EMG data from the portable device was instantaneously streamed via Bluetooth to the Biometrics software (Biometrics DataLOG version 3, Gwent, UK) for preliminary visual inspection. Muscle quiescence was quantified as less than 2% MVC EMG amplitude which was collected during the assessments. This was indicated on the live stream as two horizontal lines which marked the area where the EMG amplitude surpassed ±2% MVC.

Statistical Analysis

All dependant variables were compared between the control group (n=10) and the control leg (n=11, non-stretched) in a two way repeated measures ANOVA for the within subject factor of time (week 0, week 6) and the between subject factor of group (control group, non-stretched leg of trained group). There were no significant differences found between the control group and the non-stretched leg of the experimental group, therefore all statistical comparisons were based upon the non-stretched leg as the control ($p=0.12$).

A four way repeated measures ANOVA for the within subject factor of time (week 0, day 5, week 3, week 6, week 7) and between subject factors of condition (stretched, non-stretched), muscle (LG, MG) and position (junction, belly) was executed to evaluate changes in muscle fascicle length and pennation angle. A three way repeated measures ANOVA for the within subject factor of time (week 0, week 3, week 6, week 7) and the between subject factors of condition (stretched, non-stretched) and muscle (LG, MG) was used to assess changes in muscle depth and tendon depth. A five (time: week 0, day 5, week 3, week 6, week 7) by two (condition: stretched, non-stretched) repeated measures ANOVA was used to evaluate the effect of stretch training on AT length, MVC, VA and the dependant variables collected from the DXA scans (area, bone mineral content, bone mineral density, fat mass, lean mass, total mass, fat percent and normalized lean mass). Ankle angle during stretch, pain and calf circumference recorded during the 30 stretch sessions were assessed with a one way repeated measures ANOVA for the within subject factor of time. For all repeated measures ANOVA where the assumption of sphericity was violated ($p<0.05$) according to Mauchly's Test of Sphericity, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. Where an interaction was significant Tukey's post hoc analysis was used for variables with greater than two groups, and a

Student's paired T-test or one way ANOVA was used for those variables with two groups. Significance was considered when $p \leq 0.05$. Data are presented as mean ± SD in tables and mean ± SE in figures.

2.2 Results

During 30 days of stretch training the ankle joint became more flexible as evident by the decrease in angle from session 1 to session 30 with a significant increase in range of motion (ROM) achieved at day 17 and continued to day 30 ($p<0.001$) (Figure 2.2a). The calf circumference measured immediately after each of the 30 stretching sessions increased from session 1 to session 30 with a significant enlargement from session 1 observed at session 4 ($p=0.02$), session 13 ($p=0.03$), session 15 ($p=0.03$) and session 18 to session 30 ($p<0.001$) (Figure 2.2b).

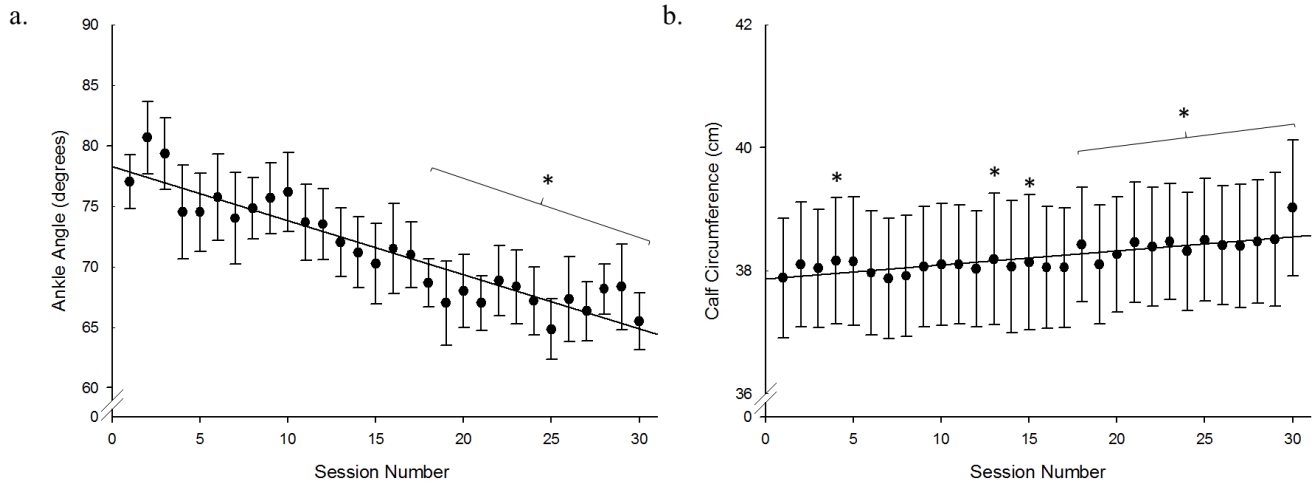

Figure 2.2: Ankle angle and calf circumference measured at each of the 30 stretching sessions. Ankle angle was measured in degrees where 0° was maximal dorsi flexion and 180° was plantar flexion with the leg and foot in a horizontal plane. Calf circumference was measured in millimetres and converted to centimetres (cm). Values are mean ± SE. Significance from the first stretching session (session 1) is denoted by (*), $p<0.05$.

Although stretch training increased ankle flexibility and calf circumference, there were no significant interactions or main effects evident for bone density or bone mineral content, lean mass, or fat mass of the total body measured with DXA.

The interaction of time by condition for muscle depth measured from ultrasound was significant ($F=2.534$, $p=0.04$) and there was also a main effect for time ($F=13.786$, $p<0.001$), and condition ($F=10.031$, $p=0.002$). The muscle depth was significantly larger at day 5 ($p=0.009$), week 3 ($p<0.001$), week 6 ($p=0.009$) and week 7 ($p=0.002$) compared to week 0 and across all time points the stretched condition was greater than that of the non-stretched (Figure 2.2).

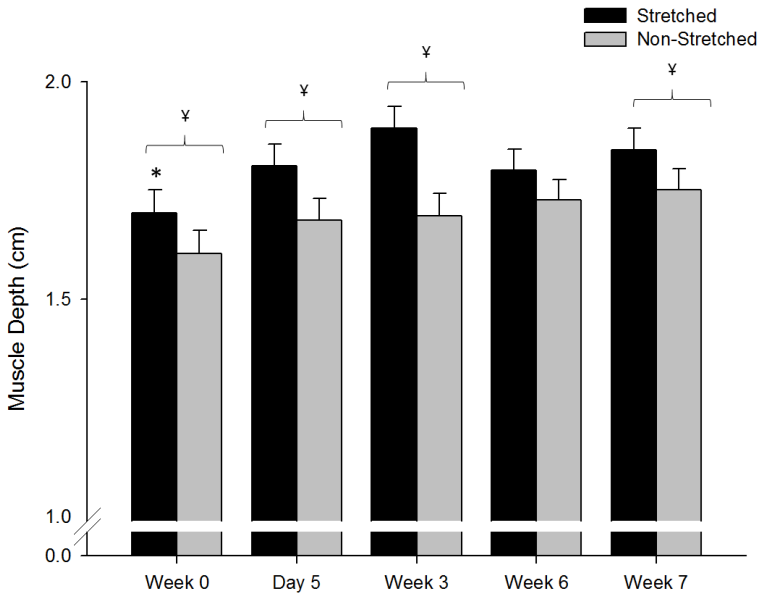

Figure 2.3: Time by condition interaction of muscle depth. Depth was measured in centimetres (cm). Significance denoted by (*) for the within effect of time and indicates an increase in muscle size for all time periods compared with week 0. Significance for the between subject effect of condition is denoted by ($^{¥}$) and indicates difference between the stretched and non-stretched leg. Values are mean ± SE, $p< 0.05$.

Although there was an increase in muscle depth, MVC of the plantar flexors did not change (baseline stretched; 114.04 N ± 11.36, non-stretched; 111.72 N ± 14.31) ($p=0.90$) and

VA did not differ over the stretch training intervention (stretched; 95.51% ± 3.34, non-stretched; 96.04% ± 2.11).

There was a significant three way time by muscle by position interaction ($F=3.478$, $p=0.01$) and time by condition by muscle interaction ($F=2.342$, $p=0.02$) for muscle fascicle length. The LG and MG fascicle length in the junction increased significantly at week 3 ($p<0.001$), week 6 ($p<0.001$) and week 7 ($p<0.001$) when compared to the week 0 and although the LG fascicle length was unchanged at day 5 ($p=0.09$) the MG fascicle length at day 5 was significantly longer than week 0 ($p<0.001$). In the belly of the muscle the LG fascicle length was also longer at day 5 ($p=0.01$), week 3 ($p<0.001$), week 6 ($p<0.001$) and week 7 ($p<0.001$) compared to week 0, and in the MG, week 6 was significantly different than week 0 ($p=0.03$). For fascicle lengths the two way interaction of time by position ($F=4.702$, $p<0.001$) and time by muscle ($F=3.673$, $p<0.001$) were significant and there were main effects for time ($F=8.490$, $p<0.001$) and position ($F=45.837$, $p<0.001$). The fascicle lengths at the junction were significantly shorter than the belly ($p<0.001$) and the muscle fascicles of both the LG and MG were longer at week 3 ($p<0.001$), week 6 ($p<0.001$) and week 7 ($p<0.001$) when compared to week 0 (Figure 2.4).

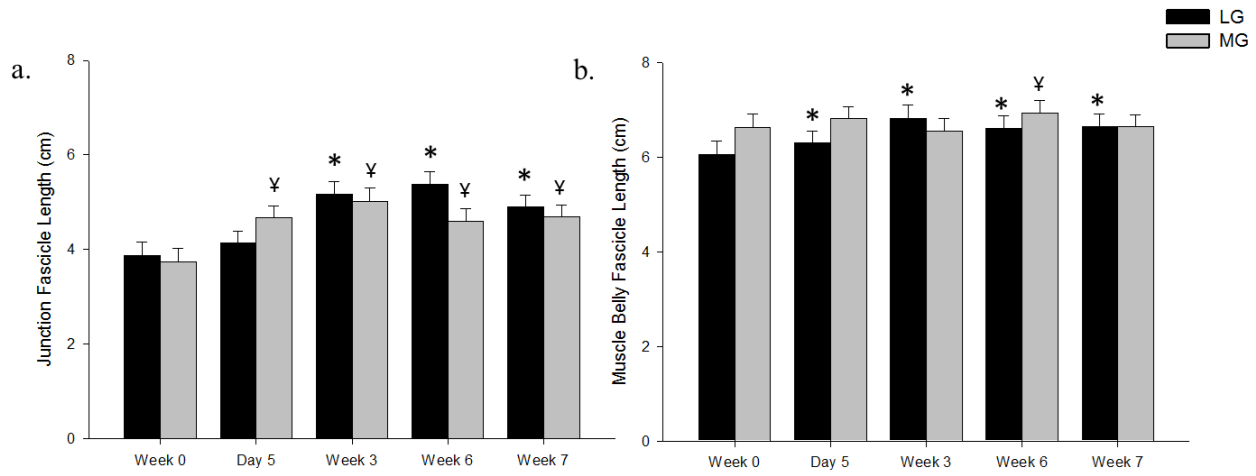

Figure 2.4: Time by muscle by position interaction for fascicle length over the 7 week stretch training for fascicles at the muscle tendon junction (a) and the muscle belly (b). Fascicle length was measured in centimetres (cm). Significant increase in fascicle length of the lateral gastrocnemius (*) from week 0 (baseline) whereas significant increase in length of the medial gastrocnemius is denoted by ¥ compared to week 0 (baseline). Values are mean ± SE, $p<0.05$.

