Proximal vs. Distal Fascicle Behavior within the Biceps Femoris Long Head at Different Muscle Activation Levels

by

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Hamstring strains have been shown to occur more often in the long head of the biceps femoris (BFLH) than the semitendinosus and semimembranosus, with most injuries occurring in the proximal half of the BFLH. Muscle modeling has shown significantly greater tissue strains in the more proximal regions compared to more the distal regions of the BFLH. These data suggest there are regional differences in fascicle behavior between the proximal and distal fascicles of the BFLH during contractions. The purpose of this study was to assess the magnitude of shortening of the proximal and distal fascicles in the long head of the biceps femoris under different muscle activation levels. This study tested the hypotheses that 1) the proximal fascicles will be longer, and 2) the proximal fascicles will undergo greater shortening than the distal fascicles when the muscle is at various activation levels.

Subjects were 11 young (age: 21.3±1.8 yrs., height: 167.9±10.0 m, and mass 65.9±10.6 kgs.) males and females who resistance trained and were non-collegiate athletes. Longitudinal ultrasound images were taken of the BFLH at rest (hip and knee at 0°) and during sustained isometric contraction levels of 10, 25, 50, and 75% MVIC, while prone on a dynamometer with

hip and knee flexed to 45°. BFLH and ST/SM activation during ramp trials were correlated and used to predict BFLH activation during subsequent submaximal trials. Through a combination of linear regression analyses and repeated measures ANOVAs, the results showed that the proximal fascicles were longer (3.24 cm, $P<.001$) and shortened ~44% more than their distal counterparts, on an absolute level ($P<.001$). The presence of a region by condition interaction also showed the proximal fascicles had significant incremental shortening from the passive to 50.4% activation, while the distal fascicles only had significant incremental shortening from passive to 20.6% activation ($P<.05$). Once normalized to the resting lengths, the analysis of strain showed both regions underwent significant shortening ($P<.001$), however, the proximal fascicles did not undergo more shortening than the distal fascicles ($P=.72$).

The results of the group data do not agree with heterogeneous architecture behavior in previous literature. However, qualitative analyses of individual subjects show the presence of two types of heterogeneous regional fascicle behavior that was not present when averaged as a group. Some subjects showed greater strain magnitudes in the proximal or distal regions, while some subjects had seemingly equal amounts of strain in both regions. Also, some subjects reached much higher magnitudes of strains than other subjects, ranging from .09 to .33.

In conclusion, the data support our first hypothesis that the absolute lengths of the proximal fascicles are longer and shorten more than the distal fascicles. The proximal fascicles had greater absolute shortening, however, once normalized to resting fascicle length the proximal fascicles did not undergo more strain than the distal fascicles, i.e. the *behavior* differences between regions was not present in strain measurements. Thus, our second hypothesis was supported by the absolute length changes but rejected by the normalized length changes. The "qualitative" individual subject heterogeneity depicts the need for further investigation on the possibility of strain variability between subjects and within the BFLH itself, as well as the need for this type of investigation during more dynamic movements. Further knowledge of this commonly strained muscle’s regional behavior during dynamic movements could provide clinical evidence of proximal hamstring strain predisposition.

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Proximal vs. Distal Fascicle Behavior within the Biceps Femoris Long Head at Different Muscle Activation Levels

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Chapter 1: Introduction

Hamstring strains commonly occur in sports involving sprinting (Hoskins 2005) and have been found to occur most often in the long head of the biceps femoris (BFLH) (De Smet et al. 2000; Connell et al. 2004; Malliaropoulos et al. 2010). Recent research has also found the proximal region of BFLH to sustain more injuries than the distal region (Askling et al. 2006; Silder et al. 2008). During sprinting, the hamstring muscles undergo active lengthening, eccentrically contracting, to slow the lower leg down after forward swing. In the late swing phase of sprinting the hamstrings are stretched to their greatest extent compared to standing upright lengths (Thelen et al. 2005). Though all of the hamstrings produce their greatest length changes during this time, the BFLH was shown to undergo the most stretch compared to the semimembranosus and semitendinosus, while actively slowing down the movement of the swinging limb. Motion capture of hamstring strain injuries determined that the injury occurrence was during the late swing phase (Heiderscheit et al. 2005; Schache et al. 2010). Stretching the muscle has also shown to have influence on total torque production in the hamstrings (Lunnen et al. 1981; Mohamed et al. 2002). As hip flexion angle increased, which stretched the hamstrings, the hamstrings were able to produce greater torque. The highest torque producing positions were similar to those that occur during the late swing phase in sprinting (≈hip flexion 70° knee flexion 45°).

In passive states, the muscle fascicles of the BFLH have been shown to undergo a significant amount of stretch as the hip flexion angle increases, with knee flexion angles held constant, from resting length (Chleboun et al. 2010). Either the eccentric contraction to slow down the swinging leg or the stretch the hamstring muscles undergo during the late swing phase could be the determinant of hamstring strains. However, muscle strain during eccentric contractions has been shown to be the primary determinant of muscle damage (Lieber and Friden 1993). The high frequency of strains in the proximal BFLH in the late swing phase of sprinting could be due to the active lengthening by the BFLH. Though we have an understanding of how the hamstrings function during sprinting, we still do not know why hamstring strains are occurring more in the proximal region of this muscle.

Regional behavior differences in muscles have been shown in biarticular muscles (Ahn et al. 2003; Blemker et al. 2004; Rehorn et al. 2010). The American toad showed heterogeneous strain within the semimembranosus across varying hopping distances (Ahn et al. 2003). The proximal and central segments of the semimembranosus exhibited higher strain compared to the distal segments throughout all hopping distances. Muscle modeling of the biceps brachii found non-uniform strain patterns throughout the muscle (Blemker et al. 2004). The variation in strain throughout the muscle is believed to be due to the differing fascicle lengths and curvatures located in the biceps brachii, a biarticular muscle (Blemker et al. 2004). Regional strain variation has also been seen in the BFLH through MRI and muscle modeling (De Smet et al. 2000; Silder et al. 2010; Rehorn et al. 2010). Imaging of the proximal region of the BFLH found larger amounts of strain in the regions closest to the proximal musculotendinous junction compared to more distal regions (Silder et al. 2009). Muscle modeling of the entire BFLH predicted non-uniform strains within the muscle (Rehorn et al. 2010). The proximal half of the BFLH reported greater strains than the distal half, and was attributed to the narrower width of the aponeurotic tendon in this region compared to the distal half. Although variation of strain has been observed in animals and predicted through modeling techniques assuming variant fascicle lengths, we do not have evidence of heterogeneous regional (proximal vs. distal) fascicle behavior in active human muscles, particularly in the long head of the biceps femoris, the most common hamstring muscle injured during sport-related activities.

Hypothesis

Hamstring strains are shown to be more prevalent in the BFLH, particularly in the proximal region. Though length changes of the fascicles of the BFLH due to lengthening of the muscle have been shown by ultrasound, we do not have an understanding of regional variations under active contraction conditions. We hypothesize that 1) the fascicles in the proximal half of the BFLH will be longer than the distal fascicles, and 2) the proximal fascicles will undergo greater shortening than the distal fascicles when the muscle is at various activation levels.

Statement of Purpose

The purpose of this study was to assess the magnitude of shortening of the proximal and distal fascicles in the long head of the biceps femoris under activation levels ranging from passive to 67% maximum muscle activation.

Significance

Determining regional fascicle behavior in the long head of the biceps femoris shows how the biceps femoris muscle functions during contraction in greater detail. Heterogeneity of fascicle behavior could mean the two regions of the muscle do not produce the same amounts of force or undergo similar amounts of strain during stretch. This study could enhance our understanding of this possible mechanism behind strain injuries to the proximal region of the biceps femoris muscle.

Delimitations

1. All subjects will be healthy, with no history of hamstring strains.
2. Subjects will be young adults between the ages of 18-25.

3. Subjects must resistance train at least 3 times per week.
4. Subjects must have no excessive amount of adipose tissue on their lower extremities, which would not allow for adequate image quality i.e. visually examine and measure fascicle lengths.
5. Testing will be only in isometric contractions due to the inability of capturing longitudinal ultrasound images of full fascicles on moving limbs.

Limitations

1. Isometric contractions differ from eccentric contractions seen in the late swing phase of sprinting and BFLH injuries, however previous research has shown similarities between fascicle lengths measured in the two contraction types (Reeves et al. 2003). We are also assessing lengths at different muscle activation levels, so that we may have a better understanding of fascicle behavior over a spectrum of activations.
2. Individual fascicle lengths can vary the further proximal or distal the measurements are taken, but we will be taking 2 fascicle length measurements within each region to give a regional representation.

Operational Definitions

1. Isometric Contractions – Contractions in which the muscle-tendon unit length does not change. However, during these contractions the muscle fascicles undergo a shortening contraction. In the text, isometric contractions will refer to a shortening contraction of the muscle fascicles.
2. Proximal/Distal Region – the BFLH will be divided into 2 equal halves (proximal and distal) and measurements of fascicles will be of those that originate in each region.

3. Muscle Fascicle – Several muscle fibers bundled together and surrounded by perimysium represent a muscle fascicle, measured from the superficial muscle tissue to deep aponeurotic tendon.
4. Muscle Belly – the thickest portion of the muscle, usually in the middle of the muscle, measured from the superficial muscle tissue to deep aponeurotic tendon.
5. Musculotendon Unit – The combined muscle length and the tendon attaching the muscle to bone.
6. Aponeurotic Tendon – A deep fibrous connective tissue that acts as an extension of the external tendon, to muscle fascicles insert.
7. Pennation Angle – The angle at which muscle fascicles insert onto the deep aponeurotic tendon.

Chapter 2: Review of Literature

The purpose of this study was to assess the magnitude of shortening of the proximal and distal fascicles in the long head of the biceps femoris under different muscle contraction levels. This review of literature will cover: 1) The occurrence and location of muscle strains 2) Hamstring muscle function, 3) Evidence of heterogeneity of fascicle behavior in biceps femoris, and 4) Summary.

Occurrence and Location of Muscle Strains

The hamstrings are the most injured muscles in athletes who participate in sports that involve sprinting, particularly: soccer, sprinting, and rugby (Woods et al. 2004; Brooks et al. 2006; Haggled et al. 2008). These injuries cost athletes playing time and require varying recovery periods. Of the hamstrings group, several previous studies have pinpointed the biceps femoris long head (BFLH) as the most injured muscle (De Smet et al. 2000; Connell et al. 2004; Malliaropoulos et al. 2010). Furthermore, studies also show the proximal section of the BFLH to sustain the most injuries compared to their distal counterparts (De Smet et al. 2000; Askling et al. 2006; Silder et al. 2008). Determining a more definitive location of hamstring strains is essential in developing a better understanding of how and why strain injuries are occurring.

Hamstring injuries are highly prevalent throughout many different sports and across many nations. These injuries are responsible for sidelining players, and require much attention in rehabilitation (Hoskins 2004). For example, out of 796 hamstring injuries that occurred in football players, 749 (94%) were strains (Woods et al. 2004). Hamstring strains also accounted for 12% of all the injuries that were sustained by all players during a 2-year period. These high occurrence rates are not only prevalent in football. Previous research on 296 rugby players also reported a high occurrence of hamstring injuries (Brooks et al. 2006) Of the 164 cases of hamstring muscle injuries, 68% occurred during sprinting. Further, a total of 96 of these injuries were hamstring strains. The sustained hamstring strains resulted in an accumulation of 1484 (704) days of absence from play of all the athletes. The amount of time players spent off the field means replacing players during that period, which can be costly. These injuries do not discriminate, and occur in both sexes. Reports of both female and male soccer players showed that out of 847 documented injuries, 112 were hamstring injuries (Haggled et al. 2008). The hamstrings have been more highly diagnosed with muscle strains than any of the other muscles injured in these sprinting related sports. But, of more significance, the BFLH has been recently pinpointed as more commonly injured than any of the other hamstrings (Connell et al. 2004; De Smet et al. 2000; Malliaropoulos et al. 2010).

Determining exactly which muscle of the hamstrings is most commonly injured could make an impact on how we treat, or even preempt the occurrence of these strains. The BFLH has been well documented as having the highest occurrences of hamstring strains (Connell et al. 2004). Research on 60 Australian football players using MRI showed 87% of subjects sustained an injury to their BFLH. These results were much higher than injuries of the ST and SM, which collectively only accounted for 5 injuries. Research in other sports such as hockey, football, and track athletes found similar results of the high frequency of BFLH strains compared to the other hamstring muscles (De Smet et al. 2000). Out of a total of 15 injuries, 6 were isolated BFLH strains, and 5 were primary with a secondary injury of the semitendinosus. Ultrasound techniques on muscle injuries have become more prevalent, and have shown the BFLH as the predominantly injured muscle (Malliaropoulos et al. 2010). Of 90 injuries assessed by ultrasound, 68 (75.6%) were located in the BFLH. Though recent research has shown hamstring strains occur more often in the BFLH than any other muscle, further study is needed to figure out why strain injuries are occurring more often in this particular muscle and whether the muscle injuries are sustained in a particular region within the BFLH.

Injuries in the hamstrings have commonly been referred to as high hamstring strains, depicting that the proximal portion of the hamstrings as the site of injury. MRI studies have begun to focus in on the proximal location of hamstring injuries within the muscles (Silder et al. 2008; Askling et al. 2006). Imaging on 14 athletes, who had been previously diagnosed with a hamstring injury that required at least two weeks' worth of time out of their sport, showed ≈85% of the subjects sustained BFLH-only hamstring injuries, and 1 subject had dual BFLH and ST injuries (Silder et al. 2008). Following these results, they found that ≈57% sustained a proximal BFLH injury, with ≈35% of those being multiple site injuries, totaling 14 proximal strains to the BFLH. High hamstring strains of BFLH injuries are also well documented in sprinters, who stated that they occurred when they were near or at their maximal speeds (Askling et al. 2006). MRIs of the injuries of 18 sprinters showed all injuries occurred in the BFLH and ≈55% were sustained in the proximal portion of BFLH. Previous findings were also consistent with these, reporting that 11 injuries in the BFLH, of which ≈54% were sustained only in the proximal portion. These observations were made using the origin short head of biceps femoris on the femur as the boundary for proximal and distal halves (De Smet et al. 2000). Research studies have shown the proximal portion of the BFLH seems to be the primary site of strain injuries occurring during sprinting, however, we currently do not have a full understanding of why hamstring strain injuries occur more often in this region of the muscle.