The pennation angle in the stretched condition was larger ($p=0.01$) than the non-stretched (condition main effect, $F=3.304$, $p=0.04$). The average pennation angle for the LG was smaller ($p<0.001$) when compared to the MG and there was a main effect for muscle ($F=56.474$, $p<0.001$). In the junction of the LG the pennation angle decreased at week 6 ($p=0.02$) but increased in the junction of the MG at week 7 ($p=0.04$) when compared to week 0 ($F=2.389$, $p=0.05$). In the muscle belly the pennation angle of the LG decreased significantly at day 5 ($p=0.005$), week 3 ($p=0.004$) and week 7 ($p=0.003$) when compared to week 0 with no change in the pennation angles in the MG muscle belly. The pennation angle in the muscle belly was larger than in the junction at week 0 ($p=0.001$) and at week 6 ($p=0.002$). At week 3 in the muscle belly the pennation angle was significantly smaller ($p=0.006$) than week 0. The pennation angle in the junction was significantly greater at day 5 ($p=0.02$) when compared to week 0. However, there was a main effect for position ($F=45.837$, $p<0.001$) and the pennation angle at the junction was smaller than the belly ($p=0.001$) (Figure 2.5).

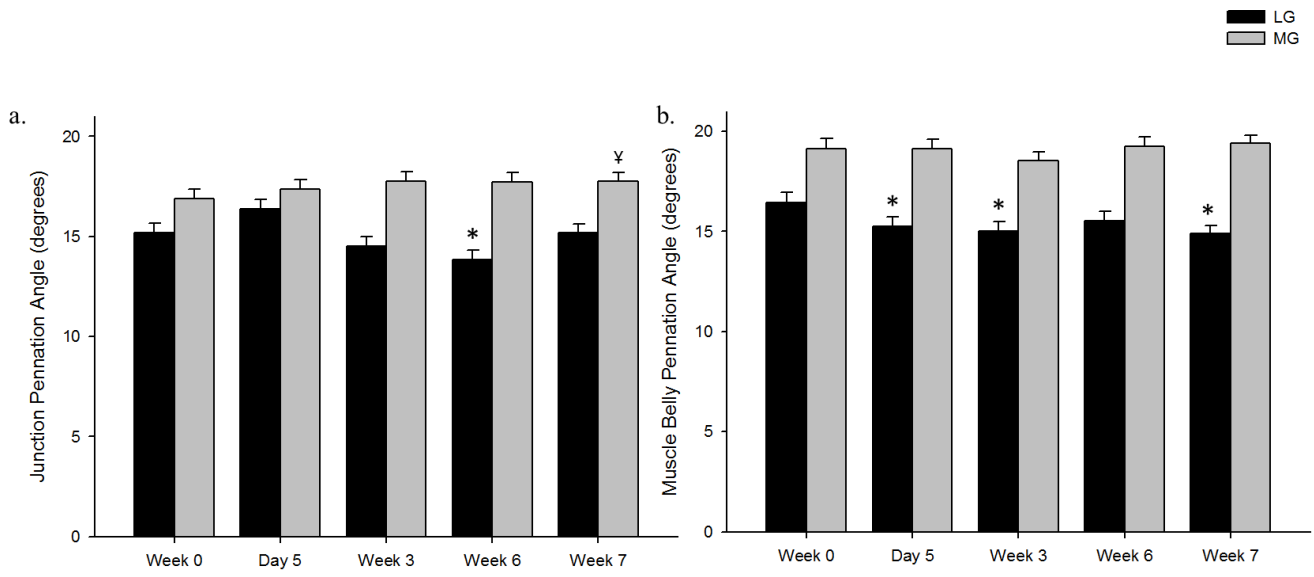

Figure 2.5: Time by muscle by position interaction for pennation angle of the lateral gastrocnemius (LG) and medial gastrocnemius (MG) at the muscle tendon junction (a) and the muscle belly (b). Pennation angle was measured in degrees. Significance from week 0 (baseline) of the lateral gastrocnemius is denoted by (*) and the medial gastrocnemius is denoted by ($^{¥}$). Values are mean ± SE, $p<0.05$.

The AT length was unchanged over the 7 weeks of training. The two way time by condition was not significant ($F=0.181$, $p=0.95$) and there were no main effects for time ($F=1.046$, $p=0.39$) or condition ($F=0.139$, $p=0.71$) for length. The AT depth was smaller ($F=100.26$, $p<0.001$) at the superior end of the tendon compared with the middle ($p<0.001$) and the inferior portion ($p<0.001$). Also, the middle AT depth was smaller than the inferior ($p<0.001$). However, there were no significant three ($F=0.264$, $p=0.97$) or two way interactions, and no main effects for time ($F=1.387$, $p=0.25$) or condition ($F=0.037$, $p=0.85$) for tendon depth (Table 2.1).

Table 2.1: Tendon properties. Comparison of the tendon properties in the stretched and non-stretched condition and at the superior, middle and inferior measurement points. Length and depth measured in centimetres (cm). The superior tendon depth was smaller than the inferior and middle and is denoted by (**) while the middle tendon depth was smaller than the inferior and is denoted by (*). Data are mean ± SD, $p<0.05$.

	Tendon Length (cm)	**Tendon Depth (cm)**	
Stretched	23.18 ± 0.68	**Superior**	0.16 ± 0.02**
		Middle	0.23 ± 0.02*
		Inferior	0.39 ± 0.02
Non-stretched	23.53 ± 0.71	**Superior**	0.16 ± 0.01**
		Middle	0.24 ± 0.02*
		Inferior	0.38 ± 0.01

2.3 Discussion

The gastrocnemii contract in tandem to produce plantar flexor force; however, in previous studies of resistance training adaptations in muscle size, pennation angles and length of fascicles were reported to be non-uniform between the LG and MG (Abe, 2000, Kearns et al., 2001). The main finding of the present study was that six weeks of overloaded stretch training increased muscle depth as well as fascicle length at both the MTJ and muscle belly in the MG and LG but the extent of adaptation was greater in the lateral head. Maximal force was unaffected and this was attributed to a compensation of pennation angle decreasing over the course of the 30 stretch training sessions. This is the first study in humans to demonstrate that with sufficient loading stretch training increased the size of the muscle with a concomitant increase in the length of the muscle fascicles and decrease in pennation angle which differs

between the LG and MG. These changes resulted in similar plantar flexor force output remaining constant over the course of the training period.

Muscle growth in this study was not due to an eccentric training response because this requires active muscle contraction. The use of instantaneous EMG during the stretch training sessions enabled on-line muscle activity monitoring. EMG during all training sessions was less than 2% of the MVC, thus the adaptations in fascicle lengths and muscle depth are not consequential to eccentric contractions but rather are a result of stretch training

As a muscle lengthens, there is unfolding of the collagen elastic network that lies in parallel to the muscle until non-contractile tissue like the perimysium maximally extends. At that point, there is a rapid rise in muscle stiffness and any further extension of the muscle would be unlikely (Purslow, 1989). Increased passive muscle tension induced by stretching the non-contractile components of the MTU results in prostaglandin creation through G-protein linked cascades (Vandenburgh et al., 1995). Prostaglandin F2 (PGF2) signalling enhances protein production (Dix & Eisenberg, 1990) through via the mammalian target of rapamyosin (mTOR) pathway (Markworth & Cameron-Smith, 2011). Although the exact mechanism through which mTOR influences skeletal muscle growth is equivocal (Hesselink et al., 2006), it is essential to translation, enabling protein synthesis (Drummond et al., 2009). Ingestion of amino acids is also known to increase protein synthesis (Drummond et al., 2009) and the post stretch supplementation might have stimulated these pathways. Amino acid supplementation has not previously been used in stretch training studies and this combined with making use of the principle of overload may have contributed to the observed increase in muscle depth.

The principle of overload is well established for resistance training but has received little attention in stretch training in humans. The necessity of overload was identified in the seminal

stretch training studies of animals (Barnett et al., 1980, Holly et al., 1980, Sola et al., 1973), but with extreme overloading there is risk of damaging structural elements of the sarcomere and impairing force production (Dolezal et al., 2000, Norrbrand et al., 2008, Williams & Goldspink, 1971). In this study the MVC was unaffected over the six weeks as was the AT. The tendon is considered part of the series elastic components of the MTU and transmits force to bone. The elastic portions of the tendon adjust initially in response to static stretching but thereafter remain static to sustain tension through the MTU (Proske et al., 1993). Thus, the effect of sustained stretching is transferred to the series elastic components within the muscle such as the aponeurosis, perimysium and inter-sarcomeric proteins. Stretch training has been reported to increase the compliance of these structures and, without compensation within the muscle, the force of the MTU would decrease (Blazevich et al., 2014). Ankle angle during stretch gained 15% flexibility between day one of stretch training and day 30 which aligns with the universal report that joint ROM increases as a result of stretch training (Blazevich et al., 2014, Guissard & Duchateau, 2004, Hayes et al., 2012, Nakamura et al., 2012). This also indicates more compliance in the MTU; however, the decrease in pennation angles observed in the LG of the stretched leg likely compensated to maintain muscle force even with increased ROM, lengthened fascicles and no change to the tendon.

At baseline in the MTJ and muscle belly the packing density of the MG was greater than the LG resulting in greater pennation angle and a higher contribution to plantar flexion force from the medial head over the lateral head (Fiebert et al., 1998). The packing density of the LG is less than the MG meaning that each fascicle must transmit an increased amount of force to the tendon compared to a fascicle in the MG. This architecture, combined with the "twisted" attachment of the LG fascicles to the AT (Edama et al., 2014) would provide additional torsional

force and passive tension from the lateral head but not the medial head. Stretching these fascicles would lengthen and unfold the twists in the LG, decreasing the contribution of this torsion to passive tension and overall force causing the plantar flexion force to decrease. As the stretch training progressed, the pennation angle of the LG but not the MG was significantly reduced when compared to baseline indicative of muscle specific adaptation of pennation angle and was the likely means through which the overall force of the plantar flexors was maintained during this study. The decrease in pennation angle indicates an even greater portion of force from each muscle fascicle, thus force was unchanged because the pennation angle decreased to adjust for the significantly lengthened LG fibres in the MTJ.

The fascicle will lengthen by adding sarcomeres in series (Goldspink et al., 1974). In this study, both the LG and MG fascicles were longer after stretch training, with the longest fascicles noted in the LG; again indicative of a differential adaptation in the LG induced by stretch training compared with the MG. At baseline the LG muscle fascicles were longer in both the MTJ and muscle belly compared with MG suggesting that prior to stretching the fascicles in the lateral head were comprised of a greater number of sarcomeres in series. Fascicles with more sarcomeres in series have a greater amount of non-contractile elastic tissue, such as Titin which is sensitive to changes in muscle length brought about by stretching and as mentioned previously, can be damaged by overstretching. The metabolic route through which sarcogenesis is stimulated is mediated by the consequences of passive lengthening under tension, including damage to Z lines. Therefore, the additional number of sarcomeres in series prior to stretch training in the LG may have created a unique environment for stretch training to preferentially effect the LG at the MTJ. There is evidence from animal studies (Dix & Eisenburg, 1990) that the majority of sarcogenesis occurs at the MTJ (Williams & Goldspink, 1971), due to the

congress of mRNA and myosin heavy chain (MHC) polysomes (Dix & Eisenburg, 1990). The MHC mRNA accumulation is determined by the large sarcomeric spaces at the end of stretched fascicles, which allow for regional synthesis of contractile proteins, rapid sarcomere assembly and extension of myofibrils (Dix & Eisenburg, 1990) and therefore lengthening of the muscle fascicle at the junction.

At the level of the stretching sarcomere, the elastic portion of the protein Titin extends causing non-linear increases in passive tension as the molecule unfolds (Ottenheinjm, 2010, Ranatunga, 2001). Titin extends from the thick filaments to the Z line and in animals, stretching has been shown to disrupt these Z lines (Khan, 1986) in a process called streaming; this phenomenon has also been observed in eccentric training in humans (Friden & Lieber, 2001). Based on the eccentric model, these signs of disruption can be indicative of adaptive remodelling in the cell and will also induce a signalling cascade that leads to greater protein production (Clarkson & Sayers, 1999). This protein is preferentially incorporated into the Z line material (Khan, 1986) and through this, streaming widens the Z line, encourages focal thickening and expansion (Khan, 1986) and potentially increases the LG and MG length. As the length of the LG fascicles was greater than the MG initially there were more Z lines which would create sites of damage after stretching and greater stimuli for protein incorporation, thus lengthening the LG muscle fibres to a greater extent than the MG at the MTJ.

This was the first study to report an increase in fascicle length following stretch training. Previously, the muscle belly was the primary site of investigation and there was minimal application of the principle of overload (Blazevich et al., 2014, Nakamura et al., 2012). Following stretch training muscle fascicles in the belly increased 5.1% whereas those in the junction were 25% longer. The 5.1% increase in fascicle length was also very similar to the 5.6%

increase in muscle depth recorded at week six when compared to baseline. As muscle size and length are correlated with fascicle length (Abe, 2000, Kearns et al., 2001) the increase in depth and circumference measured in this study was indicative of the progressive increase in muscle size over stretch training. The muscle belly also was the site where the muscle fascicles in the MG surpassed the length of the fascicles in the LG by week six. This finding demonstrates the necessity of evaluating the response to stretch training the gastrocnemii separately between the LG and MG. The LG adapted to stretch training through an increase in fascicle length associated with a decrease in pennation angle, likely as a mechanism to maintain force production. While the MG also increased the length of the muscle fascicles, however this translated to an increase in muscle depth.

In conclusion, six weeks of stretch training of the LG, MG and AT stimulated muscle specific adaptations in the LG and MG not previously reported in stretch training studies. In the LG fascicle length significantly increased as a result of sarcomeres added in series with the greater change evident at the MTJ compared with the muscle belly. The pennation angle in the LG decreased likely as a compensatory mechanism to maintain plantar flexion force and adapt to higher tensile load being placed between the contractile and non-contractile elements of the tendon which showed no stretch adaptation. In the MG muscle belly fascicle length increased similarly to the muscle depth, and were longer than the LG, leading to the conclusion that after stretch training the architecture of the LG adapts to maintain force while the architecture of the MG adapts to increase muscle size.

The lack of adaptation in the tendon indicates that stretch training with a load acts primarily on the contractile component of the MTU and causes hypertrophic adaptation, likely through stimulating an inflammatory response, upregulating protein synthesis and increasing

protein incorporation into the sarcomeres. However, in this study because the force was unchanged over the stretch training protocol, the muscle was not stretched to the point of negative adaptation through damage of overload. Furthermore, it was necessary to maximally extend the stretch limits of the MTU by applying the principle of overload in order to induce adaptations in skeletal muscle architecture.

Chapter 3: Question 2

3.0 Background

When muscle is lengthened or shortened subsequent force production can be impaired and the extent of the impairment is dependent on how far the muscle deviates from optimal length. Maximal contractile force is generated at muscle lengths which enable ideal overlap of actin and myosin and maximize the contribution of contractile and non-contractile elements to total tension development. The relationship of length and tension is well documented (Herzog & Leonard, 2002, Kaufman et al., 1989) and can be demonstrated through acute stretching (Behm et al., 2001, Fowles et al., 2000). Lengthening of sarcomeres through stretch promoted less overlap of actin and myosin, decreased the contribution of active tension to total tension which increased the reliance on the descending limb of the length tension relationship for force generation (Rassier et al., 1999, Trajano et al., 2013, Ter Keurs et al., 1978). This resulted in an acute depression of force generation immediately post stretch, and both voluntary and involuntary force, stimulated through evoked twitches were affected.

Acute static stretching increased passive tension of a resting muscle (Proske & Morgan, 1999) and damaged contractile components which resulted in an increased reliance on the elastic components of the sarcomere to resist the stretch and prevent overstretching (Whitehead et al., 2001). In chronic stretch training this lead to disruption of Z lines, altered calcium kinetics and compromised membranes (Barnett et al., 1980, Holly et al., 1980, Vandenburgh & Kaufman, 1979). However, in animal studies this disruption was reported to be a strong growth signalling mechanism and culminated in the addition of sarcomeres in series which lengthened muscle fibres and increased muscle size (Dix & Eisenburg, 1990, Holly et al., 1980, Sola et al., 1973). The evidence that chronic stretch training had the same impact as acute stretching on the length

tension relationship was equivocal (Akagi & Takahasi, 2014, Blazevich et al., 2012, 2014, Guissard & Duchateau, 2004, Hayes et al., 2012, Kubo et al., 2001) and to date, no study has demonstrated that stretch training in humans alters the length tension relationship. This is partly due to the difficulty in assessing sarcomere lengths non-invasively. Recent advances in ultrasonography allow for quantifications of muscle fascicle lengths across different joint angles *in vivo* (Blazevich et al., 2014, Nakamura et al., 2012).

There have been no reports of changes in resting fascicle lengths of human muscle after stretch training (Blazevich et al., 2014, Nakamura et al., 2012). The length of a muscle fascicle is representative of its collective sarcomere lengths (Rassier et al., 1999). For this reason, the length tension relationship that exists for the sarcomere can be applied to muscle fascicles (Leonard & Herzog, 2010). If stretch training increases the length of the muscle fibres then muscle force should be likewise increased, until a point where fascicles become too long and thus the relationship is shifted to the descending curve of the length tension relationship. The muscle and tendon act as a unit and when the tendon is stretched or compressed through manipulation of the joint the muscle compensates through alterations in architecture including the fascicle lengths and pennation angles to sustain force. This creates an opportunity to make inferences about the impact of stretch training on the length tension relationship of the MTU, fascicles and sarcomeres through observation of muscle architecture and force at different joint angles.

Stretch training can dampen reflex responses and muscle activity; however, this too is equivocal (Blazevich et al., 2012, 2014, Guissard & Duchateau, 2004, Hayes et al., 2012, Kubo et al., 2001) and has been linked to changes in the viscosity and length of the MTU. Magnusson et al., (1996) reported that increased stretch tolerance arises from alterations in Ia afferent

feedback from the stretched muscle, which might also influence muscle activity. However, Blazevich et al., (2014) and Hayes et al., (2012) reported no neural adaptation or changes in muscle activity after six weeks of stretch training and suggested that mechanical adaptation such as reduced passive stiffness of the non-contractile components of the MTU was the primary factor in the increase in ROM. The reduction in passive stiffness would also decrease the resistance to stretch, and subsequently lead to a reduction in the contribution of passive tension to force development and a decrease in force overall as the length tension relationship is compromised.

The purpose of this study was to examine the effect of stretch training on the neural, muscle architectural, and force characteristics of the plantar flexors at five different lengths that were imposed on the MTU by changing the ankle angle. It was hypothesized that stretch training would attenuate the length tension relationship of the gastrocnemius AT MTU by decreasing the reliance on the passive tension contribution to total tension and shifting the sarcomeres into less than optimal overlap. This would result in reduced voluntary force without changing reflex activity. It was further hypothesized that twitch contractile times would be faster and there would be increase muscle size and length of muscle fascicles.

3.1 Method

Subjects

Twenty two healthy males aged 19-30 years were recruited for this study. Twelve took part in the stretching intervention, however one did not complete the stretching protocol so data are based on 11 subjects in the experimental group (22 years ± 2, 179.45 cm ± 8.33, 76.77 kgs ± 15.15) and 10 subjects as a separate control group (22 years ± 4, 179.03 cm ± 10.93, 83.93 kgs ± 15.98). The subjects in both the training and control group were moderately active as defined by

the Canadian Physical Activity Guidelines of at least 150 minutes of moderate to vigorous activity per week (Canadian Society for Exercise Physiology, 2015). Activities in the training group included sports such as basketball and badminton, resistance training, track and field, ballet and longboarding. In the control group, subjects participated in soccer, basketball, badminton, volleyball, swimming, resistance training and cross country running. The exclusion criteria for this study included individuals who had current lower body injury, were under the age of 19 or over the age of 30, had neurological or musculoskeletal disorders, or were unable to read or speak English fluently. The non-dominant leg was stretched while the dominant served as an internal control. Dominance was determined by asking the subjects which leg they preferred to kick a soccer ball with (Hoffman, 1995) and all subjects reported their right leg to be dominant, therefore the left leg underwent the stretching intervention. For the duration of the protocol, subjects were asked to maintain the same fitness and nutrition regimes with the addition of the stretching and the post stretch protein drink. Informed written consent was obtained prior to participation in this study. All procedures were approved by the University of British Columbia Behavioural Research Ethics Board (H13-03377) and conformed to the Declaration of Helsinki.

Experimental Set Up

Assessments

During the seven week protocol, four assessments were recorded at week 0 (baseline), week 3, week 6 and one week following the conclusion of the stretching intervention at week 7. During these assessments muscle depth, fascicle lengths and pennation angles were measured with ultrasound (GE LOGIQ E9, Connecticut USA) with a ML6-15 probe (4.5-15.0 MHz linear array, 13 x 58 mm foot print, 30 frames per second 50 mm field of view, 8 cm depth of field LOGIQview, GE©), muscle activity with EMG (Coulbourn, Allentown, Pennsylvania), voluntary

force and twitch contractile properties with a dynamometer (Biodex Medical Systems, New York, USA), and voluntary activation assessed with the twitch interpolation technique.