Distinctions have been documented of the higher incidence of hamstring muscle strains in the BFLH than the other hamstring muscles (De Smet et al. 2000; Connell et al. 2004; Malliaropoulos et al. 2010). Furthermore, it has been reported that the majority of these injuries are sustained in the proximal region compared to the distal region (De Smet et al. 2000; Askling et al. 2006; Silder et al. 2008). With such a significant frequency of hamstring strains, particularly in the proximal portion of the BFLH, it is clear we need a better understanding of what makes this muscle so susceptible to injury. Examining hamstring muscle function during injuries and in similar positions to those found in sprinting could provide insight into the mechanism of hamstring strains.

Hamstring Muscle Function

Determining when, particularly phase of movement and timing within phase, hamstring muscle strains occur could allow for preparation and training to decrease the frequency of injury. The behavior of hamstring muscles during injuries has been studied as an injury occurred (Heiderscheit et al. 2005; Schache et al.2010). These studies show the late swing phase, when the hamstrings are actively lengthening, to be the time of injury. Also, studies have shown evidence of the hamstrings developing the most amounts of torque in more lengthened positions, which mimic the muscle lengths that occur during active lengthening found in the late swing phase of sprinting (Lunnen et al. 1981; Mohamed et al. 2002). To understand why hamstring strains are occurring so often in sprinting, we have to examine the phase of movement the hamstrings are being stretched and the activity of the hamstrings when they are being stretched.

When and in what positions do hamstring strains occur

Understanding when the hamstrings are stretched during sport related movements could allow us to develop a better approach in prevention and analysis of hamstring injuries. The full sprinting gait involves stretching and shortening of the hamstring muscles (Thelen et al. 2005; Yu et al. 2008). During the late swing phase in sprinting, the hamstrings' responsibility becomes that of slowing the swing of the lower leg down, which is done by active lengthening or eccentric contractions (Yu et al. 2008). Along with these eccentric contractions occurring in the hamstrings, the hamstrings were also shown to be at their peak stretch lengths during the late swing phase. Peak muscle lengths also coincided with the largest amount of muscle activity, 2 to 3 times greater than stance phase activation. This increased stretch of the hamstrings during the late swing phase has been noted by other research (Thelen et al. 2005). While 14 athletes sprinted on treadmills, hamstring mechanics were obtained through motion capture and simulations. A 3-d model created from the data and allowed for computation of joint angles and muscle-tendon lengths. The model showed the semitendinosus and semimembranosus were stretched an average of 8.1% and 7.4%, respectively, past their standing upright lengths during late swing phase while the BFLH were stretched the most at 9.5% past standing upright length. The greatest stretch of the muscles occurred during the late swing phase while the hip was flexed approximately 65° and the knee was approximately 45°, with the BFLH reaching a length of 1.1 times its normal resting length during standing. The increased stretching of the BFLH during the late swing phase could be a strong determinant of hamstring strains.

While normally it is very difficult to obtain data from an injury as it occurs, 2 studies were able to do so (Schache et al. 2010; Heiderscheit 2005). Both studies showed that during an acute hamstring strain the hamstrings were stretched to their greatest lengths at the proposed time of injury, and it occurred while the muscles were eccentrically contracting. Significant differences between the pre- and post- injury trials muscle mechanics were noted in one study as: peak force, lengthening velocity, and negative work (Schache et al. 2010). Specifically, peak force (F/kg) was greatly increased during the late swing phase during the injury trial, from

46.54 (4.28) in the pre injury trials to 49.56 during the injury, lengthening velocity (m/s) was reduced from 0.52 (0.08) to 0.30, and negative work (J/kg) -0.69 to -0.20. While this study provides evidence of mechanics of a hamstring strain during sprinting, showing the hamstrings of the injured leg had a reduction in capacity to do negative work due to the lower peak lengthening velocity, this study did not determine or predict a timing of injury. In a similar study, a hamstring strain injury was observed during motion capture and the timing of the injury was predicted. The predicted timing of injury was during the late swing phase, when the hip was flexed to approximately 69° and the knee was flexed to 58° (Heiderscheit 2005). In this study, the BFLH, ST, and SM models during the late swing phase reached peak lengths of 12.2%, 9.8%, and 10.4% greater than those of their upright positions, respectively. These results lead to the conclusion that the BFLH was the most susceptible to injury during the late swing phase of sprinting, when the magnitude of stretch is the greatest. To further understand how the hamstrings function at different lengths, we will examine the muscle in more controlled settings than in sprinting movements.

Hamstring function during stretch

The hamstrings are biarticular muscles that span both the hip and knee joints. They undergo stretching by flexion of the hip and extension of the knee joints. By increasing the overall length of the hamstrings close to those found in sprinting, researchers found that maximal isometric torque increased, and the hamstrings required less muscular activity to produce submaximal torques compared to shorter positions (Lunnen et al. 1981, Mohamed et al. 2002). Measured hamstring muscle activity, by EMG, and hamstring torque, on a dynamometer, with varying hamstring lengths through flexion of the hip produced significant changes from extended positions (Lunnen et al. 1981). The research showed flexing the hip 45°

to 90°, while holding the knee at a constant flexion of 45°, generated a significant increase in the torque production during isometric contractions compared to less stretched positions. Muscle activity was also shown to decrease as hip flexion increased during submaximal effort contractions at the same target torque levels. When the hamstrings were at a more lengthened position, hip flexed 90°, the hamstrings were only 30% as active as the shorter position, hip 0°. The significant advantages of the muscle to produce more torque and sustain a submaximal level of torque with less muscle activity were reproduced in a more recent study (Mohamed 2002). A torque production of 716.1 (47.1) kg cm was found at hip position of 90° and knee position of 45°, and was significantly higher than the 246.6 (30.5) kg cm reported with the hip at 0° flexion. A decrease in EMG activity as the hip was flexed past 90° and knee flexed at 0°, compared to hip flexion of 0° and knee flexion of 90°, was found as subjects attempted to sustain a target torque. Both Lunnen's and Mohamed's investigations provide more knowledge of how muscles function when in lengthened versus relaxed states. Their results indicated that as hip flexion increased, the hamstrings could produce more torque and required less muscle activity to sustain submaximal torques, as compared to positions with less hip flexion. Muscles can produce greater amounts of force when in a stretched state due to the non-contractile elements' (i.e. tendons, connective tissues, etc.) addition of passive tension onto the active tension created by the muscle's contractile elements (Mohamed et al. 2002). Since the active muscle and passive tension of the tendon both contribute to the knee flexor torque produced while in lengthened states known to be injurious, it is important to investigate the contractile and passive elements of the musculotendon unit.

The isometric force a muscle produces is dependent upon the muscles length, which is determined by the position of the joints the muscle covers (Gordon et al. 1966). This is commonly referred to as the muscles length/tension relationship. When muscles are stretched to very long lengths, or in a much shortened state, muscles produce low amounts of torque or force. When a muscle is placed in an "optimal" length, it can produce much higher torques or forces. At the level of the muscle's contractile unit, the sarcomere, the optimal length is achieved when the maximum number of cross bridges between myosin and actin is present (Gordon et al. 1966). When a muscle is stretched past its optimal length, tendons can be stretched, creating a passive tension that increase torque or force produced when it recoils due to its elasticity. However, there is subject by subject variability in how the force-length curve is expressed, and variability varies from muscle to muscle (Winter & Challis 2010). The overall concept of the sarcomere length-tension relationship has been predicted in BFLH sarcomeres as a function of changes in hip and knee joint angles (Chleboun et al. 2001). The length/tension relationship of BFLH sarcomeres has been estimated using sarcomere lengths in cadavers with hip and knee in 0° conditions, correlated with measured BFLH fascicle lengths in a variety of joint positions while in a passive state (Chleboun et al. 2001). Based on the figure below, the optimal sarcomere length occurred when the hip and knee are flexed to 90°, with the shortest length at hip 0° flexion and knee 90° flexion, and longest length at hip 90° flexion and knee 0° flexion (Figure 1). These estimations would likely place the BFLH sarcomere lengths during the late swing phase of sprinting (when the hip is in ≈65° flexion and knee is in ≈45° flexion) beyond the optimal range on the descending limb of the graph.

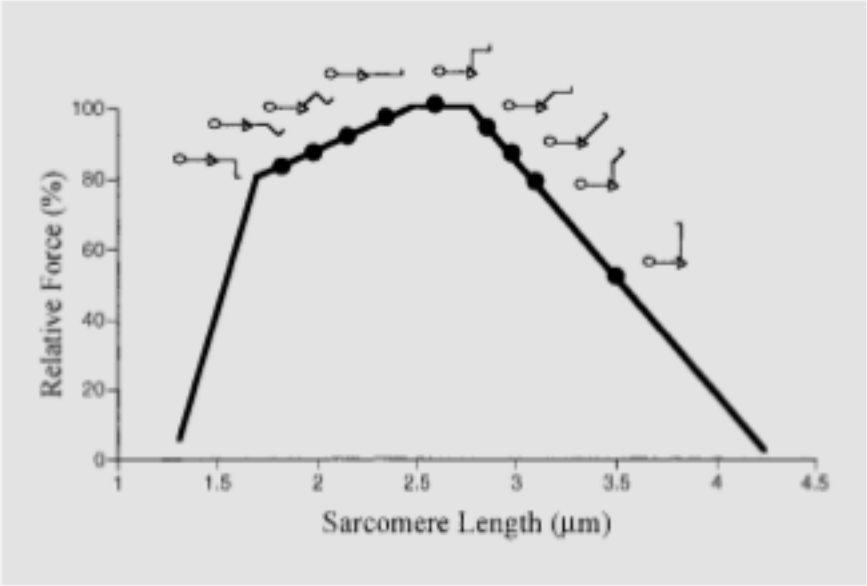

Figure 1. Estimated sarcomere length/tension curve of the biceps femoris long head (Chleboun et al. 2001). The optimal sarcomere length was at hip 90° and the knee 90°. The shortest and longest sarcomere lengths were at hip 0° and the knee 90°, and hip 90° and the knee 0°, respectively.

BFLH fascicles were shown to undergo significant length changes as hip flexion angles increased by ultrasounding the entire BFLH during passive hip and knee flexion (Chleboun et al. 2001). When the hip flexion angle was increased from 0° to 45° and 90°, with the knee flexion angle held at 45°, the fascicles showed a significant amount of stretch (Figure 2).

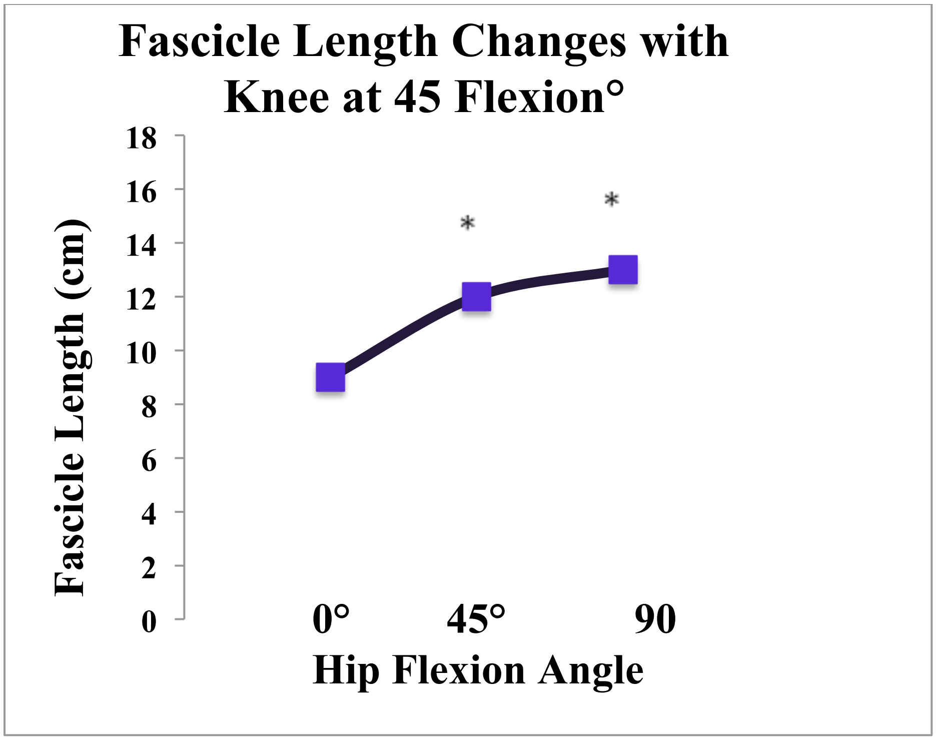

Figure 2. *Fascicle length changes due to hip flexion angle.* This graph depicts the results found by Chleboun et al. 2001 on fascicle length changes due to increase of flexion angles in the hip. The change in fascicle length from hip flexion 0 ° to 45 ° and 90 ° were significant ($p<0.05$).

Fascicle lengths were more sensitive to hip flexion with the knee position held constant than knee flexion with the hip position held constant, due to the larger moment arm at the hip vs. knee for the BFLH. More fascicle length sensitivity to hip flexion angle increases agree with previously estimated BFLH muscle length changes due to the larger hip vs. knee moment arm (Thelen et al. 2005). These data indicate even with a shortening of the BFLH from ~45° knee flexion during the late swing phase, hip flexion to ~70° would lead to a large stretch of the fascicles, similar to the length seen at hip flexed 90°. The significant stretch of fascicles as the entire muscle is stretching during the late swing phase could be a reason why hamstring strain injuries occur in the BFLH.