Prior to force, the ultrasound measures were collected with the subject laying prone, the feet were allowed to rest at 120º as measured by a goniometer (JAMAR® Easy Read™, Patterson Medical, Mississauga, Ontario, Canada). Using ultrasound with a RSP6-16D 3D/4D probe (5.6-18.4 MHz linear array, 38.4 x 44.5 mm footprint, volume: 37.4 mm x 29º field of view) the MTJ was identified. True position of the MTJ was determined in the 3D setting through simultaneous capture of longitudinal, cross sectional and frontal position. The MTJ was defined as the position at which the muscle receded to tendon in all three of these views. Aquasonic clear ultrasound transmission gel (Parker Laboratories, Inc., New Jersey USA) provided acoustic contact between the probe and skin. At the skin this position was marked using an echo-absorptive marker. A longitudinal scan of the MG and LG gastrocnemius was performed using the ML6-15 probe in LOGIQview setting. Each gastrocnemii muscle was scanned separately from the MTJ to the insertion on the femur over the widest part of the muscle along the medial aspect. The ankle was rotated to 90º and the soles of the feet were restricted against the base of the bed so the subject could passively maintain the ankle angle as measured by a goniometer. The MTJ was identified, and the scans of the muscles were repeated at 90°. To prevent an order effect, the ankle angles of 90° and 120° were randomized as were the order of the legs.

MVC was collected on the Biodex dynamometer and Spike 2. The subject was seated with the foot secured to the foot plate of the dynamometer while the other leg hung freely. The order of testing between the control and stretched leg was randomized between subjects. Straps were attached across the chest and shoulders of the subject. After the foot was positioned with

the medial malleolus aligned with the centre of rotation of the Biodex it was secured to the plate with ratchet straps. For the stimulation protocol, the ankle angle was set to 90º. Force was produced on the Biodex dynamometer, sampled at 496Hz, converted from analogue to digital format (Power 1401, Cambridge Electronic Design, Cambridge UK) and displayed in Spike 2 software (Cambridge Electronic Design, Cambridge UK).

The skin was exfoliated and prepared for surface electrodes with coarse 70% isopropyl alcohol swabs. Self-adhesive bipolar surface electrodes (4 mm, rectangular Ag/AgCl) were attached over the medial aspect of the muscle belly of the MG, LG, SOL, and TA with an inter-electrode distance of 2 cm. Ground electrodes were placed over the lateral and medial malleolus of the ankle, and the lateral and medial epicondyle of the femur. EMG was sampled at 1024Hz amplified (x1000), filtered (8 Hz - 1 kHz) (Coulbourn, Allentown, Pennsylvania) and converted from analog to digital format (1401, CED, Cambridge, England). Two 4.5 x 4.5 cm Carbon stimulation electrodes were placed on the patella and inferior to the popliteal fossa to stimulate the tibial nerve. Supramaximal stimulation intensity was established by increasing intensity until the M wave plateaued after which the intensity was increased a further 10%. Three stimulations were applied prior to, during and immediately post MVC with an inter-stimulation interval of one second. Once this amplitude was decided, the ankle angle was randomized to one of five positions: 80º, 90º, 100º, 110º, 120ºand MVC with the twitch interpolation protocol was repeated at all angles and in both legs.

30 Stretching Sessions

Stretching occurred five times per week for six consecutive weeks. Stretching was organized into blocks of five stretches with two days of rest between each block of five. The incline leg press sled was loaded with 20% of the highest MVC achieved across all ankle angles

for each session (TuffStuff PPL-960 45°Leg Press, TuffStuff Fitness Equipment, Chino, CA) and the load was increased at the start of each block of stretching by 5% above the previous week to adhere to overload. To ensure stretch rather than contraction muscle activity was measured with EMG and subjects were reminded to relax. When muscle activity exceeded 2% of the MVC, the subject had ten seconds to relax the muscle. If they could not, the stretching session was stopped, ten minutes of rest was given, and the stretch attempt was repeated.

During each stretch session muscle activity and ankle angle were monitored online with a portable surface EMG device (Biometrics DataLOG P3X8, Gwent, UK). Following skin preparation on the vastus lateralis, biceps femoris, LG, MG, Sol, and TA. Electrodes (Biometrics SX230; Gwent, UK) with an inter-electrode distance of 20mm were placed according to SENIUM Guidelines (Merletti & Torino, 1999). High conductivity electrode cream (Signa Creme, New Jersey, USA) was used with the reference electrode (R200, Biometrics), which was positioned on the lateral malleolus of the fibula. The cables from the electrodes were placed into the data logger (9.5 x 15.8 x 3.3 cm; 380 g) and the portable EMG unit was placed next to the subject. An electronic goniometer was attached over the ankle joint so the inferior portion aligned with the midline of the foot, and the superior portion aligned with the midline of the shank. Signals were sampled at 1,000 Hz, amplified (1,000x), band-pass filtered (20-450Hz), and displayed in real time on a computer screen visible to the subject.

Experimental Procedures

All baseline measures were collected on the first day of stretch training prior to the start of the first stretching session. Subjects self-reported caloric consumption and physical activity in the three days prior to the first assessment. At the completion of the six weeks, these measures were collected for the three days prior to the end of the protocol.

Stimulation intensity was established with the ankle at 90º and then passively rotated to the first of five randomized positions. Subjects were instructed to contract their plantar flexors as hard and fast as possible to reach the isometric MVC then hold it for 5 sec. Supra-maximal pulses were elicited prior to, during and after the MVC to assess voluntary activation. Maximal isometric dorsi flexion was also executed at each ankle angle. All procedures were executed for both the trained and untrained leg. The order of testing was randomized between subjects and the ankle angles were randomly assigned within each leg being tested.

At each stretch training session a Likert scale was administered to self-report pain from 0-10 where 0 represented no pain and 10 represented the highest pain ever felt. Warm up was conducted prior to each session on a mini-rebounder (Tempo Fitness, Cottage Grove, WI). Subjects maintained a low intensity bounce for three minutes while focusing on maximal plantar flexion at each push off. Then subjects performed 15 calf raises on a custom built step (61 cm x 15 cm x 20 cm) which enabled the heels to drop toward the floor in dorsi flexion, thus stretching the plantar flexors, before raising to full plantar flexion on both legs. Electrodes were attached to the non-dominant leg of the subject and the subject sat on the leg press machine so their hip was flexed to 90º as set by the angle of the back rest. The ball of the foot was placed on the calf raise portion of the leg press machine, and using both legs the subject pressed the weight up so both legs were extended. On ‘go’ the subject removed their dominant leg from the leg press, the three minute timer was started and the subjects were encouraged to relax their plantar flexors so the ankle of the non-dominant leg could rotate to dorsi flexion, thus stretching the calf. Muscle activity was monitored for the duration of the stretch and subjects were given visual feedback of the EMG activity which was used to encourage relaxation of the stretched and monitored muscles. The stretch was held for three minutes and at one minute the ankle angle was recorded

from the goniometer. At three minutes, the subject replaced their dominant foot on the leg press, and both legs guided the weight back to the resting locks. Within 10 minutes of completing the stretch, the subject drank a protein and water mixture consisting of eight ounces of water, and 0.25g protein powder per kilogram of body weight (Natural Whey Isolate, 25 g protein per scoop, Kaizen Canada, Calgary, AB).

Data Analysis

MVC, VA, EMG, twitch contractile properties and reflex data were analyzed off line using custom scripts in Spike 2 (Cambridge Electronics Design, Cambridge UK). MVC was measured at each ankle angle and VA was assessed according to Jakobi & Rice (2001) and was based on the formula: voluntary activation (%) = (1- (interpolated twitch/resting twitch))*100).

Root mean square (RMS) EMG was measured over 0.5 second intervals, rectified and smoothed for each muscle (LG, MG, SOL, TA) over the 3 second MVC. Artifact from the stimulation was removed using a high pass cut off and not included in the EMG analysis. EMG was normalized to the peak to peak amplitude of the M wave. Twitch contractile properties including time to peak tension (TPT), half relaxation time (HRT), contraction duration (CD), and peak tension (PT) were based on the twitch immediately prior to contraction (resting twitch) and the twitch immediately post contraction (potentiated twitch). M wave and H reflex latency were measured as the time between the stimulus and the onset of the neural response. H/M ratio was the positive to negative peak to peak amplitude of the H reflex over the same measure of the M wave.

To quantify self-reported perception of pain on each testing session the distance between the point of origin on the left and the marked position to the right was recorded in millimetres.

Muscle depth was assessed offline using OsiriX Dicom Viewer software (OsiriX 5.5, Switzerland). OsiriX measurements were calibrated to the measurements inherent to the B1500 ultrasound by using a predetermined 1 cm distance on the dicom image. An intra-measurement reliability of 0.01cm was established using the line and spline trace and 0.1º for the angle measurements. Muscle depth was considered the vertical distance from the horizontal upper aponeurosis to the horizontal lower aponeurosis across the widest part of the muscle and was measured from both the LG and the MG. Muscle fascicle length was measured from the lower aponeurosis to the upper with a spline trace where any deviations from linear could be accounted for and included within the trace pattern. Where the muscle fascicle met the lower aponeurosis the pennation angle was measured using the angle function. Researchers were blinded to the subject, condition and assessment number during analysis. Three fascicles and pennation angles were isolated from the MTJ and three from the muscle belly in both the LG and MG, stretched and non-stretched condition and at 90º and 120º (Figure 3.0).

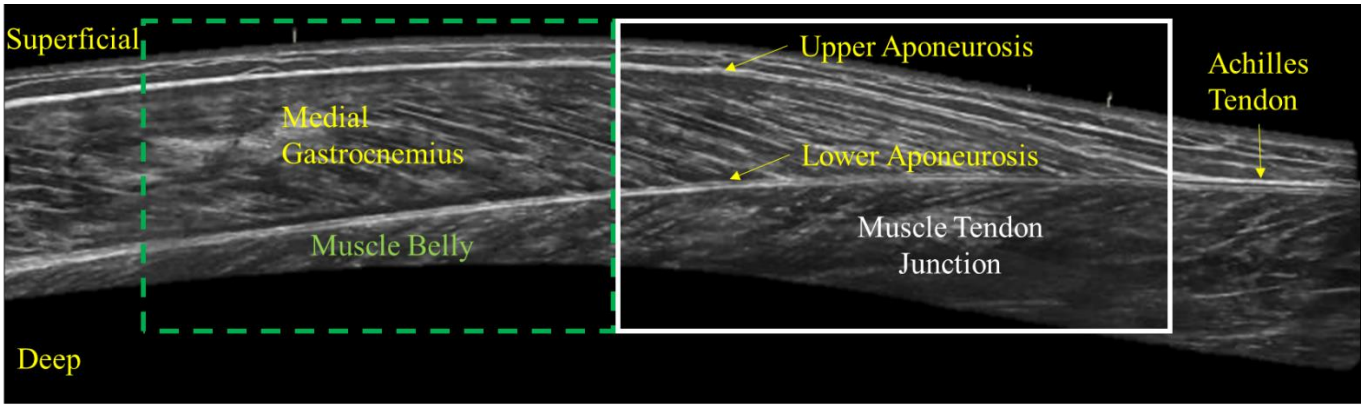

Figure 3.0: LogiQview Scan of the Medial Gastrocnemius. Muscle fascicles are visible throughout the MG and measured from the muscle tendon junction and muscle belly. Image was collected with a LOGIQview scan from the MTJ to the popliteal crease with a ML6-15 probe (4.5-15.0 MHz linear array, 13 x 58 mm foot print, 30 frames per second, 50 mm field of view, 8 cm depth of field LOGIQview, GE©)

Statistical Analysis

All dependant variables were compared between the control group (n=10) and the untrained dominant leg which served as the internal control for the experimental group (n=11, non-stretched) in a two way repeated measures ANOVA for the within subject factor of time (week 0, week 6) and the between subject factor of group (control, non-stretched leg). There were no significant differences found between the control group and the non-stretched condition, therefore all statistical comparisons were based upon the non-stretched leg as a control.

A four way repeated measures ANOVA with the within subject factor of time (week 0, week 3, week 6, week 7) and the between subject factors of condition (stretched, non-stretched), ankle angle (80 º, 90 º, 100 º, 110 º, 120 º) and muscle (LG, MG, SOL, TA) was conducted for the dependant variables EMG, reflex measures including H reflex and M wave latency and peak to peak amplitude, and H/M ratio with the modification that muscle was measured across three levels (LG, MG, SOL) rather than four. Twitch contractile properties including TPT, HRT, CD and PT were also analyzed with a four way repeated measures ANOVA; however, twitch position (resting, potentiated) was used rather than muscle as the fourth level in the ANOVA. Finally, a four way time by condition by ankle angle (90º, 120º) by muscle (LG, MG) repeated measures ANOVA was used to assess depth.

MVC and VA were analyzed using a three way repeated measures ANOVA for the within subject effect of time (week 0, week 3, week 6, week 7) and the between subject factors of condition (stretched, non-stretched) and ankle angle (80º, 90 º, 100 º, 110 º, 120 º), as were muscle fascicle lengths, and pennation angles but these variables were only assessed at two ankle angles (90º, 120º). Pain over the thirty stretching sessions was assessed with one way repeated measures ANOVA (time: session 1-30).

When the ANOVA returned a significant interaction or main effect, Tukey's post hoc analysis was performed. If there were fewer than two levels a Student's paired T-test or one way ANOVA was conducted. For all repeated measures ANOVAs where the assumption of sphericity was violated ($p<0.05$) according to Mauchly's Test of Sphericity, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. The alpha level was set to 0.05 and significance was considered if $p\leq0.05$. Values in tables are mean ± SD, and in figures are mean ± SE.

3.2 Results

Six weeks of stretch training significantly decreased force production in the stretched leg. Force was 3.3% less at week 3 ($p=0.03$), 6.4% less at week 6 ($p=0.008$) and 4.9% less at week 7 ($p=0.04$) when compared to week 0 in the stretched condition ($F=3.955$, $p=0.01$). Similar to the stretched leg the non-stretched leg also demonstrated a 7.4% decrease in force at week 7 ($p=0.004$) when compared to week 0 (Figure 3.1).

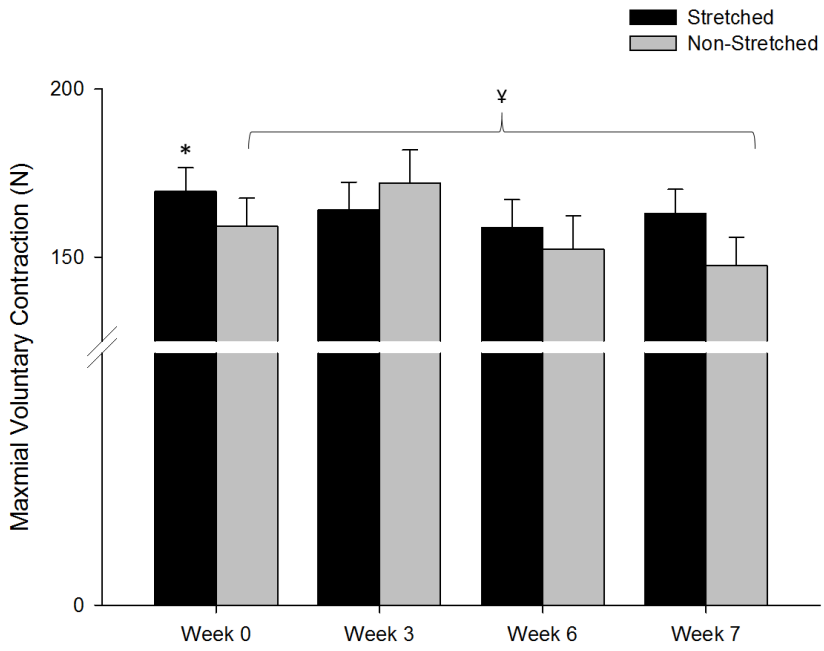

Figure 3.1: Maximal voluntary contraction time by condition interaction. Maximal voluntary contraction was measured in Newtons (N). Significance in the stretched condition from all other bars is denoted by (*) and significance in the non-stretched condition of week 0 compared to week 7 is denoted by (^{Y}). Values are mean ± SE, $p \leq 0.05$.

There was a main effect for time ($F=13.083$, $p=0.001$) and ankle angle ($F=10.051$, $p<0.001$) for MVC. At week 0, prior to stretch training MVC was significantly greater compared week 6 ($p=0.04$) and week 7 ($p=0.008$). The MVC was also greater at longer muscles lengths when the ankle was positioned at 80º compared with 100º ($p=0.001$), 110º ($p<0.001$) and 120º ($p<0.001$). The stretch induced reduction in strength was not due to a reduction in voluntary activation which was ~95.48% ± 0.92 over the course of the stretch training program.

Over the seven weeks there were increases in the muscle fascicle lengths (n=2640) which resulted in a main effect for time ($F=36.182$, $p<0.001$). The muscle fascicles were significantly longer at day 5 ($p<0.001$), week 3 ($p<0.001$), week 6 ($p<0.001$) and week 7 ($p<0.001$) when compared to week 0. An increase in muscle fascicle length would normally be associated with a

decrease in pennation angle; however, the pennation angle (n=2640) was unchanged at day 5, week 6 and week 7 when compared to week 0. The only time that the pennation angle was less than week 0 was at week 3 ($p=0.05$). The pennation angle in the stretched condition was also greater than the non-stretched (condition main effect; $F=11.104$, $p=0.001$). At 90 º compared to 120 º fascicle length ($p<0.001$) and pennation angle ($p<0.001$) were longer and smaller, respectively (ankle angle main effect; $F=61.647$, $p<0.001$).

The EMG of the stretched MG was greater than the non-stretched condition ($p<0.001$) although the LG ($p=0.09$) and Sol ($p=0.56$) did not differ between conditions. The EMG of each muscle differed from all others in each condition. Overall, the stretched condition demonstrated greater EMG than the non-stretched (condition main effect $F=5.806$, $p=0.02$). The normalized EMG amplitude was significantly less at week 3 ($p=0.001$), week 6 ($p<0.001$) and week 7 ($p=0.004$) when compared to week 0 (time main effect $F=11.617$, $p<0.001$, Figure 3.2a). The muscle by condition interaction was also significant ($F=3.274$, $p=0.04$, Figure 3.2b).

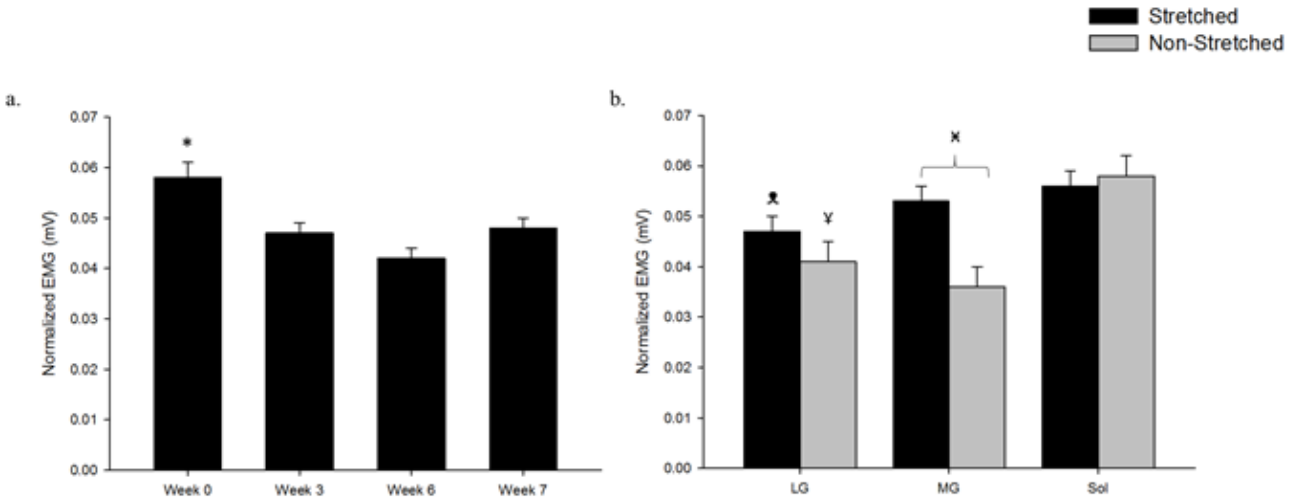

Figure 3.2: Time main effect for normalized electromyography (a) and muscle by condition interaction (b). Significance from all other time points in (a) is denoted by (*). In (b), ($^{\bullet}$) indicates the lateral gastrocnemius (LG) electromyography (EMG) was less than the medial gastrocnemius (MG) and soleus (Sol) in the stretched condition; (^{Y}) indicates the LG EMG was greater than the MG and less than the Sol in the non-stretched condition; (^{x}) indicates the MG EMG was less in the non-stretched condition when compared to the stretched. Root mean square (RMS) EMG amplitude was normalized to the peak to peak amplitude of the muscle compound action potential (M wave). Values are presented as mean ± SE, $p<0.05$.

For the twitch contractile properties of TPT, HRT, PT and CD there was a time by condition ($F=2.374$, $p=0.01$) and time by ankle angle interaction ($F=1.901$, $p=0.05$). The HRT and CD decreased significantly at week 3, week 6 and week 7 when compared to week 0 in the stretched condition (Figure 3.3a). There was a main effect for time ($F=5.838$, $p=0.02$). The TPT decreased significantly at 7 weeks compared to week 0 ($p=0.03$), week 3 ($p=0.007$) and week 6 ($p=0.04$) (Figure 3.3b).