Hamstring strain injuries could be due to the force required to slow down the leg during the late swing phase, or due to the stretch the BFLH undergoes during the active lengthening. In eccentrically contracting muscle, strain has been shown to be the determining factor of muscle damage instead of force (Lieber et al. 1993). Muscle damage was measured in the tibialis anterior of rabbits through two different strain amounts, 12 and 25% of muscle fiber lengths, and at two different timings, beginning immediately and being delayed 200ms after. By varying the starting time of the strain, the force was significantly higher in the delayed start test. Comparisons of pre- and post contractile properties of the muscle (time to peak twitch tension, rate of rise of twitch and tetanic tension, etc.) showed no significant differences between strain timings. However, there were significant differences in post-contractile properties between strain magnitudes ($p < .001$). These data suggest the large amount of stretch the BFLH undergoes during the late swing phase of sprinting is the primary factor of muscle damage, not the force or torque it produces to slow down the swing leg.

The hamstrings are at their greatest lengths and undergo an active lengthening during the late swing phase of running (Heiderscheit et al. 2005; Thelen et al. 2005; Schache et al. 2010). Stretching the hamstring muscles to increased hip and knee flexion angles allows the muscle to produce more force than in more shortened positions (Lunnen et al. 1981; Mohamed et al. 2002). The higher generated force can be due to a combination of the optimal muscle length and the added recoil of the elastic tendon (Lieber et al. 1993). The hip and knee positions seen in the late swing phase of sprinting seem to stretch the BFLH's sarcomeres, and possibly fascicles, beyond their optimal length, which has been predicted through cadavers and ultrasound imaging (Chleboun et al. 2010). Excessive stretch in the fascicles could be a reason for hamstring strains in the BFLH. The limitation of current simulation studies and investigations of muscle torque and forces in varying conditions is neither evaluates what happens in the hamstrings in vivo, nor do they address regional differences within the muscle. Regional architectural differences within the muscle may help to address the disparity in muscle strains within the proximal vs. distal regions of the biceps femoris long head.

Evidence of Heterogeneity of Fascicle Behavior in Biceps Femoris

The importance of relating changes in muscle architecture, at the fascicle level, to muscle function hasn't previously been investigated as much as fiber type and force length relationships. Recent research has provided insight into architectural differences that occur in several muscles of various animals undergoing active contractions (Ahn et al. 2003; Soman et al. 2004, McGowan et al. 2007). However, in humans, most of the work has been performed on passive state muscles (Chleboun 2010), human cadavers (Kellis et al. 2010), or through muscle modeling (Blemker et al. 2004; Rehorn et al. 2010). Understanding the architectural differences in muscle may help to explain the higher occurrence of strains in the proximal region compared to the distal region of the BFLH.

While muscle models can give noninvasive force and excursion data, it is incorrect to apply strain in a homogenous fashion throughout the entire muscle (Ahn et al. 2003). The semimembranosus (SM) of the American toad showed significantly higher strains in the proximal and central regions than the distal regions, during varied hopping distances. By using sonomicrometry crystals attached within the muscle in proximal, central, and distal segments, the proximal segments strained as much as -15.6 (5.3)%, while the distal segments strained only -6.5 (3.2)% when the muscle underwent active lengthening. The strain percentage also was significantly greater in the central segment, as much as -23.9 (10.0)% compared to distally, as much as -9.5 (5.7)% during supra-maximal contractions. These results indicate that differences in fascicle behavior result in heterogeneous amounts of strain along the muscle. However, these results haven’t been shown across all muscles.

In contrast to the findings in American toads (Ahn et al. 2003), the pectoralis of pigeons during flight and the vastus lateralis (VL) of tammar wallabies during level and incline hopping were shown to have uniform strain throughout the muscle (Soman et al. 2004; McGowan et al. 2007). A study of the pectoralis in American pigeons showed higher strains in the proximal segment of the anterior sternobrachial fascicles compared to the distal segment, an average difference of 6.2%, and the posterior sternobrachial segment strained 30% less than the anterior and mid segments, but they found when regional strain was averaged across the entire muscle and of all subjects, the differences between regions were not significant (Soman et al. 2004). Homogenous strains have also been found within the VL of tammar wallabies during incline and level hopping. Proximal and distal sections of the VL on 3 wallabies found the proximal fascicles strained to a greater extent compared to the distal fascicles (18±5.4% vs. 14.7±4.1% during level hopping and 4.9±3.7% vs. 2.8±3.1% during incline hopping) but the amount was not significant ($p>.05$) (McGowan et al. 2007). While both studies showed uniform strain within the muscle, it is important to note that neither the pectoralis, nor the VL are biarticular muscles. The nature of biarticular muscles in humans, like the hamstrings, could prove similar to the findings of those in American toads (Ahn et al. 2003).

Architectural variations in the BFLH of humans have been found in cadavers (Kellis et al. 2010). Dissection of 4 human cadavers showed distinctive architectural differences within the BFLH, namely the fascicle lengths and pennation angles. Starting distally, measurements were taken at 25, 40 (mid belly 1), 60 (mid belly 2), and 80% of the full muscle length. The proximal fascicles had a 10.8% difference in length compared to their distal counterparts.

Fascicle pennation angles were found to be significantly larger proximally (80%) than distally (25%), 23.96 (3.82) ° and 17.78 (1.95)°, respectively. Also of note, the muscle thickness was much larger proximally, 2.71 (0.27), than found distally 1.32 (0.20) cm. The greater size of the proximal portion of the muscle was believed to be necessary to house the larger size of fascicle lengths and pennation angles.

Regional differences of fascicle lengths within human muscle could provide further understanding of the non-uniform strains found in active biarticular muscles. Application of fascicle length variations has been applied to muscle models of the biceps brachii and BFLH (Blemker et al. 2004; Rehorn et al. 2010). Models of the biceps brachii were created from dynamic MR imaging of low load elbow flexion, which reported differing fascicle lengths and pennation angles (Blemker et al. 2004). These models predicted non-uniform strains in along fiber stretch, from 1.0 at the tendon to 1.6 at the proximal and distal regions of the mid-belly of the muscle, throughout the muscle during eccentric contractions at 15% muscular activation. Although this is a different type of muscle and the muscle fascicle orientation is different from the BFLH, it is similar to the BFLH in that it's a biarticular muscle. Muscle modeling and MR imaging of the BFLH have also shown non-uniform strains, with the proximal region predicting the most amount of tissue strain (Rehorn et al. 2010; Silder et al. 2010). Dynamic MR imaging of the BFLH under eccentric contractions showed significant increase of strain in the regions closest to the proximal musculotendinous junction (MTJ). From 0 to 1.0 cm distal to the MTJ, the muscle sustained significantly greater amounts of strain than 1.0 to 4.0 cm distal to the MTJ ($p<0.05$). Muscle modeling of the full BFLH also predicted heterogeneous fiber strain within the muscle (Rehorn et al. 2010). Fiber strains within the muscle belly closest to the proximal MTJ were predicted to reach 1.64 (0.15) times the resting length, whereas the muscle belly closest distal MTJ were 1.54 (0.53) times resting length. Muscle modeling of biarticular muscles predicted non-uniform strain within the muscle, showing that regional behavior of the biceps brachii and BFLH is heterogeneous (Blemker et al. 2004; Silder et al. 2010; Rehorn et al. 2010). These MR studies do not, however, measure fascicle behavior or strain, but tissue behavior and strain. Muscle modeling, with its inherent assumptions, may not accurately reflect in vivo fascicle behavior.

Given the non-uniform strains in the SM of the American toad (Ahn et al. 2003), within the proximal region of the BFLH in humans (Silder et al. 2010), and the heterogeneity of fascicle lengths in the BFLH of humans (Kellis et al. 2010), it seems biarticular muscles are significantly different than uniarticular muscles. Muscle modeling has provided theoretical evidence that biarticular muscles, such as the BFLH, do not respond to stretch or strain uniformly throughout the entire muscle. The limitation of current studies on human fascicle length behavior is that these have all measured tissues and not fascicles (Silder et al. 2009), or have been performed at either: a passive state (Chleboun et al. 2001), through muscle modeling (Rehorn et al. 2010), or in cadavers (Kellis et al. 2010). Understanding how proximal and distal fascicles behave differently in active human muscles could provide a basis for further insight into why hamstring strain injuries occur so often in the proximal portion of the BFLH.

Summary

Hamstring strain injuries occur most often in the long head of the biceps femoris, particularly in the proximal region (Malliaropoulos et al. 2010; De Smet et al. 2000; Askling et al. 2006). Hamstring strain injuries occur when the BFLH is on a maximal stretch (Thelen et al. 2005; Heiderscheit et al. 2005). Under isometric contractions, the hamstrings develop the most amount torque in lengthened positions because of the active and passive elements of the muscle

(Lieber et al. 1993; Lunnen et al. 1981; Mohamed et al. 2002). At the fascicle level, BFLH fascicles lengthen more when flexing the hip compared to flexing the knee (Chleboun et al. 2010). Fascicles of the BFLH are longer in the proximal region than in distal region (Kellis et al. 2010). Muscle models of the BFLH predicted heterogeneous fiber strains within the BFLH muscle (Rehorn et al. 2010). Currently we do not have evidence of regional fascicle behavior in active human BFLH muscles. Determining heterogeneous regional fascicle behavior in the long head of the biceps femoris could provide a starting point for understanding why hamstring strain injuries occur most often in the proximal region of this muscle. Greater shortening in the proximal fascicles vs. the distal fascicles within the BFLH could cause greater strain upon the proximal musculotendon complex. The purpose of this study was to assess the magnitude of shortening of the proximal and distal fascicles in the long head of the biceps femoris under different muscle contraction levels. This study tests the hypothesis that the proximal fascicles will be longer, and undergo greater shortening than the distal fascicles when the muscle is at various activation levels.

Chapter 3: Methodology

Design

This study aims to determine the regional differences of muscle fascicle behavior in the BFLH as a relation to muscle activation at varying contraction levels when the hip and knee are flexed to 45 degrees. We hypothesized that the proximal fascicles were longer, and undergo greater shortening than the distal fascicles when the muscle is at various activation levels. Contraction levels were randomized in the form of 10, 25, 50, and 75% MVIC. This study used a within subject model to show the differences between regional fascicle behavior.

Subjects

The subjects used in this study were 11 college students (age: 21.3±1.8 yrs., height: 167.9±10.0 m, and mass 65.9±10.6 kgs.), who were currently involved in resistance training at least 3 times per week and were able to be classified as recreationally active, but participate in no collegiate sports. Subjects had no history of known hamstring injuries. All subjects were provided and required to sign a consent form approved by the IRB prior to participation in the study.

Instrumentation

All isometric contractions were performed on a HUMAC NORM Dynamometer (CSMI, model 502140, Stoughton, MA), and target contraction levels were viewable by the subject on a monitor. Ultrasound images of the long head of the biceps femoris were obtained using a GE Logiq e Ultrasound unit (General Electric, model Logiq e, Jiangsu, China) and Aquasonic Ultrasound Gel (Parker Laboratories, Aquasonic 100, Fairfield, NJ). Muscle activation levels, torque, and position were acquired using a Myopac unit (RUN Technologies, model MPRD-101

Receiver/Decoder Unit, Mission Viejo, CA) and Datapac Software (Run Technologies, Mission Viejo, CA) on a laptop.

Procedures

The subject's height, weight, BMI, self-reported activity level, and history of hamstrings injury were recorded. All subjects were required to wear compression shorts and t-shirt. The subject's initial longitudinal biceps femoris imaging was performed while the subject lies prone on a treatment table. After initial measurements were taken, subjects lay prone on a platform with their inferior iliac crest directly above the moveable arm of the platform and remained there throughout the study. The right hip and knee were flexed to 45 degrees with right shank held in the Humac Dynamometer arm, and the left leg was supported keeping pelvis balanced.

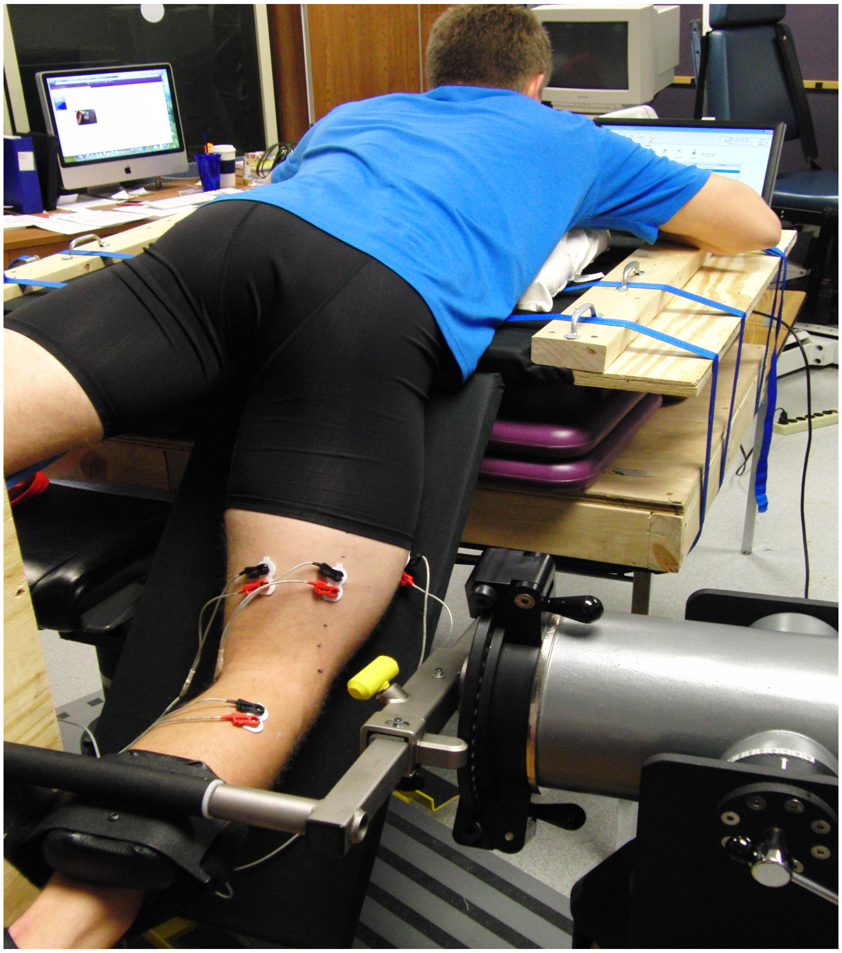

***Figure 3**. Experimental Set-up. Subjects lie prone on platform, with hip and knee flexed to 45 degrees. The left leg and hip are supported for comfort. The right shank is secured in the Humac Dynamometer arm.*

EMG.