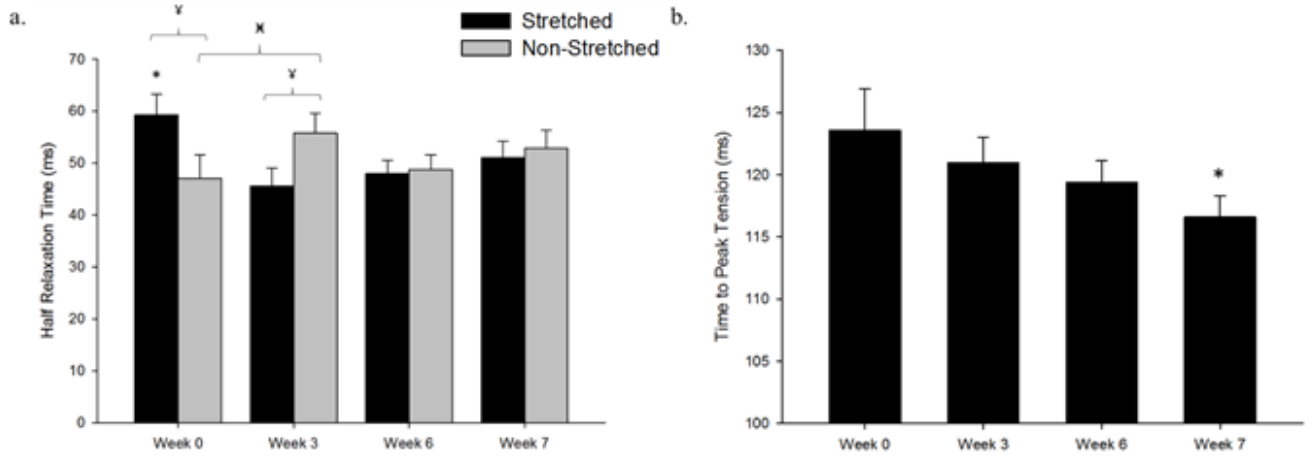

Figure 3.3: Half relaxation time (a) and time to peak tension (b) for the 7 week time period. HRT and TPT were measured in milliseconds (ms). In (a), significance from all other stretched condition time points is denoted by (*); difference between the stretched and non-stretched condition is marked by (^{Y}) and significance in the non-stretched condition is marked by (^{x}). In (b), (*) indicates week 7 is significantly lower than all other time points. Values are mean ± SE, $p<0.05$.

At ankle angles 110 º and 120 º, the PT was significantly greater at week 3, week 6 and week 7 when compared to week 0, and at 80 º and 90 º the PT was significantly less at week 3, week 6 and week 7 when compared to week 0. AT 100 º PT did not change over time. There was a main effect for ankle angle ($F=15.581$, $p<0.001$), and twitch position ($F=8.242$, $p=0.005$). The TPT, HRT, PT and CD decreased significantly from 80 º to 90 º, 100 º, 110 º, and 120 º. The PT of the potentiated twitch was significantly higher ($p=0.005$) had shorter TPT ($p=0.02$), and CD ($p=0.02$) when compared to the resting twitch.

For reflex measures, there was a significant time by condition interaction ($F=3.999$, $p=0.01$). The M wave latency decreased significantly at week 3 when compared to week 0 for the stretched condition. There was a main effect for time ($F=4.102$, $p=0.03$) and muscle ($F=12.674$, $p<0.001$). The H reflex latency increased significantly at week 3 ($p=0.04$) and week 7 ($p<0.001$) when compared to the week 0 measures, the M wave latency was longer in the Sol when

compared to the LG (p=0.006) and MG (p<0.001). The H/M ratio was significantly greater for the Sol when compared to the lateral (p<0.001) and medial gastrocnemius (p=0.001). The M wave peak to peak amplitude was greater at week 3, week 6 and week 7 when compared to week 0.

Overall, subjects reported progressively less pain due to stretching on a Likert Scale over the thirty stretch training sessions. There was a main effect for time (F=1.834, p=0.008). When compared to the first session, subjects reported significantly more pain at day two to five, seven, nine, ten and seventeen (p<0.05) (Figure 3.4).

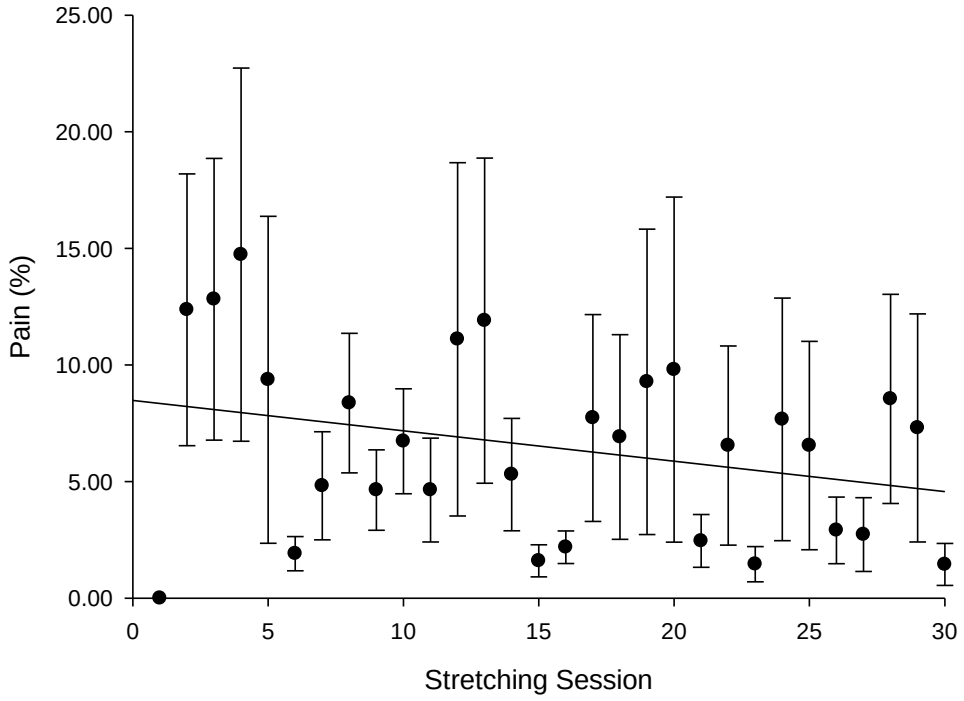

Figure 3.4: Self-reported pain for the 30 stretch sessions. Pain is represented as a percentage of the maximal potential Likert Score. Values are mean ± SE.

Six weeks of stretch training caused an increase in the muscle depth of the trained leg that was not observed in the non-stretched leg. The time by condition interaction was significant (F=2.584, p=0.04). The depth of the stretched leg at day 5 (p=0.009), week 3 (p<0.001), week 6

(p=0.009) and week 7 (p=0.002) was greater than baseline and differed from the non-stretched condition at week 3 (p=0.008) (Figure 3.5).

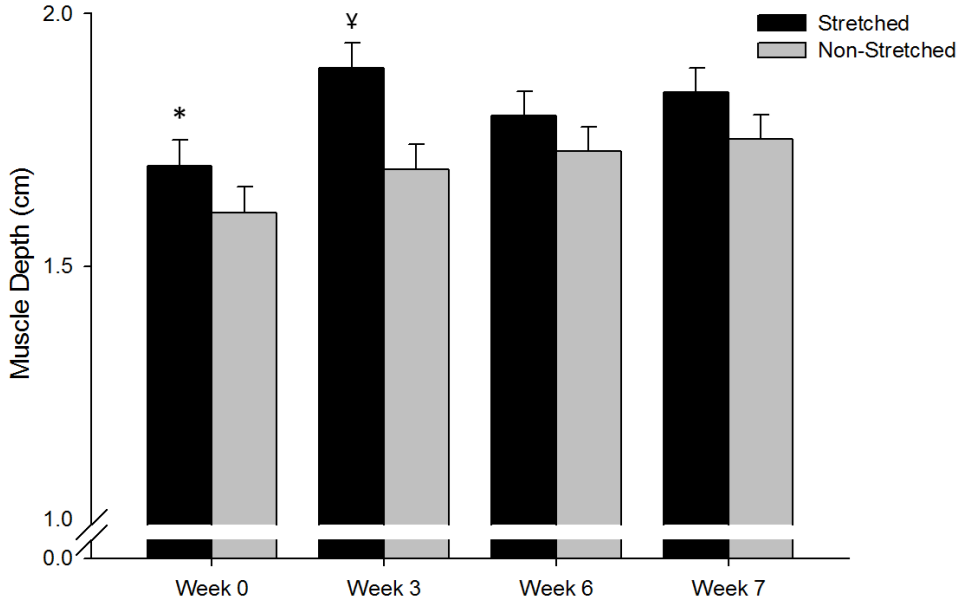

Figure 3.5: Muscle depth of the stretched and non-stretched leg. Muscle depth was measured in centimetres (cm). Significance from all other stretched condition time points is denoted by (*) while significance from the non-stretched condition is denoted by ($^{¥}$). Values are reported as mean ± SE, $p<0.05$.

3.3 Discussion

In humans, eccentric training was reported to cause a lasting shift toward longer lengths (rightward) in the optimal contraction length of a muscle (Brockett et al., 2001). A rightward shift in the optimal length for active tension production is indicative of increased compliance and can describe a disruption of sarcomeres (Proske & Morgan, 2004). The sarcomere non-uniformity hypothesis states that damage will occur only when sarcomeres are stretched beyond optimum and the damage can impair calcium kinetics and excitation contraction coupling which Endo (1973) has associated to a reduction in activation and overall decrease in tension.

Decreased activation implicates reduction in central drive and an impact of shifting the length tension curve on not only the structural but also the neural components of the MTU. The underlying cause of alterations in muscle force due to stretch training remains to be established (Blazevich et al., 2012, 2014, Guissard & Duchateau, 2004, Hayes et al., 2012, Kubo et al., 2001, Maganaris et al., 1996, Nakamura et al., 2012) in part because few studies have simultaneously evaluated neural, mechanical and structural adaptations to this training modality in accordance with the established length tension relationship.

The main findings of this study were that stretch training increased the length of muscle fascicles and decreased muscle passive tension, shifting the length tension relationship away from optimal and resulting in a reduction of force across all ankle angles. This was the first study to report decrements in force as a result of stretch training and suggest stretch training alters the length tension relationship through increasing the fascicle length and simultaneously decreasing the contribution of passive tension to total force. However, this adaptation appears to be impacted by joint angle because as the ankle was rotated from dorsi to plantar flexion the force characteristics were non-uniformly altered over each angle.

The length tension relationship of whole muscle is impacted by alterations in the mechanical factors including the viscoelastic properties of the muscle and neural factors such as altered motor control strategies or reflex sensitivity (Cramer et al., 2004). The force reduction observed in the stretched leg at all ankle angles occurred with no change in voluntary activation suggesting that stretch training adaptations are peripheral in nature and are most prominent in measures of fascicle length and pennation angle. A rightward shift in the length tension relationship after stretch training would indicate increased MTU compliance and possible damage to the sarcomeres (Proske & Morgan, 2004). If compliance increased, the contribution

of passive tension to total tension would decrease as the non-contractile components of the MTU become less stiff. This is a well-documented consequence of stretch training and has been associated with increased joint ROM (Blazevich et al., 2012, 2014, Nakamura et al., 2012, Trajano et al., 2013), which was reported in Question one of this thesis as an outcome of overloaded stretch training.