For preparation, the subject's semitendinosus/semimembranosus, biceps femoris, lateral gastrocnemius, and the vastus lateralis were palpated, shaven, scrubbed with abrasive cream, and cleaned with alcohol wipes. Two electrodes were placed on the muscle belly of each tested muscle on the right leg. A reference electrode was placed upon the anterior tibial surface. The electrodes were connected to the Datapac and laptop, and then data will be collected in Datapac Software. Torque and position data were recorded from the Humac Dynamometer to the laptop and used in the same program. The subject was instructed to perform a light contraction to ensure EMG data is being captured correctly.

Humac Dynamometer.

Subjects' hips were flexed to 45°. Then the ankle was secured in the dynamometer arm with the knee in line with the axis of rotation of the dynamometer arm, and the leg was weighed for gravity correction purposes, before beginning the protocol. Once leg weight was recorded, the knee was flexed to 45 ° and held in place by the dynamometer. Subjects were then provided an opportunity for familiarity with the target contraction levels by viewing the monitor providing visual feedback and contracting their hamstrings.

First, subjects were instructed to contract with their maximal effort for 5 seconds while EMG and torque data are captured for 2 repetitions. Next, subjects performed "ramp contractions," which involve slowly increasing contraction level until meeting 80% of their previously measured maximums, then maintaining contraction level for 1 second, and finally slowly decreasing contraction level. The ramp contractions were used to determine the relationship between the medial hamstrings and BFLH, because the BFLH electrode must be removed in order to collect ultrasound images. The maximal torque recorded was used as the basis for MVIC percentages used throughout the study. Next, subjects were instructed to perform 2 contractions at each of the randomized MVIC percentages: 10, 25, 50, and 75 for 10 seconds each. At the end of each contraction subjects were allotted 1 minute for rest. Finally, subjects performed one more MVIC, 1 concentric MVIC of the vastus lateralis, and 1 MVIC of the lateral gastrocnemius.

Ultrasound Imaging:

Subjects' BFLH were imaged longitudinally while in prone position on a treatment table. Markings were applied to the skin as a reference of the location of the distal, central, and proximal segments of the BFLH with a black Sharpie. All ultrasound measurements were performed starting at the distal musculotendinous junction and ending at the proximal musculotendinous junction. Longitudinal images were also obtained while subjects were lying on the platform during passive and target torque conditions (Figure 4.). Two ultrasound images were taken of the full length of the BFLH with no contraction, and at each contraction level with hip and knee flexed to 45°, starting approximately 2 seconds when the subject is instructed to maintain a steady state contraction. No images were taken while the subject was performing MVIC due to the inability of the subject to maintain a 100% effort while scanning the muscle.

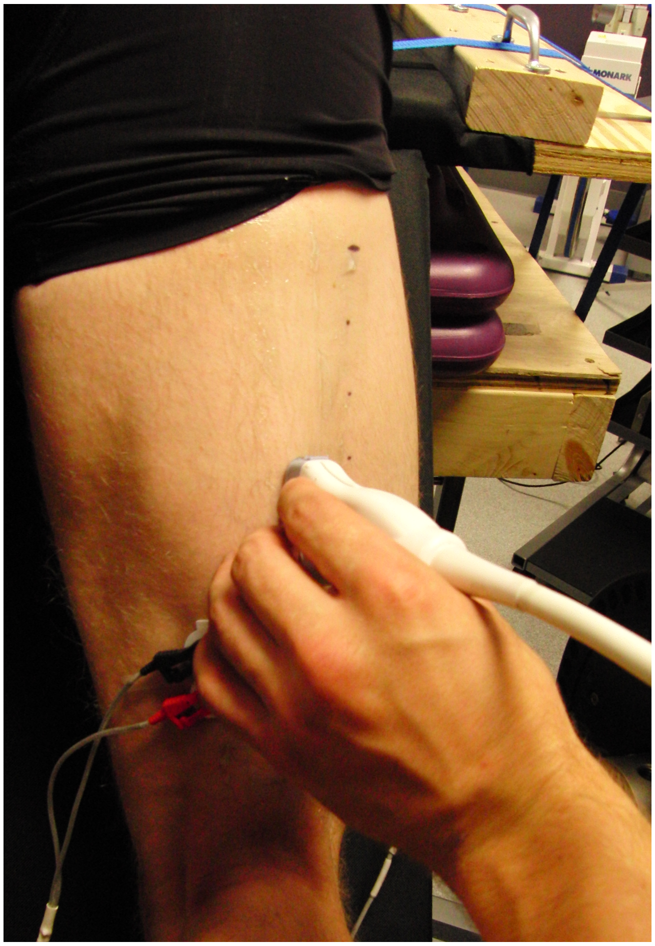

Figure 4. *Imaging of subject's BFLH while hip and knee flexed at 45°.* A track line is made on the skin of the BFLH observed when subjects are lying prone on treatment table and subsequently used in submaximal trials to notate the lateral border of the BFLH.

Data Reduction

Measurements of proximal and distal fascicle lengths were performed on the GE Ultrasound Computer. The full length of the muscle was measured twice on each image, starting at the most proximal point of the muscle before the musculotendinous junction, and ending at the most distal point of the muscle before the musculotendinous junction. The muscle was then divided into 2 regions, proximal and distal. Two fascicles were measured on each half of the biceps Femoris (Figures 5 - 8). The measurements started at the fascicle's superficial origin and end at the fascicle's insertion onto the deep aponeurotic tendon.

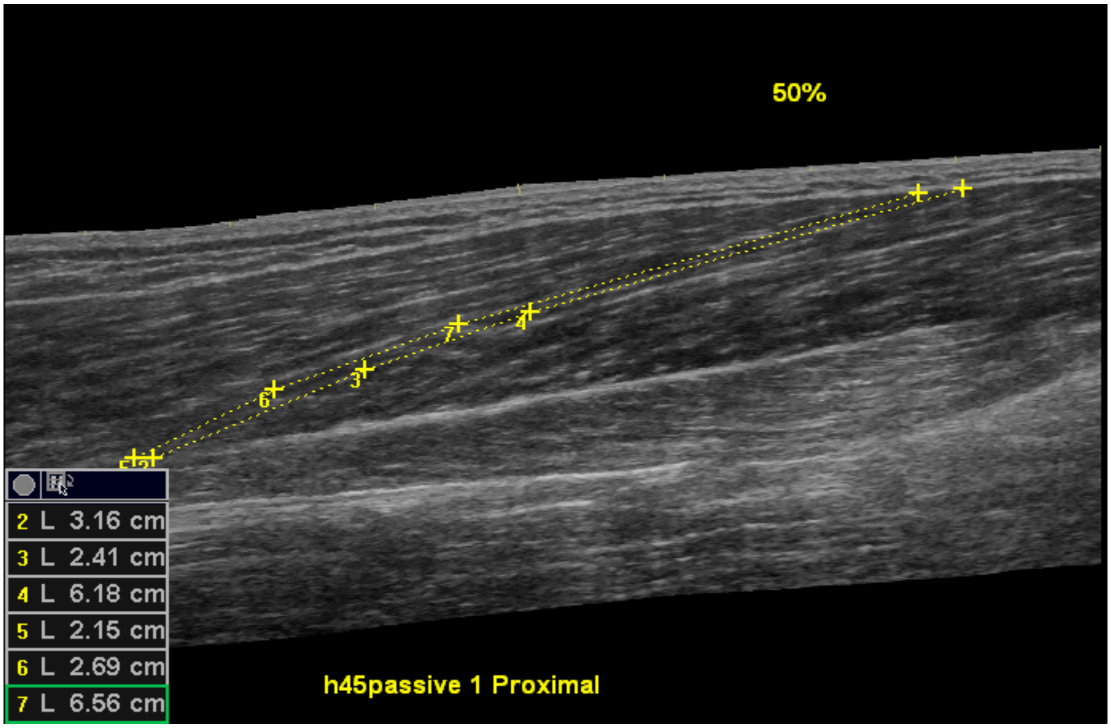

Figure 5.

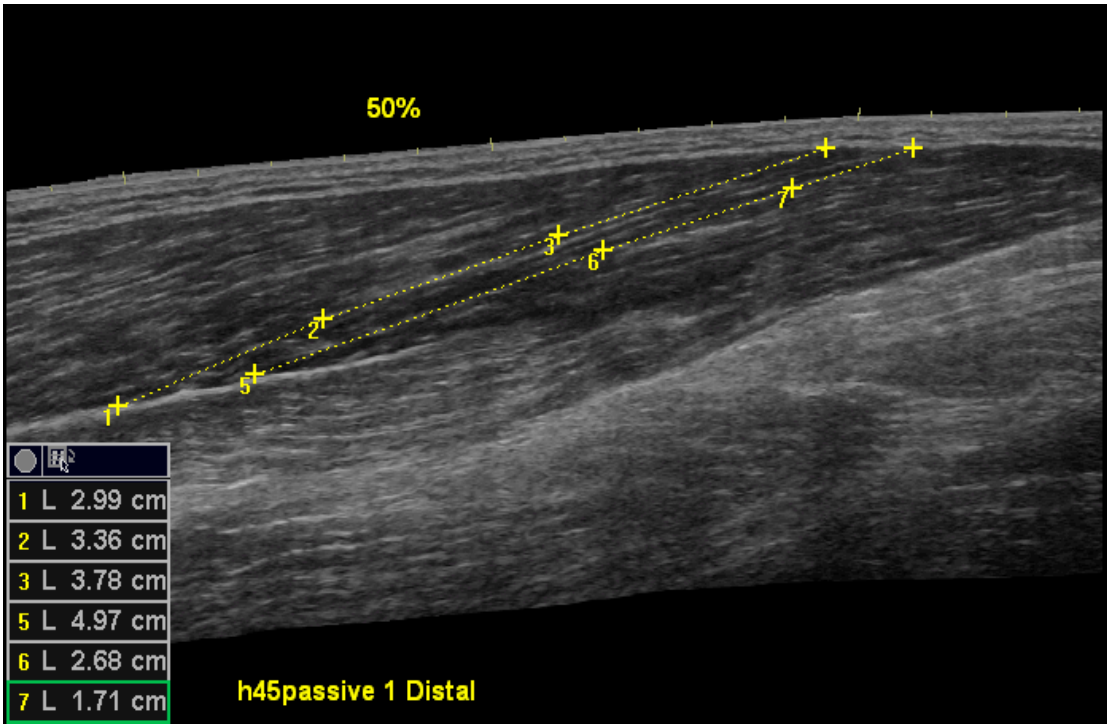

Figure 6.

Figures 5 &6. *Measurement of regional fascicle lengths imaged in the passive position (hip & knee 45°).*

There were two fascicles measured in each region: proximal (Figure 5.) and distal (Figure 6.). The fascicles were determined to be in each region based on which half (split by the 50% marking on the above image) of the muscle the majority of the fascicle length lies in.

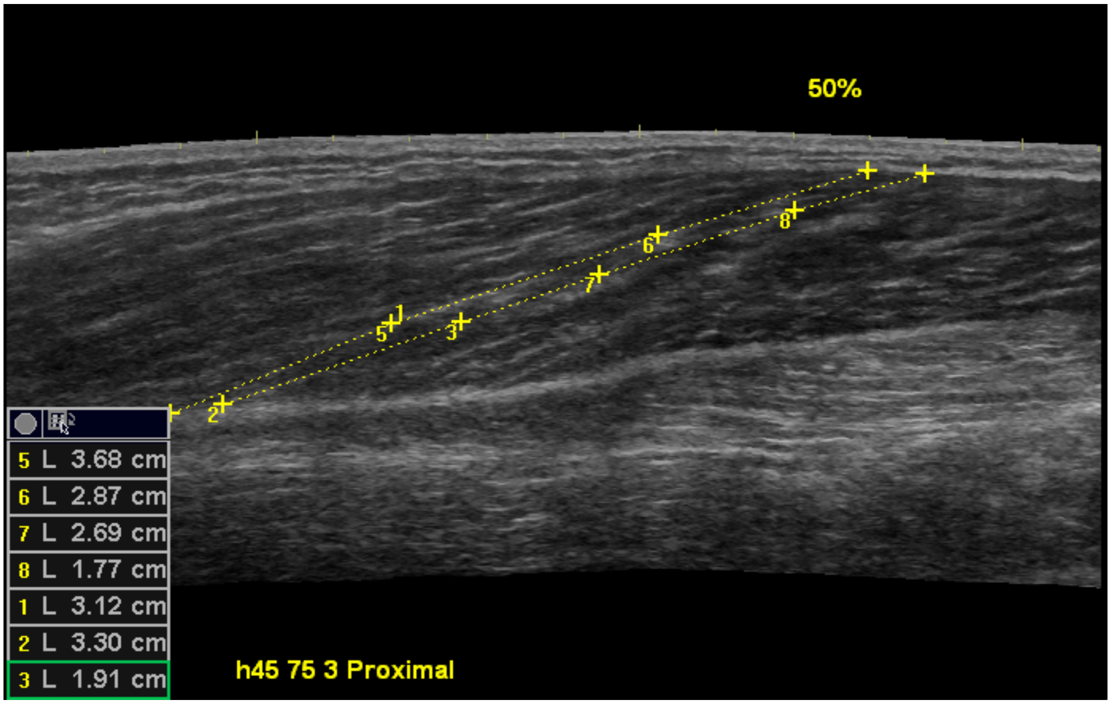

Figure 7.