Stretch training in animals (Dix & Eisenburg, 1990) and eccentric training in humans (Brockett et al., 2001) stimulated an increase in the number of sarcomeres in series resulting in shorter average sarcomere lengths at a set fascicle length or joint angle (Proske & Morgan, 2004). This allowed the muscle to operate at longer lengths while avoiding the descending limb of the length tension curve and a resultant decrease in force. The descending limb is the portion of the length tension relationship where sarcomere inconsistencies in strength develop and damage to sarcomeres is most likely (Gordon et al., 1996). The weakest sarcomeres in a myofibril become progressively weaker during stretching, until the sarcomere yield point is reached (Morgan, 1990). At this point, the sarcomeres lengthen rapidly until actin and myosin no longer overlap and tension in the passive structures, like Titin, balance those sarcomeres that still have overlap; however when the stretch persists, such as in stretch training, this process is repeated in the next weakest sarcomeres (Proske & Morgan, 2004). In eccentric training this process has been reported to cause disrupted sarcomeres, membrane damage, impaired excitation contraction coupling and a fall in tension directly related to shifting of the length tension relationship (Brockett et al., 2001, Proske & Morgan, 2004).

Increased sarcomeres in series and avoidance of the descending limb of the length tension relationship could have sustained the force levels across all ankle angles in this study if the muscle fascicles remained the same length throughout the six weeks of stretch training; this is a

possible explanation as to why previous work in stretch training did not report decreases in force (Guissard & Duchateau, 2004) or increases in muscle fascicle lengths (Blazevich et al., 2014, Nakamura et al., 2012). However, the muscle fascicles lengthened significantly in this study through mechanisms described in Question one of this thesis and the impact on force was not clearly delineated until plantar flexion force was quantified across various ankle angles.

When the muscle fascicles were in a shortened position (plantar flexed ankle angles) unfavourable overlap between actin and myosin was created. Without shifting the length tension relationship, this would translate to decreased maximal force even with the same amount of activation. In this study, if the length tension relationship shifted rightward due to stretch training, as reported in eccentric training (Brockett et al., 2001, Proske & Morgan, 2004), the length at which the sarcomeres would begin to produce force would be shifted rightward as well, to longer lengths and recorded as a decrease in force at more plantar flexed ankle angles. This was observed at joint angles of 100º, 110º and 120º, providing evidence that stretch training impacts the length tension relationship similarly to eccentric training and is also indicative of increased MTU compliance.

At 80º (dorsi flexed) voluntary force decreased at week six while muscle fascicle length was significantly enhanced. A dorsi flexed ankle angle stretches the muscle fibres and increases the contribution of passive tension to total tension as the non-contractile units of the muscle fascicles and sarcomeres were stretched. A rightward shift of the length tension relationship would allow the dorsi flexed ankle angle to sustain higher force over longer lengths. The rise in passive tension in this position also compensated for the increased length of the muscle fascicles at the onset of training but as the stretch training progressed the passive tension of the entire MTU was reduced resulting in a lesser contribution to total tension. Over time this would

decrease the ability of the muscle to produce force and at week six force was reduced at 80°. At larger ankle angles where there is a lesser contribution of passive tension to total tension to begin with, the reduction in force was evident earlier.

The 3.3% decrease in force across all ankle angles was accompanied by a reduction in pennation angle at week three; however, the pennation angle did not differ from baseline at the end of the stretch training and force was decreased by 6.4%. Smaller pennation angles are associated with longer muscle fascicles and allow a greater percentage of force transmission per fascicle to the tendon (Fukunaga et al, 1997). As stretch training shifted the length tension relationship rightward and sarcomeres were added in series optimal length for force production would be longer but the contractile components of the sarcomeres could be damaged and therefore be of a lesser quality to produce force. This would cause a greater reliance on the passive tension producing components of the sarcomere to increase total tension (Morgan, 1990). The smaller pennation angles at week three could have been an attempt to transmit more force per fascicle, compensating for injured sarcomeres and increased MTU compliance. Force still decreased from baseline, but not as severely as at week six where no change in pennation angle was recorded and MTU compliance was the greatest indicating pennation angle compensation is important for sustaining force in accordance to stretch training and the length tension relationship.

To date an increase in muscle size has not been observed following stretch training in humans; however, animal studies have reported muscle hypertrophy following stretch training (Barnett et al., 1980, Holly et al., 1980, Sola et al., 1973). Six weeks of extensive overload stretching in this study resulted in a 10.3% increase in muscle depth at three weeks which was long before the accepted time frame for resistance training induced hypertrophy, but within the

timeline for eccentric training. Increase in muscle size following eccentric training is associated with repetitive damage to muscle fibres generated by supramaximal loading of the muscle and shifting to the descending limb of the length tension relationship. The initial phase of damage can include disrupted sarcomeres, altered calcium kinetics, and damaged cellular membranes (Friden & Lieber, 2001, Peake et al., 2005) all of which stimulate immune and metabolic adaptations which lead to protein incorporation at the damaged sites and signalling of hypertrophy (refer to Question 1) (Higbie et al., 1996). Muscle size is associated with force in a semi-linear relationship (Dix & Eisenburg, 1990) but this was not evident following stretch training suggesting that the muscle underwent hypertrophic adaptation but this did not translate to an increase in muscle force as expected from resistance training.

The length tension relationship will eventually shift back to it's original position in accordance with optimal sarcomere length (Leonard & Herzog, 2010); an effect noted after just two days of rest post eccentric training (Brockett et al., 2001). The stretch training was applied daily for five consecutive days making the leftward shift most relevant after the two days of rest given weekly in this study and was observed in the pain scale recorded at the onset of each stretching session. Day 7, 17, 22, and 27 were indicative of the pain created from the sessions immediately after the two days of rest (6, 16, 21, and 26). If healing and restoration of the length tension relationship had occurred over the rest days, the stretch training would be more painful as the sarcomere lengthened and shifted to the descending limb of the length tension relationship, where damage is known to occur. Furthermore, the sessions immediately after rest days were reported to be have the least pain associated from the previous stretch session performed prior to the two days of rest.

The normalized EMG to M wave amplitude also decreased over the stretch training period and this has previously been reported for acute bouts of static stretching and eccentric training (Behm et al., 2001, Fowles et al., 2000). An acute decline in force and muscle activity was also reported through reflex mediated responses of type IV nociceptor afferents in situations when pain is present (Farina et al., 2005, Rutherford et al., 1992). Subjects reported consistent levels of discomfort during each stretch training session but progressively less muscle pain over the course of the six week training intervention. Although the decrease in self-reported pain might be associated with increased tolerance to the stretch (Magnusson et al., 1996) decreased motor unit discharge rates and muscle activity have been reported during movement when pain is present (Farina et al., 2005). In this study, pain from the previous stretch session may have decreased the EMG amplitude collected at the assessments. It remains more likely however, due to no change in the VA that the EMG decline was resultant to the force decline which was mediated through peripheral rather than central mechanisms.

A decrease in H-reflex amplitude has been reported after stretch training in humans (Guissard & Duchateau, 2004) indicating a reduced transmission from the motor neuron pool. The H/M ratio was unchanged in this study; however, when taken separately the M wave amplitude increased at week 3, 6, and 7 while the H amplitude decreased at each time point, although not significantly. The M wave amplitude is affected by the excitability of the sarcolemma (Farina et al., 2005) and modifications to the membrane ionic activity (Pensini et al., 2002) where the H reflex is associated with the excitability of the motor neuron pool (Guissard & Duchateau, 2004). In animals, stretching damages the sarcomeres in-part through increased extracellular calcium from disruption of the sarcoplasmic reticulum (Dix & Eisenburg, 1990). It was likely that the observed M wave potentiation could be due to an imbalance in calcium which

would increase the coupling ability of the myosin light chain (Tillin & Bishop, 2009) and alter membrane ion activity as reflected by the increase in M wave amplitude. This would also impact the twitch contractile properties as intracellular calcium is responsible for the potentiation observed in evoked twitches after a pre-conditioning contraction (Tillin & Bishop, 2009). In animal studies, detachment rates of the actin and myosin cross bridges were also identified in slowing relaxation times which was due to prolonged attachment of the contractile units of the sarcomere (Raisser & Herzog, 2005). Following 30 stretch training sessions the faster relaxation times suggest a faster release of the contractile components, likely due to the increased length of the sarcomeres induced by stretching and less overlap of actin and myosin. Increased sarcomeres in series would further increase the rate of contraction as longer fibres with smaller pennation angles, like those found in this study after stretch training, transmit force more quickly (Monti et al., 2001) and would result in faster time to peak tension of the twitches.

A curious finding in this study was that the non-stretched leg, similar to the stretched leg, increased in size but decreased in force at seven weeks. These adaptations suggest that the non-stretched leg compensated for the abrupt increase in muscle size and decrease in force of the stretched leg. Gait was not measured in this study; however, the plantar flexors were significantly weaker after just three weeks of stretching, and studies have suggested adaptation of bipedal gait to unilateral weakness (Kerrigan et al., 1998, Sousa et al., 2015). Thus, the untrained leg likely experienced modest training through adaptive overload and there was a compensatory increase in size and strength to match the stretched trained leg and promote a more typical gait pattern. This warrants further investigation and could expose a structural cross education of the legs based upon adaptive gait patterning.

The results of this study indicate adaptation to stretch training creates increased muscle size but decreased force and was unlike that of resistance, or even eccentric training. This uncoupling was attributed to alterations to the length tension relationship and the excessive lengthening of the individual muscle fascicles without a concomitant increase in pennation angle. The length tension relationship is pivotal in generation of force and when altered through stretch training the impact on force production was more prominent at ankle positions which promote less passive tension and compromise actin and myosin overlap. The decrease in EMG is a normal adaptation to decrease in force and the changes to the reflex activity, and twitch contractile properties suggested peripheral adaptation as they are likely the result of altered calcium kinetics, membrane sensitivity and actin and myosin overlap. The next step in human stretch training induced force adaptation is defining the time course and mechanism of force decrease and gaining an indication of whether the principle of overload can be successfully applied to stretch training to increase muscle size without compromising the length tension relationship.

Chapter 4: Discussion of Thesis

The purpose of this thesis was to evaluate the effect of a stretch training regime on human skeletal muscle hypertrophy in the gastrocnemius AT MTU. It was necessary to investigate this purpose over two separate chapters to clearly examine muscle hypertrophy and the differential adaptation of the LG and MG architecture to overloaded stretch training at a fixed ankle angle and the separate effect of stretch training on muscle force in accordance to the length tension relationship over multiple ankle angles.

Question one evaluated whether six weeks of stretch training of the plantar flexors induced muscle hypertrophy of the gastrocnemius and altered muscle fascicle length and pennation angle non-uniformly across the LG and MG but left the tendon unaffected. Further the purpose was to examine whether changes in size and architecture enhanced plantar flexion force. I hypothesized that stretch training would be a strong enough stimuli to increase muscle depth and force without altering the tendon length or depth. It was also hypothesized the muscle fascicles would lengthen and pennation angles would decrease at both the MTJ and muscle belly, but this response would be non-uniform across the LG and MG.