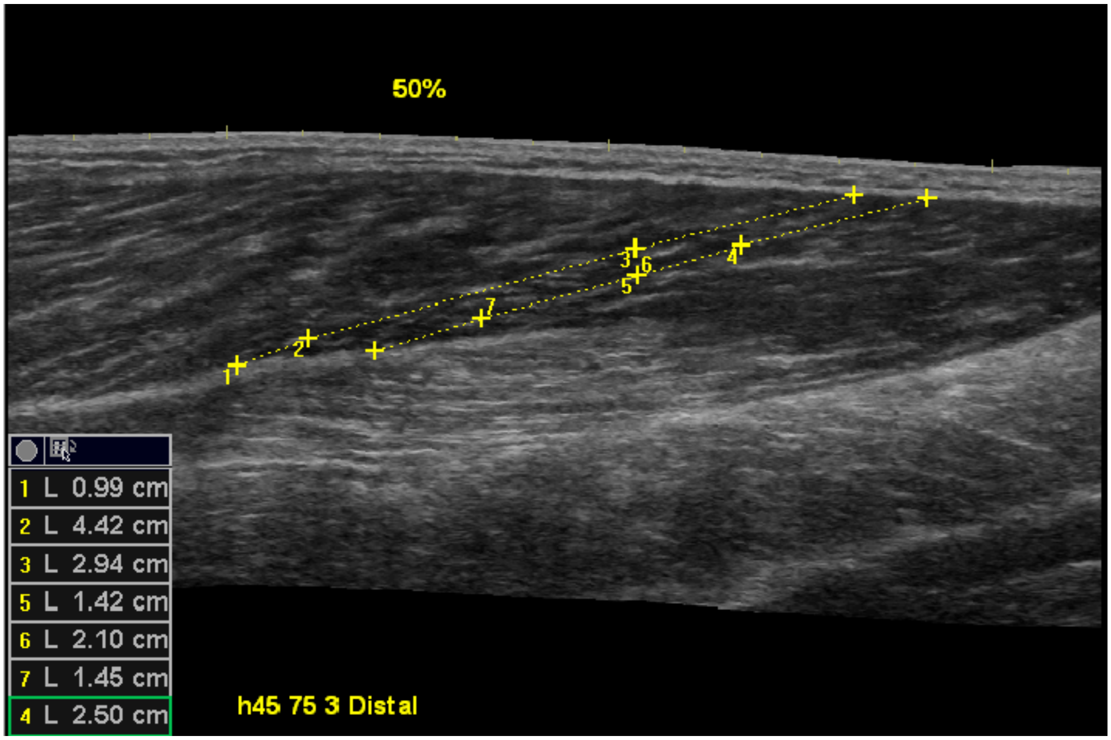

Figure 8.

Figures 7 & 8. *Measurements of regional fascicle lengths in the 66.7% activation condition.* The same process for fascicles measurements performed in the passive conditions was performed in each image obtained during all conditions. When possible, measurements were made of both images obtained during passive state and each active condition.

BFLH activity was estimated by correlating the semitendinosus and BFLH activity recorded during the ramp trials, when the BFLH electrode was still on the muscle. The activity levels were plotted on a graph in the Excel Spreadsheet, semitendinosus as the predictor variable, and a best-fit trend line was used to quantify the strength of the relationship between activation levels. The power regression equation of the trend line was used to estimate each subject's BFLH activity during the subsequent target torque conditions (Figure 9.). The power equation was chosen because it provides, qualitatively, the best fit for the scatterplot of ST/SM and BFLH activation levels. Although the range of R-square values for the power regression equations was from .83 to .99, most of the subject's values were .95 and above. Average EMG activity and torques were obtained during the steady state portion of each trial, i.e. approximately the last 8 seconds, in the Datapac Software on the same Excel Spreadsheet as the muscle fascicle data.

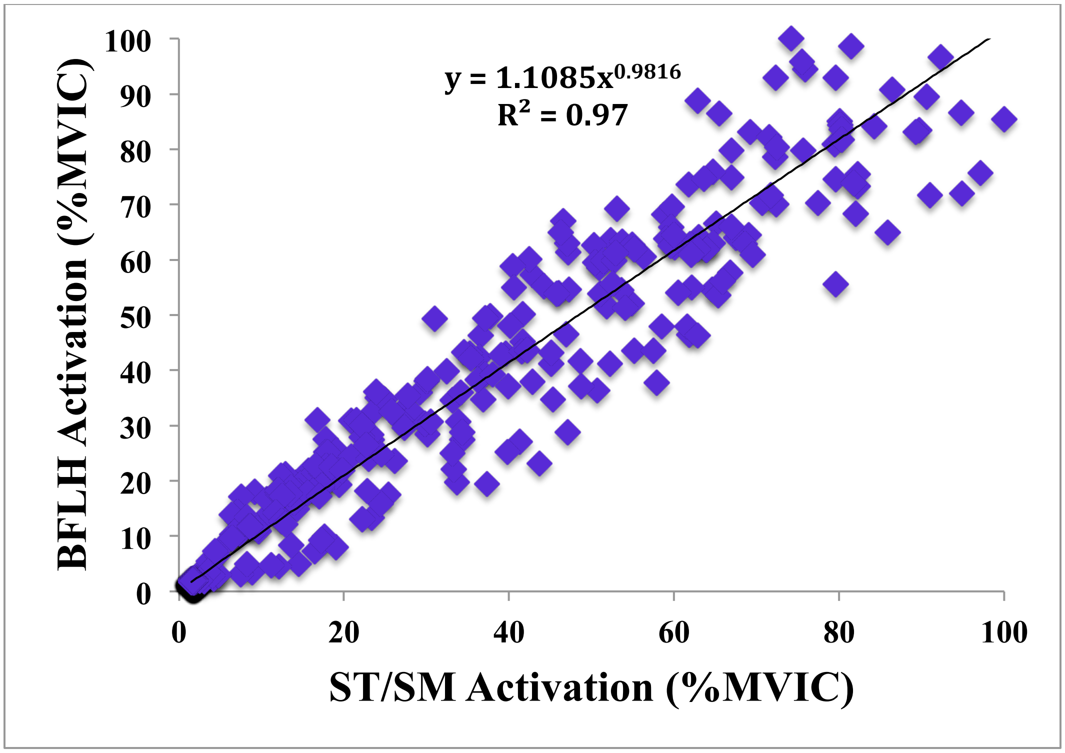

Figure 9. *Example of the power relationship between BFLH and ST/SM activation of one subject.* The equation shown was used to predict BFLH activation during the active conditions. For each subject the R-squared value ranged from .83 to .99, but the majority of values were above .95.

A 2 (condition) x 5 (contraction) Repeated Measures Analysis of Variance confirmed the presence of four significantly different predicted BFLH activation levels of 20.6, 29.9, 50.4, and 66.7% MVIC during the four isometric contraction conditions ($P<.001$). These results confirm our experimental approach of a spread of activation levels (independent variable) to assess regional BFLH fascicle behavior.

Data Analysis

We tested the hypothesis that the proximal fascicles are longer, and undergo greater shortening than the distal fascicles over a range of activation levels. We utilized a combination of linear regression and repeated measures ANOVAs to characterize the behavior of the proximal and regional fascicle behaviors across activation level. In the presence of a significant condition by region interaction, Tukey's post hoc testing was performed. All analyses were performed on the raw fascicle lengths measured and as strain (the change in fascicle lengthening normalized to the passive condition).

Chapter 4: Results

The purpose of this study was to assess the magnitude of shortening of the proximal and distal fascicles in the long head of the biceps femoris under a range of muscle activation levels. It was hypothesized that: 1) the proximal fascicles would be longer than the distal fascicles and 2) the proximal fascicles would shorten more than the distal fascicles. A linear regression analysis was performed on each region of fascicle length vs. activation independently, and then the intercepts (passive fascicle lengths) and slopes (relationships of fascicle length vs. activation level over the entire range of activation levels) of each region were compared through a combined regression analysis. Two factor (region and condition) repeated measures ANOVAs were also used to a) determine if disproportionate regional fascicle behavior was present within a subset of activation levels, i.e. region by condition interactions. In the presence of significant interactions, Tukey's post hoc testing was performed to assess the region by condition interaction. Both the linear regressions and RMANOVAs were performed on the absolute fascicle lengths and the change in fascicle lengths normalized to resting length, i.e. fascicle strain.

Linear Regression Analysis: Regional Absolute Fascicle Length vs. Activation Level

The regression relationship between the proximal fascicles was significantly different than zero ($P<.001$), with $R^2 = 0.49$ (Figure 10.). The relationship between the distal fascicles was also significant ($P<.001$), with $R^2 = 0.37$ (Figure 10.).

Passive Fascicle Length – The linear regression analysis of the intercepts showed the proximal fascicles were significantly longer than the distal fascicles, 12.53 cm vs. 9.30 cm respectively (Figure 10.). The proximal fascicles were significantly longer by 3.24 cm (t-value 36.87, $P<.001$).

Regional Fascicle Behavior – Comparisons of the slopes of each linear regression showed no significant differences between the proximal and distal regions, -0.045 vs. -0.032 respectively (Figure 10.). The non-significant difference between slopes was -0.013 (t-value -1.56, P=.12).

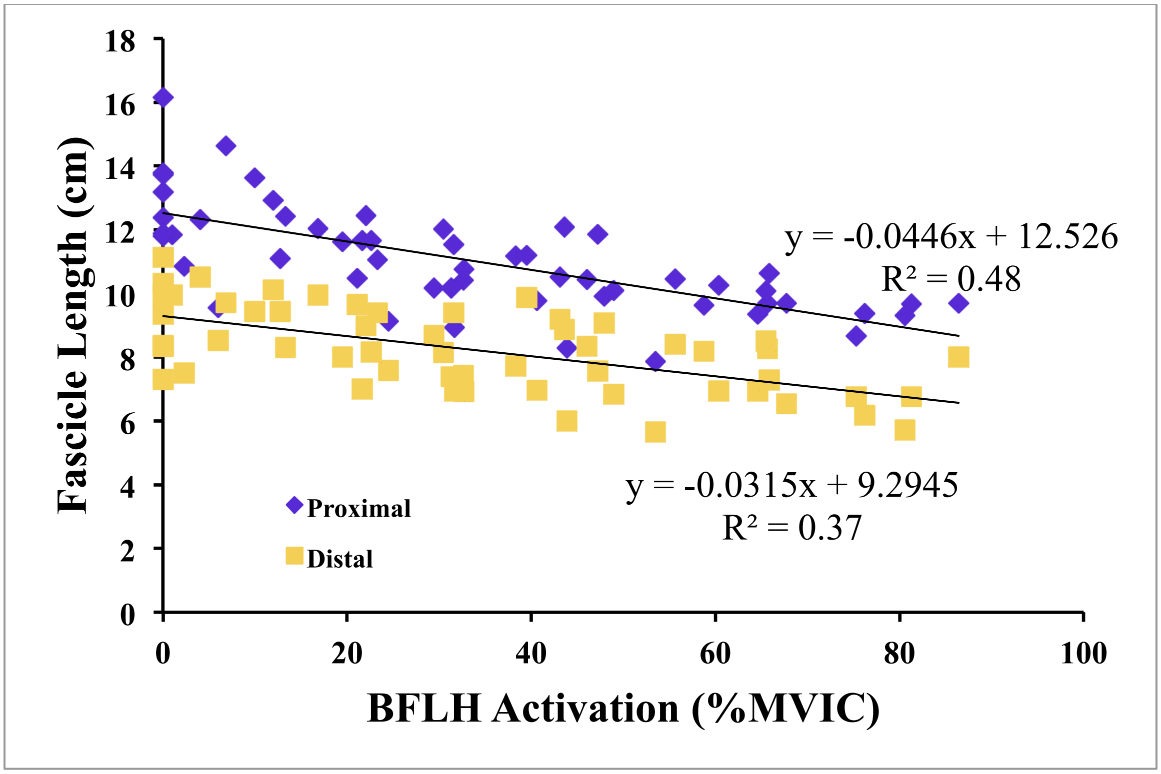

Figure 10. *Absolute regional fascicle length vs. activation level of all subjects.* The linear regression analysis showed the proximal fascicles are longer than the distal fascicles by their intercepts (12.52 vs. 9.29, P<.001), but the slopes (-0.0446 vs. -0.0315) of the proximal and distal regions were not significantly different (P=.122).

Repeated Measures ANOVA: Regional Absolute Fascicle Length vs. Activation Level

Region Main Effects – The RMANOVA results showed main effects for region (P<.001). The proximal fascicles were longer than the distal fascicles, regardless of activation level

(Figure 11.). The proximal fascicles, 11.02 cm – 95% CI (10.15, 11.90) were longer than the distal fascicles, 8.23 (7.47, 8.99) throughout all of the activation levels by 2.79cm – 95% CI (2.06, 3.53) (P<.001).

Regional Fascicle Behavior – The RMANOVA showed a significant interaction between region and condition (P=.003). Tukey's post hoc testing revealed that as activation level increased, the proximal fascicles had significant incremental shortening from the passive condition to 50.4% activation, while the distal fascicles only had significant incremental shortening from passive to 20.6% activation (Figure 11.). In addition, the proximal fascicles shortened significantly more, 0.85 cm, overall than the distal fascicles (P<.05).

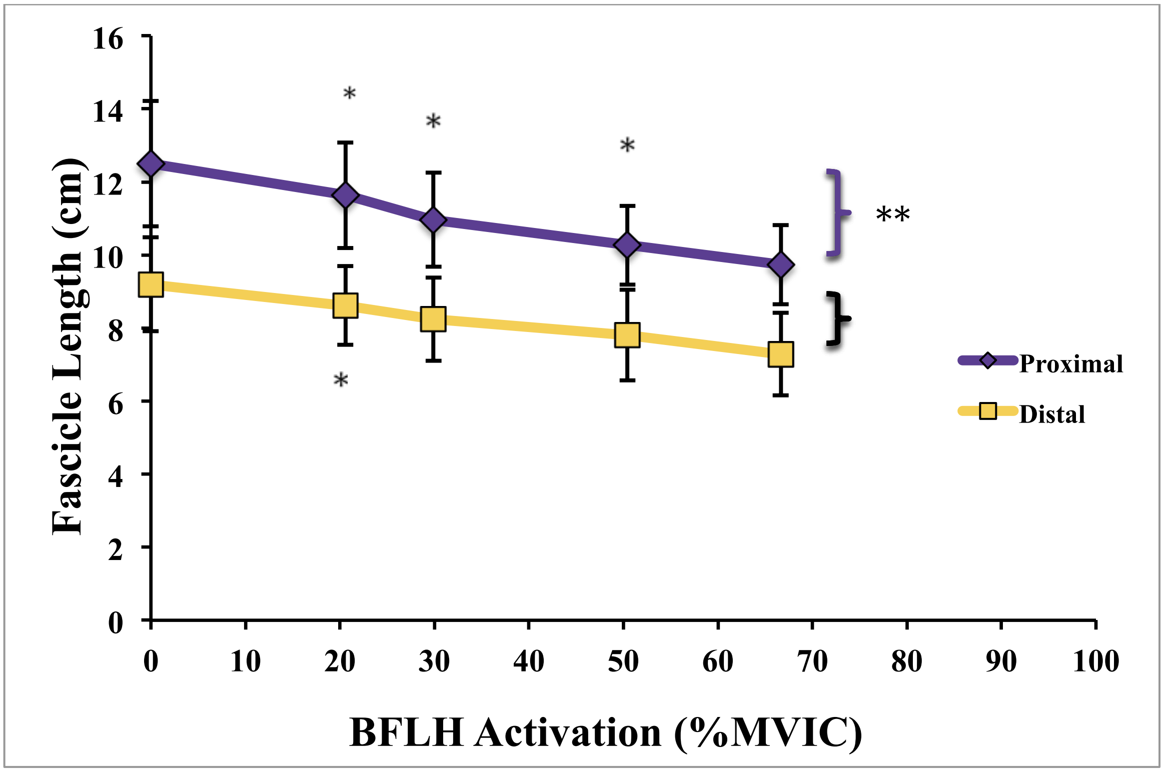

Figure 11. *Averaged absolute regional fascicle length vs. activation level across conditions.* The RMANOVA showed that the proximal fascicles were longer than their distal counterparts, regardless of activation level (P<.001). Although both regions had significant shortening at each

activation level compared to the passive condition, the proximal fascicles had significant incremental shortening from passive to 50.4% activation, while the distal fascicles significantly shortened from passive to 20.6% activation (*, P=.003). The proximal fascicles also shortened significantly more than their distal counterparts overall by 0.85cm – denoted by the larger purple than black brackets (**, P<.05).

Linear Regression: Regional Fascicle Strain vs. Activation Level

Proximal fascicle strain was significantly related to activation (P<.001), with $R^2 = 0.66$ (Figure 8). Distal fascicle strain was also significantly related to activation (P<.001) with $R^2 = 0.58$ (Figure 12.)

Regional Fascicle Strain – The regression analysis of strain showed no significant difference between the proximal and distal slopes, 0.003 vs. 0.003. The difference was -9.58E-05 (t-value -0.23, P=.816). The intercepts were not different, as both were 0 in the passive condition (Figure 12.).

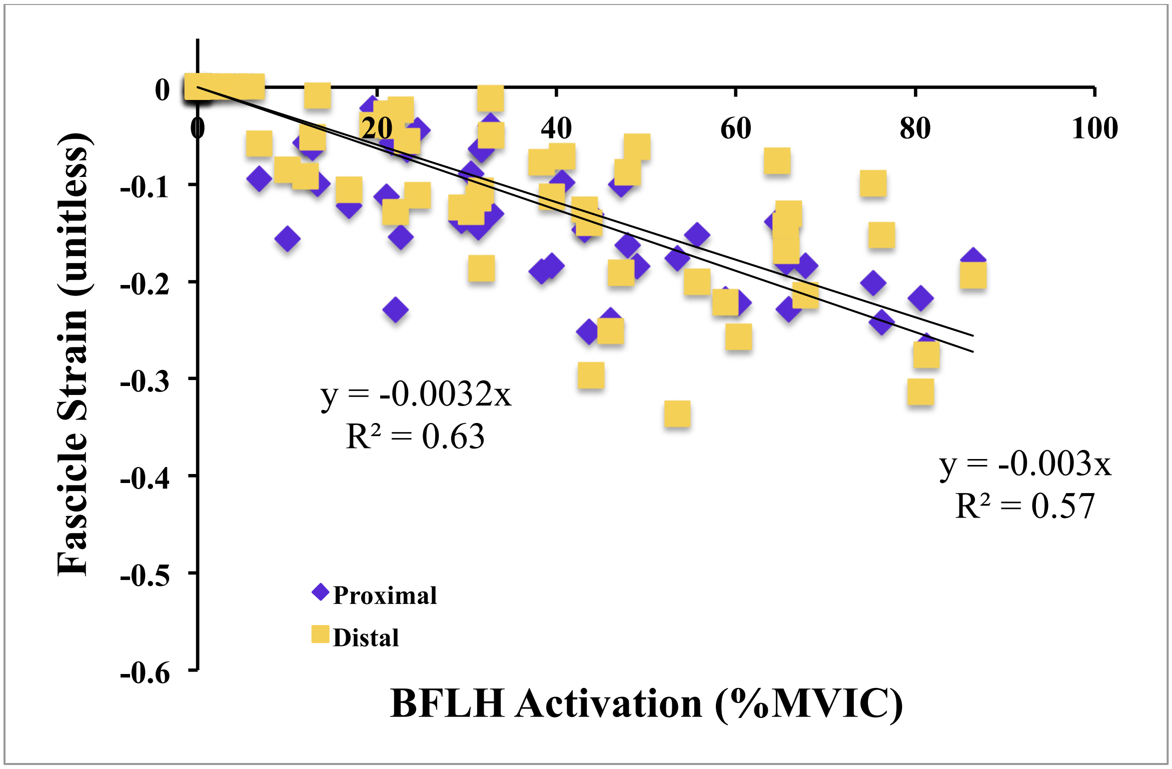

Figure 12. *Regional fascicle strain vs. activation level of all subjects.* The relationships between fascicle strain (change in length normalized to passive condition) vs. activation level of both proximal and distal regions were not significantly different (P=0.816).

Repeated Measures ANOVA: Regional Strain vs. Activation Level

Regional Fascicle Strain Behavior – The RMANOVA results showed no main effects for region (P=.49), and no significant interaction between region and condition (P=.72). The results did show, however, main effects for condition (P<.001). Both regions underwent significant incremental and overall fascicle strain as activation level increased, compared to the passive condition (Figure 13.).

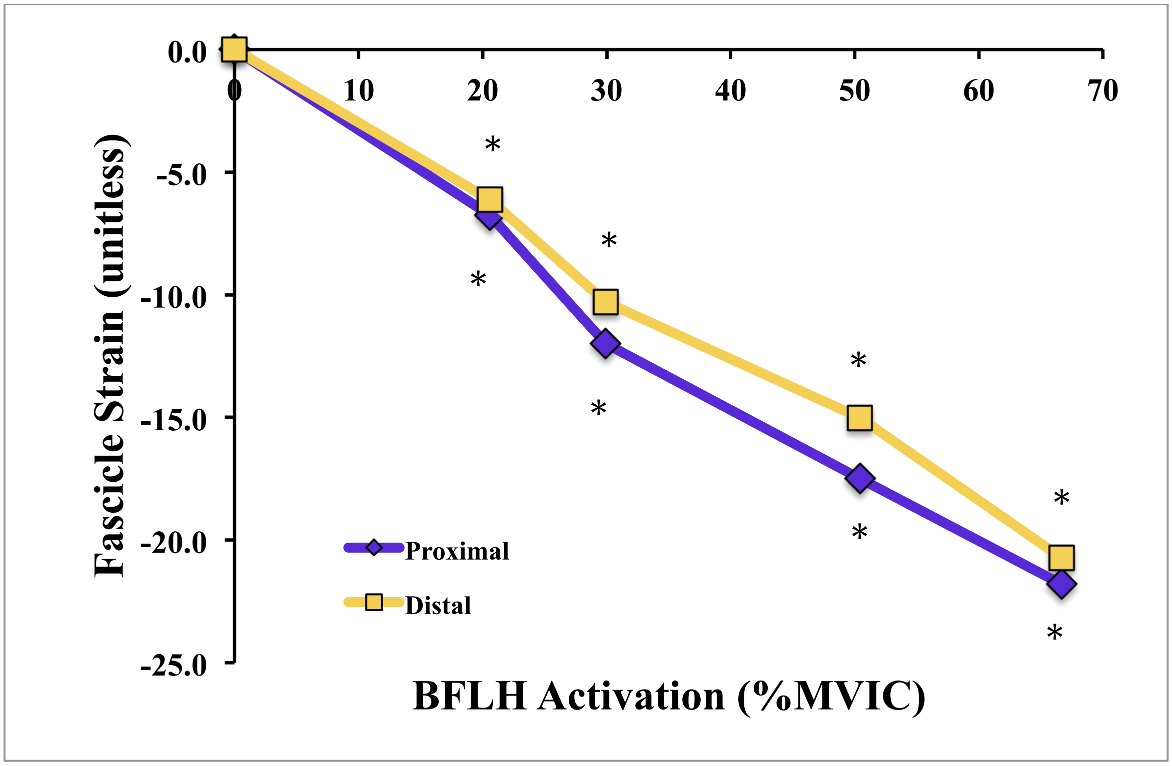

Figure 13. *Averaged regional fascicle strain vs. activation level across conditions.* While both regions' fascicles underwent significant strain as activation level increased (* - denotes P<.05), the difference of strain magnitudes between regions was not significant (P=.72).

Summary of Results

The results of this study investigating regional fascicle behavior within the BFLH found that the proximal fascicles were longer and shortened more than the distal fascicles, in terms of the absolute length and absolute shortening, respectively. There was also a region by condition interaction of the absolute fascicle lengths, which showed the proximal fascicles had significant incremental shortening from the passive to 50.4% activation level, while the distal fascicles only had significant incremental shortening from the passive to 20.6% activation level. When assessing strain, both regions underwent significant incremental and overall strain. However, the region by condition interaction was not present, and the proximal fascicles did not undergo greater amounts of strain compared to the distal fascicles. Thus, once normalized to the resting fascicle length, the behavior of proximal and distal fascicle shortening was not different.

Chapter 5: Discussion

The purpose of this study was to assess the magnitude of shortening of the proximal and distal fascicles in the long head of the biceps femoris under different muscle activation levels. The hypothesis of this study was the fascicles in the proximal half of the BFLH would be longer and would undergo greater shortening than the distal fascicles when the muscle is at various activation levels. The main findings were: 1) proximal fascicles were longer (~34%) than distal fascicles regardless of activation condition 2) absolute shortening was larger in the proximal fascicles than the distal fascicles within the biceps femoris long head from 10% to 75% activation, and 3) proximal fascicle strains were not different than distal fascicle strains as evidenced by the lack of significant slope differences (regression analyses) and the RMANOVA analyzing regional strain behavior across activation levels. These data are the first, to our knowledge, results of in vivo regional fascicle behavior in the BFLH of humans. As such, the comparisons of these data to the literature are of *in vivo* to: predicted (modeling), other analyzed muscles in vivo, and cadavers. The statistical analyses were primarily of the entire group, however, we will also include comparisons of individual subjects within the current data set to help better explain the results, and possible hamstring injury implications.

Group Comparisons

Analysis of the entire study group allows the opportunity to ascertain the possibility of fascicle heterogeneity in a given sample. This section will discuss the general nature of fascicle behavior across activation levels and the results pertaining to proximal hamstring strain injuries.

Fascicle Length vs. Activation

The overall pattern of fascicle shortening with increasing activation as shown in the current study is similar to those measured in the tibialis anterior and vastus lateralis during isometric contractions (Pasquet et al. 2005; Hodges et al. 2003; Ichinose et al. 1997). In these imaging studies, the relationship of muscle fascicles shortening as activation or torque level increases was of a curvilinear pattern. Although the fascicles shorten throughout all torque levels, there was significantly greater shortening from the passive to 20% MVC compared to the other torque level increments (P<.05; Figure 14.).

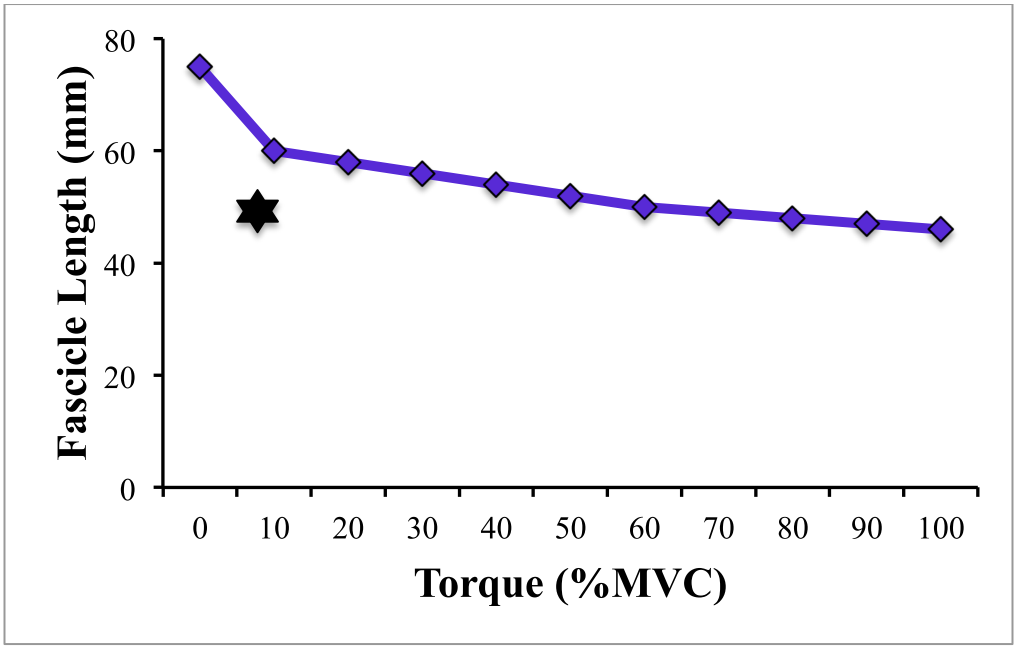

Figure 14. *Behavior of tibialis anterior muscle fascicles at increasing torque levels (Pasquet et al. 2005).* Note the curvilinear path of fascicle shortening for each muscle as torque level increases, which is similar to the results of the RMANOVA of our study (Table 3.). Star indicates significant change in fascicle length occurring only from 0 to 20% MVC (P<.05).