Question two examined the effect of stretch training on the neural, muscle architectural, and force characteristics of the plantar flexors at five different lengths of the MTU imposed by changing the ankle angle. I hypothesized that stretch training would shift the length tension relationship of the gastrocnemius AT MTU and result in reduced voluntary force without changing voluntary activation or reflex activity. I further hypothesized that the rates of the twitch contractile properties would be faster and there would be an increase in muscle size and length of muscle fascicles.

The main findings of this thesis supported the hypotheses that stretch training increased the MG and LG muscle fascicle lengths and decreased the pennation angle only for the LG. Stretch training had minimal effect on the tendon and force production at a 90º ankle angle. However, when tested over five different ankle angles, stretch training induced its greatest detriment in force production at shortened muscle lengths, when the ankle was more plantar flexed, and the reduction in force was not due to muscle activity. Through the use of instantaneous EMG monitoring during stretch training muscle activity was assessed to ensure that passive stretching occurred rather than active muscle contraction as a result of the muscle being loaded and lengthened at the same time. Thus, results from this study were attributed to passive stretch training rather than lengthening resistance exercise. The weighted stretch training protocol used also successfully applied the principle of progressive overload. This was the first study to apply this principle to human stretch training, and the first study to demonstrate muscle growth and architectural changes not previously reported as well as alterations to the length tension relationship.

The combined findings from these two central Questions specified that stretched muscles undergo hypertrophic adaptation. Even with shifting of the length tension relationship and promoting less than optimal overlap of actin and myosin, force was decreased but, muscle depth was still larger across all ankle angles. Along with increased depth muscle fascicle lengths increased which further provides evidence of hypertrophy (Abe, 2000, Kearns et al., 2001). The metabolic routes proposed in Question one as the means through which muscle size increased also applied to Question two as the stretch training was the same but the effect evident across all joint angles considered. This was the first study in humans to report longer fascicle lengths after stretch training, and the first to our knowledge to observe increased fascicle lengths at rest in

both the junction and the muscle belly. A novel technique in this thesis which strengthened the measures of the muscle fascicles and pennation angles at the MTJ was the identification of the MTJ through use of a 3D/4D ultrasound probe. This technology made the MTJ easily recognizable in three planes, thus the results were not due to probe placement or positional assignment of the MTJ.

The positions studied in Question two were selected over five specific ankle angles to target various points along the length tension curve. At these ankle angles force decreased even at 90º following stretch training. In Question one, where force was measured at 90º as well, there was no change as a result of stretch training. Placing the ankle at 90º caused the plantar flexors to be slightly stretched. This created a rise in passive tension that compensated for the lengthening of the muscle fascicles. Question one demonstrated the importance of pennation angle compensating for increased fascicle length, uncoiling of the fascicles in the LG at the attachment to the AT, and decreased passive stiffness in order to sustain force. Question one also identified muscle specific architectural adaptations in the LG and MG and demonstrated that the effect of stretch training was more evident in the LG over the MG, although both increased in muscle fascicle length. This suggests that when muscle force at different joint angles is an outcome variable the pennation angle should be considered separately in the LG and MG along with muscle fascicle lengths.

In Question two the LG and MG were not evaluated separately but given the results of Question one and the significant architectural alterations that occurred in these muscles to preserve force, a decrease in force in Question two at the same ankle angle as in Question one where force did not decrease was attributed to lack of change in pennation angles. The length

tension relationship was unable to be preserved through architectural compensation thus resulting in a decrease in force across the ankle angles.

What was difficult to explain was the decrease in EMG activity without a reduction in VA. Beyond nociceptor feedback, which may have unconsciously inhibited effort, there was no peripheral basis for a decrease in central drive. However, as the subjects reported progressively less pain across the 30 stretching sessions in Question two and no decrease in VA was recorded it was likely that muscle activity did not change as a result of central changes brought about by stretch training. The longer fascicles would not directly influence EMG activity nor would changes to the pennation angles, and in fact with the increase in muscle size one would expect greater muscle activity (Kearns et al., 2001). This indicated a potential decrease in the quality of the muscle over the course of the stretch training (Friden & Lieber, 2001). As mentioned in both Questions, damage to the structure within and surrounding the muscle fascicles was likely induced through the stretch training but also necessary to facilitate the metabolism required to increase muscle size. Damage to the extra cellular matrix from eccentric training has been linked to highly elongated sarcomeres which promote less than optimal overlap of actin and myosin and in turn less force in accordance with the length tension relationship (Lieber et al., 2003). However, the damage also alters calcium kinetics through increased intracellular calcium levels (Bryne et al., 2004). An increase in calcium impaired excitation contraction coupling and initiated inflammation (Gosselin & Burton, 2002), which would decrease the force producing capabilities of the muscle.

EMG and force production have a near linear relationship (Onishi, et al., 2000). If the force decreased due to mechanical adaptation and alterations in the length tension relationship it was likely that force per motor unit decreased and thus the EMG decreased as a compensation to

architectural and structural changes in the muscle and not the other way around. As EMG was not a primary outcome variable in Question one it is unknown whether the compensation in pennation angle observed was significant enough to sustain EMG along with the force at 90º. But, the findings from Question two indicated that EMG was likely maintained in Question one.

Chapter 5: Conclusions and Recommendations

5.0 Conclusions

Muscle depth and mass were recorded for 21 young males, ten of which served as controls, to assess whether stretch training induced muscle hypertrophy. Thirty stretch training sessions, and five experimental assessments were collected over the course of seven weeks. Ultrasonography, force dynamometry, EMG, twitch interpolation and DXA scans were utilized to collect structural measures of muscle architecture and force and neural measures of central adaptation to stretch training. Overall the primary purpose of evaluating stretch training on muscle hypertrophy, architecture and nervous system activity in human muscle was accomplished. The global hypothesis that stretch training was a great enough stimuli to induce muscle hypertrophy was accepted, as were the two hypothesis that arose from the original purpose and created two distinct Questions. The hypothesis that muscle fascicles would lengthen, pennation angles would decrease and the tendon would remain unchanged as the muscle increased in size was demonstrated in Question one and accepted. The hypothesis that stretch training would decrease muscle force and EMG activity with no change in voluntary activation through impacting the length tension relationship was accepted in Question two.

5.1 Limitations to Research

Although the results garnered from this research provided novel findings in relation to stretch training in humans, there were some limitations to the study. The muscle demonstrated hypertrophic adaptation in the stretched leg by week three. An assessment between week three and week six could have helped better identify the turning point where the greater muscle growth measured at week three rebounded to a decrease detected at week six.

The increase in calf circumference immediately following stretch training was likely a hyperemic response to inversion rather than stretch loading. To better quantify the cause of this increase in circumference measurement of the non-stretched leg would allow dissociation between stretching and inversion. Moreover, plethysmography or ultrasound during and after the stretch training would have allowed for quantification of blood flow. These measures may have provided insight into the hyperemic response and potentially inflammation. To further investigate the potential role of inflammation, inflammatory markers should have been assessed through techniques that involve a blood draw or muscle biopsy. The latter which was not feasible at the time of this study.

5.2 Directions for Future Research

The results of this study are the first to suggest stretch induced muscle hypertrophy in humans as indicated by an increase in muscle depth, circumference, mass and fascicle length; however, without a muscle biopsy to confirm an increase in the size of the muscle fibres it was difficult to conclusively state the cause of the hypertrophic response. Thus, future research in this field must include a measurement of muscle fibre hypertrophy collected through muscle biopsy in order to substantiate these fascicle findings.

Further these data recommend stretch training deviates from the resistance training model. Ideally a study should be conducted as a comparative between eccentric training and stretching induced by a passive lengthening to clearly delineate differences in stretch and eccentric induced adaptations in the nervous and muscular systems to training.

Finally, studies should evaluate females, older adults and a non-athletic population to define the applicability of these finding beyond young active males. The theme of compliance was integral to this study and with female hormones (Bryant et al., 2007) and aging (Onamabele

et al., 2006) there is an increase in compliance. These factors influence the stretch response and therefore further research is required to ascertain whether stretch training is applicable to an already tendon compliant population.

In the literature the effect of stretching is deemed beneficial for performance, range of motion, and function (Alter, 1996, Guissard & Duchateau, 2004, Stanley & McNair, 1996, Stewart & Sleivert, 1998, Wienmann & Hahn, 1997, Young & Elliot, 2001); however, some reports suggest that stretch can have detrimental effects on force production (Behm et al., 2001, Kokkonen et al., 1998). Regardless of the equivocality of scientific outcome there are 70,000 yoga instructors and over 16 million yoga practitioners in North America as of 2008. The primary perturbation in yoga is a series of sustained, deep stretches which are targeted at flexibility, posture, range of motion and improvement of strength and body composition. Interestingly, there is no literature that defines a physical benefit to yoga in terms of body composition; however, many studies identify health and psychological advantages (Hartfiel et al., 2011, John et al., 2007, Peck et al., 2005, Williams et al., 2009). If stretch induced hypertrophy is a possibility then yoga and stretching could be offered as an alternative to traditional strength training as a way to maintain and build muscle mass which is not only important in the general population but also in the aging and frail population. There are many older adults that struggle to remain independent due to age related loss of muscle mass that impairs functional ability (Fried et al., 2001). Along with normal muscle loss, a host of co-morbidities and social issues may dissuade older adults from strength training. With proper training and safety measures, it is possible that older adults might be able use stretching as an alternative to strength training and in doing so, develop or maintain muscle mass.

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Appendix A: Ethics Approval

The University of British Columbia
Okanagan
Research Services
Behavioural Research Ethics Board
3333 University Way
Kelowna, BC V1V 1V7 Phone: 250-807-8832
Fax: 250-807-8438

CERTIFICATE OF APPROVAL - MINIMAL RISK

PRINCIPAL INVESTIGATOR:	INSTITUTION / DEPARTMENT:	UBC BREB NUMBER:
Jennifer M. Jakobi	UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences	H13-03377

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Okanagan

CO-INVESTIGATOR(S):

Kaylee Larocque

SPONSORING AGENCIES:

N/A

PROJECT TITLE:

Stretch induced muscle hypertrophy in human skeletal muscle

CERTIFICATE EXPIRY DATE: March 10, 2015

DOCUMENTS INCLUDED IN THIS APPROVAL:	**DATE APPROVED:** **March 10, 2014**	
Document Name	**Version**	**Date**
Protocol:		
Overview of Literature and Methods	**01**	**March 4, 2014**
Consent Forms:		
Letter of Consent	**1**	**February 25, 2014**
Advertisements:		
Recruitment Poster	**N/A**	**March 4, 2014**
Questionnaire, Questionnaire Cover Letter, Tests:		
Lifetime Physical Activity Questionnaire	**1**	**February 25, 2014**
Letter of Initial Contact:		
Letter of Initial Contact	**1**	**February 25, 2014**

The application for ethical review and the document(s) listed above have been reviewed and the procedures were found to be acceptable on ethical grounds for research involving human subjects.

Approval is issued on behalf of the Behavioural Research Ethics Board Okanagan