Similarly in pattern, the largest fascicle length change for both regions of the BFLH in this study occurred between 0 to 20.6% activation levels in our study (Table 1.). These results combined with previous studies indicate that although the fascicles continue to shorten past ~20% activation, a large portion of the absolute length change possible for muscle fascicles is reached at low activation levels. The larger measured length changes at the lower levels of activation could due to the compression of the pad used for data collection; however, this likelihood is small due to the similar findings of the several previous studies (Pasquet et al. 2005; Hodges et al. 2003; Ichinose et al. 1997). Another possible explanation for the larger length changes in the initial activation levels is the compliance of the tendon (Ichinose et al. 1997). Greater shortening of fascicles could be occurring to make the tendon more taut during the isometric contractions in the literature and our study. Although it was not the intent of this investigation to understand why the fascicles shorten at low activation levels, this idea is certainly supported for future research.

Proximal		**Distal**	
Conditions	**Length Change (cm)**	**Conditions**	**Length Change (cm)**
0-20.6%	**-0.87**	**0-20.6%**	**-0.59**
20.6-29.9%	**-0.67**	**20.6-29.9%**	**-0.38**
29.9-50.4%	**-0.70**	**29.9-50.4%**	**-0.43**
50.4-66.7%	**-0.53**	**50.4-66.7%**	**-0.53**

Table 1. *Proximal and distal length change between activation levels.* The highlighted results show that the fascicles undergo the greatest amount of shortening from 0 to 20% activation, which is similar to previous studies.

Regional Fascicle Lengths

Our results show that the proximal fascicles are longer than the distal fascicles when no activation is present. These data are consistent with regional fascicle lengths obtained previously from cadavers (Kellis et al. 2010). Regardless of activation level, the proximal fascicles were consistently longer than the distal fascicles, which has not been previously determined in vivo. While this result shows, in part, heterogeneity of BFLH muscle fascicle lengths, it does not imply fascicle *behavior* between regions is heterogeneous.

Regional Fascicle Strain Heterogeneity

Since the proximal fascicles were longer and did shorten more than their distal counterparts, on an absolute level, we wanted to explore whether the difference was present when the shortening was normalized to the passive conditions (strain). While the current study showed clear evidence that as activation increases the proximal and distal fascicles shorten (negative strain), the proximal and distal fascicle strain behavior was not different. Thus, the significant condition by region interaction assessed on the absolute fascicle lengths was likely a symptom of the proximal fascicles being longer than the distal fascicles. These results do not agree with evidence in animals, nor muscle tissue and fiber modeling of biarticular muscles (Ahn et al. 2003; Chanaud et al. 1991; Blemker et al. 2004; Rehorn et al. 2010). While it is important to note that animal muscle fascicle strains do not necessarily indicate similarity in human muscles, nor does heterogeneity at the fiber level indicate heterogeneity at the fascicle level, these studies provide evidence to suggest the possibility of within muscle fascicle heterogeneity. Although it is possible that heterogeneity is not able to be determined at the fascicle level, as seen in these data, imaging of tissue movement and calculation of tissue strains contradicts our results (Silder et al. 2009; Fiorentino et al. 2012). Non-uniform tissue strains strictly within the proximal BFLH have been shown in both concentric and eccentric contractions. CINE phase imaging of concentric and eccentric contractions of the BFLH resulted in significantly higher first principle strains in the regions closest to the BFLH (Silder et al. 2009). Similarly, active lengthening results in significantly greater first principle strains than passive lengthening (Fiorentino et al. 2012). These results combined with the animal and muscle modeling studies suggest the presence of heterogeneity within the BFLH muscle. One reason for the absence of heterogeneity in our data could be due to the regions chosen for "proximal" and "distal" fascicle measurements in our study. The most proximal fascicles have been determined to be longer than those located closer to the muscle belly, and the most distal fascicles have been shown to be shorter than those measured there (Kellis et al. 2010). Thus, if we had been able to measure the more extremes of the two regions of the muscle, we may have observed a different outcome. We chose to measure fascicles closer to the muscle belly due to the feasibility of capturing entire, clear muscle fascicles in this region. In the more proximal area of the hamstrings near the musculotendinous junction, the gluteal muscles and greater amounts of adipose tissue are present. These tissues are more superficial than the BFLH, and thus move the proximal BFLH to the deep area of the ultrasound image. The greater adipose tissue creates a more difficult path for the sound waves to successfully penetrate and present an adequate image for measurement. It is also important to note that many of the previously determined "proximal hamstring strains" occur at the myotendinous junction of the BFLH (De Smet et al. 2000; Askling et al. 2006). It is near this point in the muscle that the fascicles begin to no longer insert on the deep aponeurosis, but insert directly into the proximal tendon (Batterman et al. 2010; and Figure 15.). Measurements of the muscle fascicles in this region are very difficult, as they become nearly parallel to the muscle's length and continue to insert on the external tendon. Other methods of imaging might provide increased quality of images and easier measurements of fascicles in this area. The inability to accurately obtain adequate images of the most proximal region of the

BFLH, and thus no fascicle measurements of this area, could mean we “missed” imaging the behavior of the most proximal muscle fascicles that are susceptible to injury.

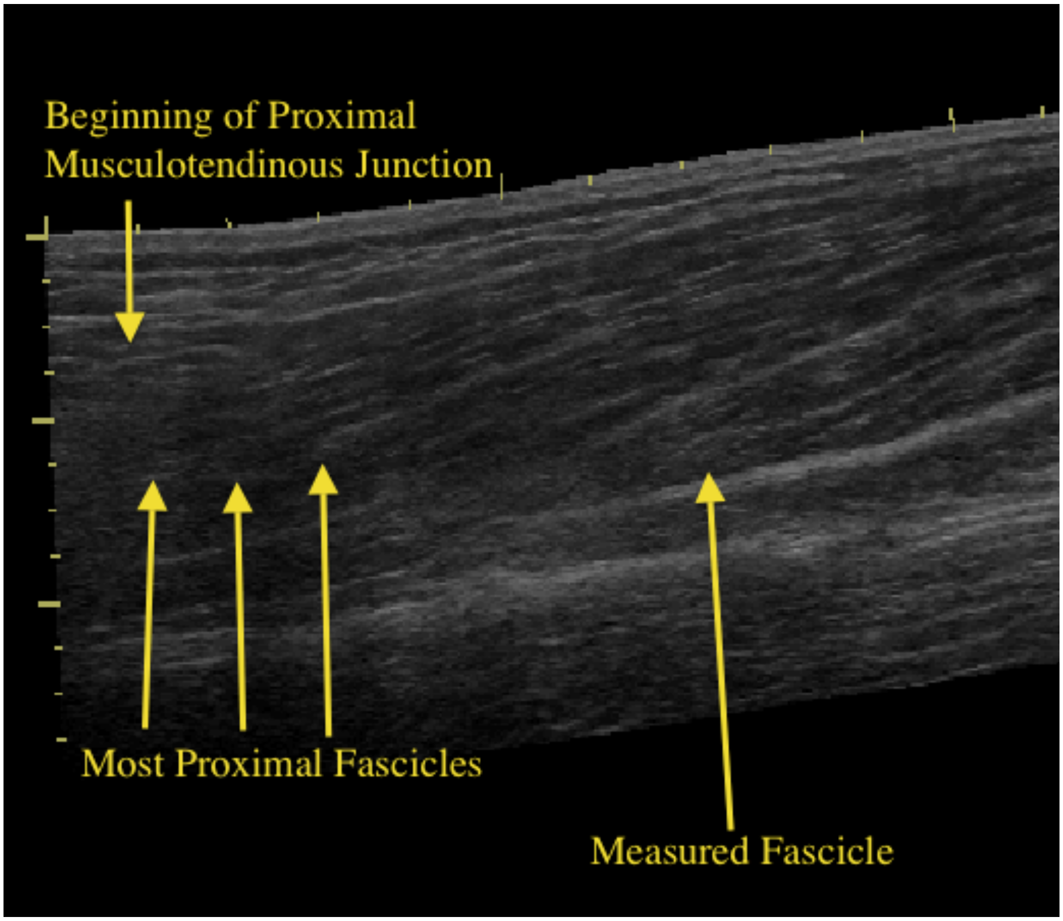

Figure 15. *Example of the most proximal muscle fascicles inserting directly into the muscle tendon and not the deep aponeurosis.* Note the difference between the measured fascicle and the most proximal fascicles. The most proximal fascicles are near horizontal, and continue on past the image into the external tendon.

Individual Subject Comparisons

While it was necessary to assess the data of the entire group together, it is possible that investigations of the individual’s characteristics will show some of the observed non-uniformity may be subject-specific. In this case, we will qualitatively compare the individual subjects regional fascicle behavior to qualitatively determine heterogeneity on a subject specific basis.

Regional Strain

The RMANOVA showed both regions undergo significant strain at each activation level, and that inter-regional fascicle strains were not significant when the entire group was analyzed. However, qualitative assessments of regional strains (both total and at each activation level) on a subject specific basis suggest that the presence and/or magnitude of heterogeneity could be subject specific.

The comparisons for maximum strain in all conditions indicate 3 types of individualistic responses to the isometric knee flexor contractions: four subjects showed greater proximal fascicle strains compared to distal fascicle strains, two subjects showed greater distal fascicle strain compared to the proximal region, and five subjects showed no appreciable difference between regions (Figure 16.). Figures 17a-c are presented to support the presence of these 3 different types of individualistic responses.

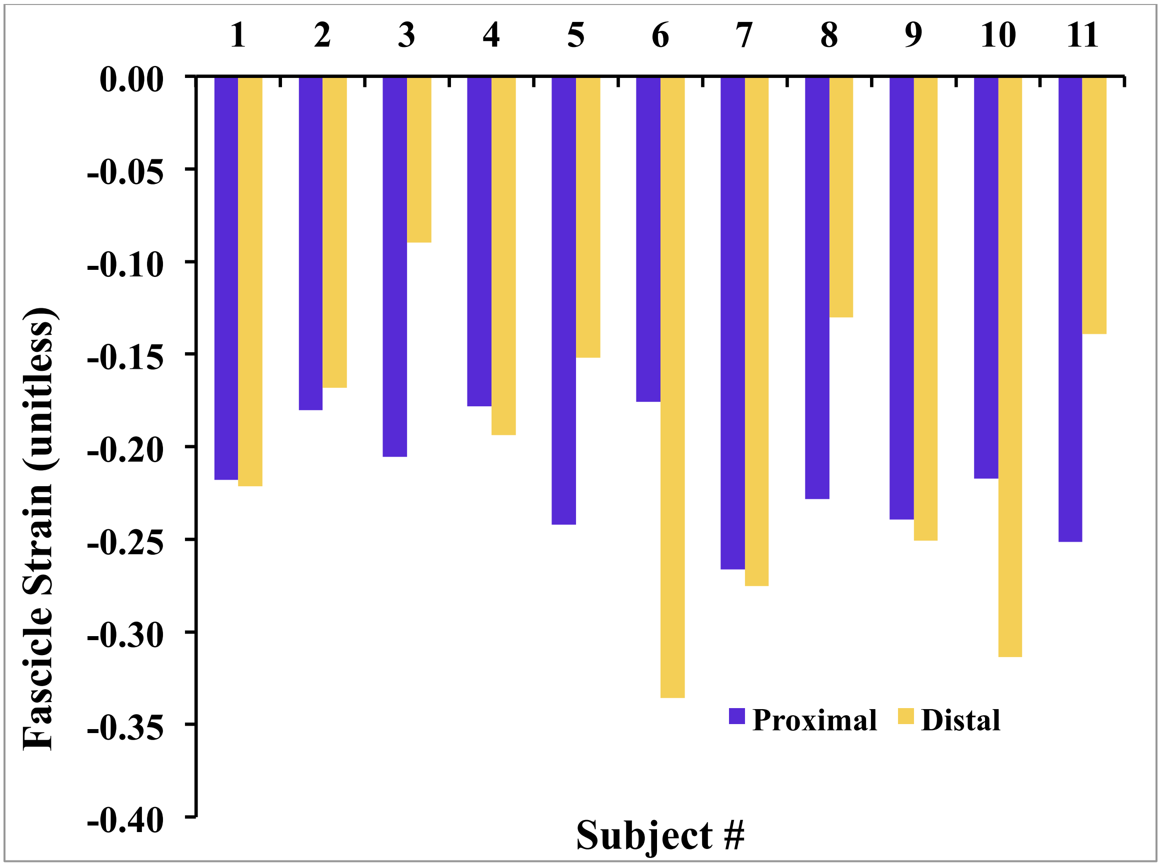

Figure 16. *Regional fascicle strains magnitudes of the BFLH at 66.7% activation level.* Note the strain magnitude differences of subjects 3, 5, 6, 8, 10, and 11. Qualitatively, these differences in strains indicate heterogeneity within individual subjects.

Of the subjects with regional differences, it seems that 4 subjects (Subject #3,5,8, & 11, Figure 16.) have markedly greater strain in the proximal vs. distal regions and at least 2 subjects (Subject #6 & 10, Figure 16.) show greater distal vs. proximal strain. Representative subjects from each response are characterized in Figures 17a-c.

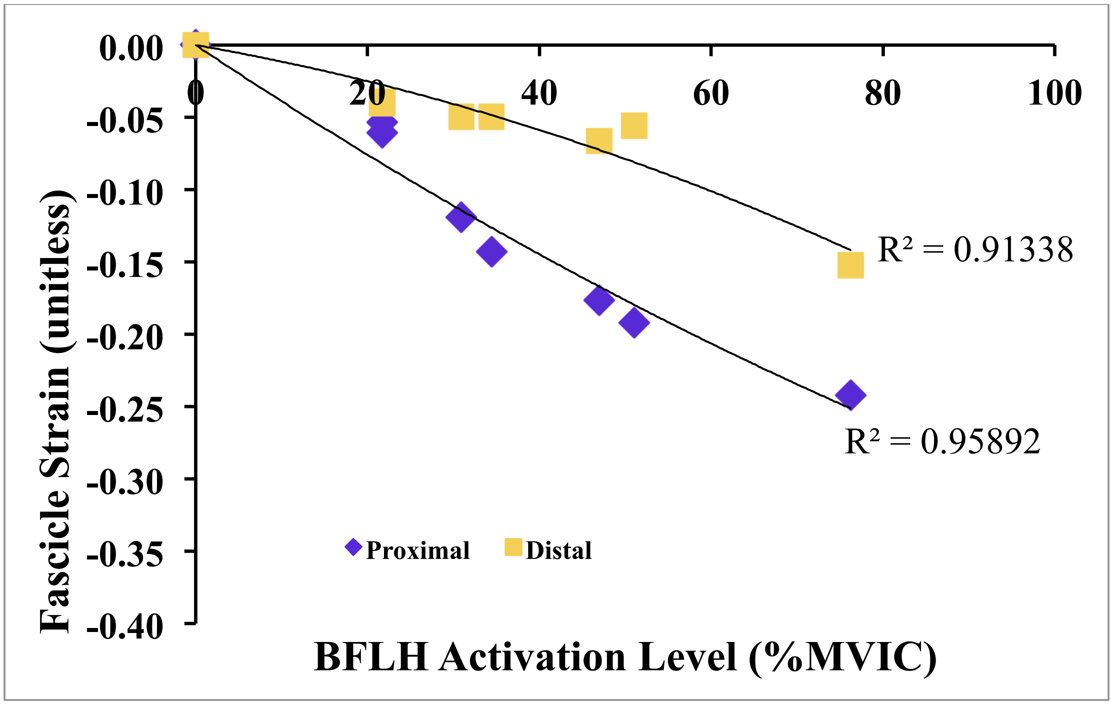

Figure 17a.

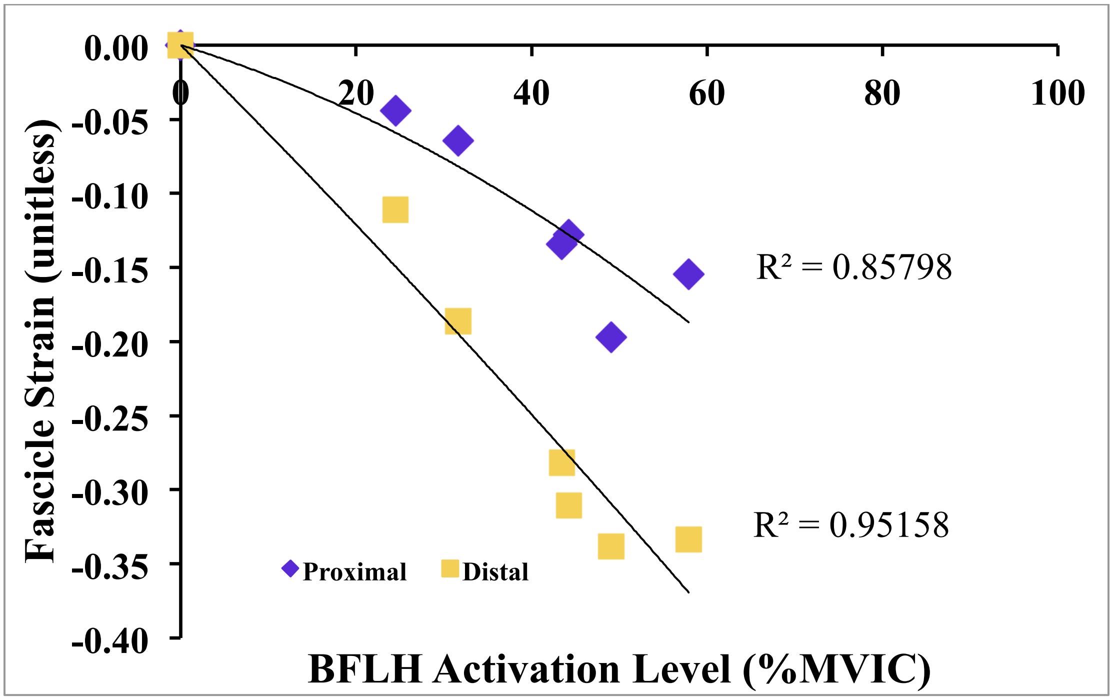

Figure 17b.

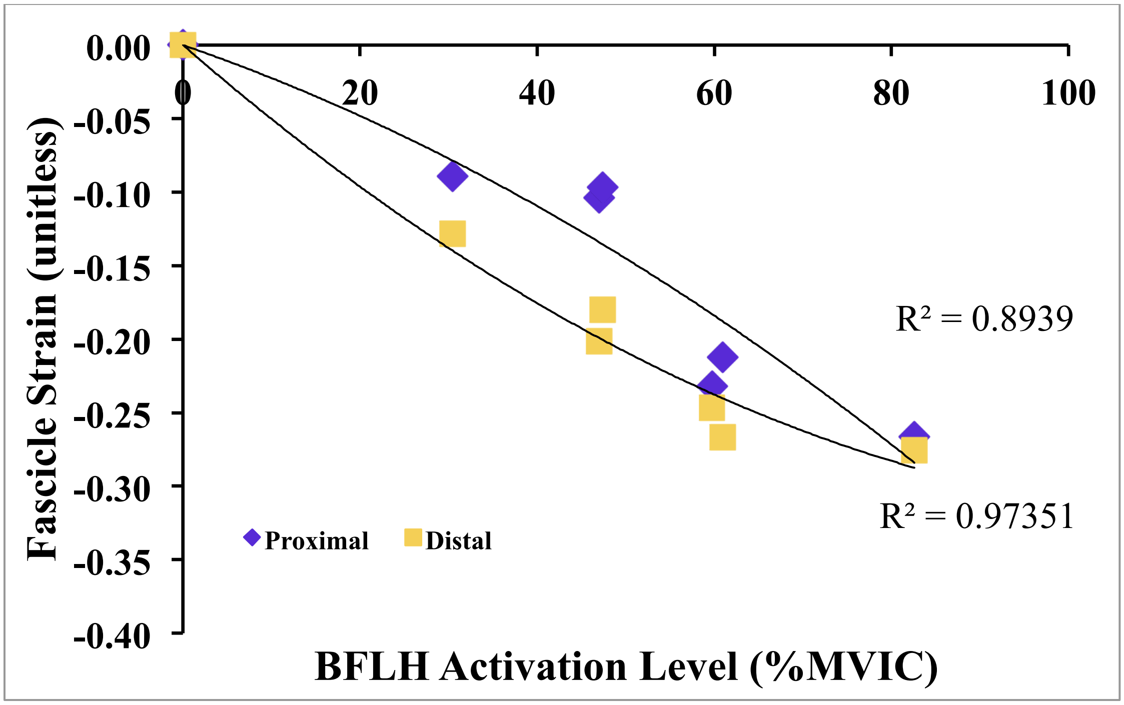

Figure 17c.

Figures 17a-c. *Selected "qualitatively heterogeneous" individual subjects' strain magnitudes across all activation levels.* Note the greater proximal (17a.) and distal (17b.) strain magnitudes and slopes of individual subjects 5 and 6, respectively. Five subjects showed no distinctive difference between regional strain values across activation levels and this is characterized with Subject #7 (17c.). On an individual basis, the R^2 values are also high, and well above those obtained on the group analysis. Both regions began with 0 fascicle strain, which is indicated by both lines beginning at 0.00.

These qualitative individualistic responses suggest: 1) the non-significant heterogeneity observed through the group analyses may not adequately represent the individualistic response because the subject specific responses varied, 2) the presence (or absence) of heterogeneity may be subject specific, 3) the magnitudes of heterogeneity (differences in regional fascicle strains) may also be subject specific, and 4) there is variability in strain magnitudes (proximal or distal) between subjects. Comparatively, the subject specific relationships between regional strain and activation level are much stronger than the group's regional relationships, with the lowest individual polynomial $R^2 = 0.86$ vs. the highest group linear $R^2 = 0.63$. Even when comparing linear relationships, the lowest individual subject's R^2 was 0.83, which is still higher than the group's regional relationships. The graphs of individuals' regional fascicle strains show not only differences between proximal and distal regions within some subjects, but variations in the magnitudes between subjects. While most subjects underwent less than .25 strain at the 66.7% activation level, others underwent up to .33 in one, or both, regions. When assessing strain as a group, it seems that the qualitative heterogeneity presented here is attenuated due to averaging. These qualitative assessments show the importance of interpreting data both in groups and on a subject-specific basis (Figure 17.).

The presence of heterogeneity on an individual basis shown in our study could be indicative of the susceptibility of the location, proximal or distal, of strain injuries within the BFLH. Given the larger strains seen, qualitatively, in either the proximal or distal regions it is possible that if an individual is at risk for injury, these data may indicate that 40% could have a proximal injury, 20% could have a distal injury, and 50% could have proximal or distal injuries. While it was not the intent of this study to predict the location of strain injuries, future longitudinal research correlating regional fascicle strain and hamstring strain injuries is suggested. The next question for this study is how do the experimental methods (isometric contractions at a range of muscle activation levels) transfer to more dynamic and injurious contraction types.

Application to Dynamic and Injurious Contractions

In this study we evaluated an isometric contraction, as such, it is reasonable to question if our results are comparable in magnitude and pattern to literature investigating other modes of contraction, specifically eccentric contractions, when hamstring strains seem to occur most often. Fascicle strain evaluated during passive and active lengthening contractions are similar to the highest strains recorded in this study (Shin et al. 2009). MR imaging of the biarticular gastrocnemius during active and passive eccentric movements has shown strains averaging .40-.50, whereas the largest strain values in our study were .33. Fascicle lengths in isometric and eccentric contractions have also been shown to be similar in a previous study (Reeves et al. 2003). The results show tibialis anterior fascicle lengths measured during isometric and eccentric contractions were not significantly different (P>.05). Although the measurements of dynamic contractions from these studies are a result of activation and musculotendon length changes, these data provide insight of the possible similarities in muscle fascicle behavior between the different modes of contraction.

The evidence of heterogeneous absolute regional fascicle behavior in this study could suggest that in dynamic activities, in which higher activation of the BFLH occurs, the proximal fascicles could be contracting (shortening) more than their distal counterparts. Coincidentally, the highest activation levels of the BFLH occur during the late swing phase in running, when the muscle undergoes its greatest lengthening (Yu et al.2008). It is during the late swing phase when the biarticular hamstrings are concentrically contracting to perform hip extension and eccentrically contracting with extension of the knee before heel contact, as seen in running study analyses (Heiderscheit et al. 2005; Thelen et al. 2005). These previously determined functions of the hamstrings during dynamic movements coupled with the heterogeneity measured, in the absolute fascicle lengths, in this isometric study indicate the need for further investigation of regional fascicle behavior during more dynamic contractions. Further investigation is needed to fully determine the similarity, or lack thereof, in fascicle behavior in the BFLH between these two modes of contraction

Limitations

While this study had a significant region x condition interaction for the absolute fascicle lengths, with power above .90, the non-significant difference with the fascicle strain data (relative lengths) may be a result of the low statistical power. The RMANOVA on fascicle strain observed statistical power to detect regional differences was low (0.10), and the power to detect the region x condition interaction was .128. Therefore, the non-significant difference could be a type 2 error.

The nature of the relationship between ST/SM and BFLH activation in this study could be affected due to the larger amount of data points at the lower activation levels. The greater amount of data points at the lower end could skew the relationship and may explain why we had higher R^2 values with the power equation vs. the linear equation. However, when evaluating the group analyses based on the ST/SM activation data, the interpretation of the results did not change.

The choice of ultrasound imaging for this study came with known limitations. While the ultrasound we used does allow for panoramic longitudinal imaging, the imaging is of two dimensions: length and depth. This complicates muscle fascicle measurements, as entire muscle fascicles lengths are not always in the same planar space being imaged but may come into and out of view of the transducer. Misinterpretations of individual entire fascicle lengths are possible, as one cannot always discern whether in line fascicles are not the same. Also, small measures of shortening and/or lengthening could be occurring outside the scope of the ultrasound transducer. The more proximal fascicles that do not insert onto the deep aponeurosis but continue through the musculotendinous junction could be more susceptible to injury, as the gradual transition from pure muscle to free tendon has been noted to occur in this region (Batterman et al. 2010).

Heterogeneity, as defined in our study, was the difference in fascicle shortening between the proximal and distal regions of the BFLH. It is possible that heterogeneous shortening exists *within* a fascicle. MR imaging of the medial gastrocnemius (Shin et al. 2009) and muscle modeling of the biceps brachii and BFLH (Blemker et al. 2004; Rehorn et al. 2010) have shown significant differences of within fascicle and fiber strains during active and passive muscle contractions and within multiple regions of the muscles. While we did not evaluate within fascicle strain, this type of strain heterogeneity could provide more insight on the mechanisms of hamstring injuries and should be explored in vivo with future studies.

Optimally, we would have liked to model the fascicle force in the BFLH, rather than use predicted BFLH muscle activation to monitor the state of the muscle. Activation alone does not adequately represent the behavior of the muscle, and does not perfectly relate to force. Modeling fascicle force would have taken into account the activation and fascicle length changes. However, models with this magnitude of detail also could have more error inherent to the assumptions made with this model. Future research could investigate regional fascicle force through other imaging techniques, or through modeling, and provide more insight to the relationship between regional fascicle behavior and muscle behavior.

Summary

The results of the study group show that while the proximal fascicles are longer than their distal counterparts regardless of activation level, the proximal and distal fascicles do not undergo heterogeneous amounts of strain during isometric contractions at varying muscle activation levels. The individual analyses provide qualitative evidence of fascicle strain heterogeneity between the proximal and distal fascicles within some subjects, as well as varying amounts of total fascicle strain between some of the subjects. These data support our initial hypothesis of longer proximal vs. distal fascicles, but reject our hypothesis that the proximal fascicles would shorten more (have high strain magnitudes) than the distal fascicles. While the group analyses do not provide insight into the possible mechanisms behind proximal BFLH hamstring strains, the variations between and within individuals provide an interesting result that should be further investigated. These data and the review of literature support the need for future studies of regional fascicle strains during more dynamic contractions, using both group and individual analyses.

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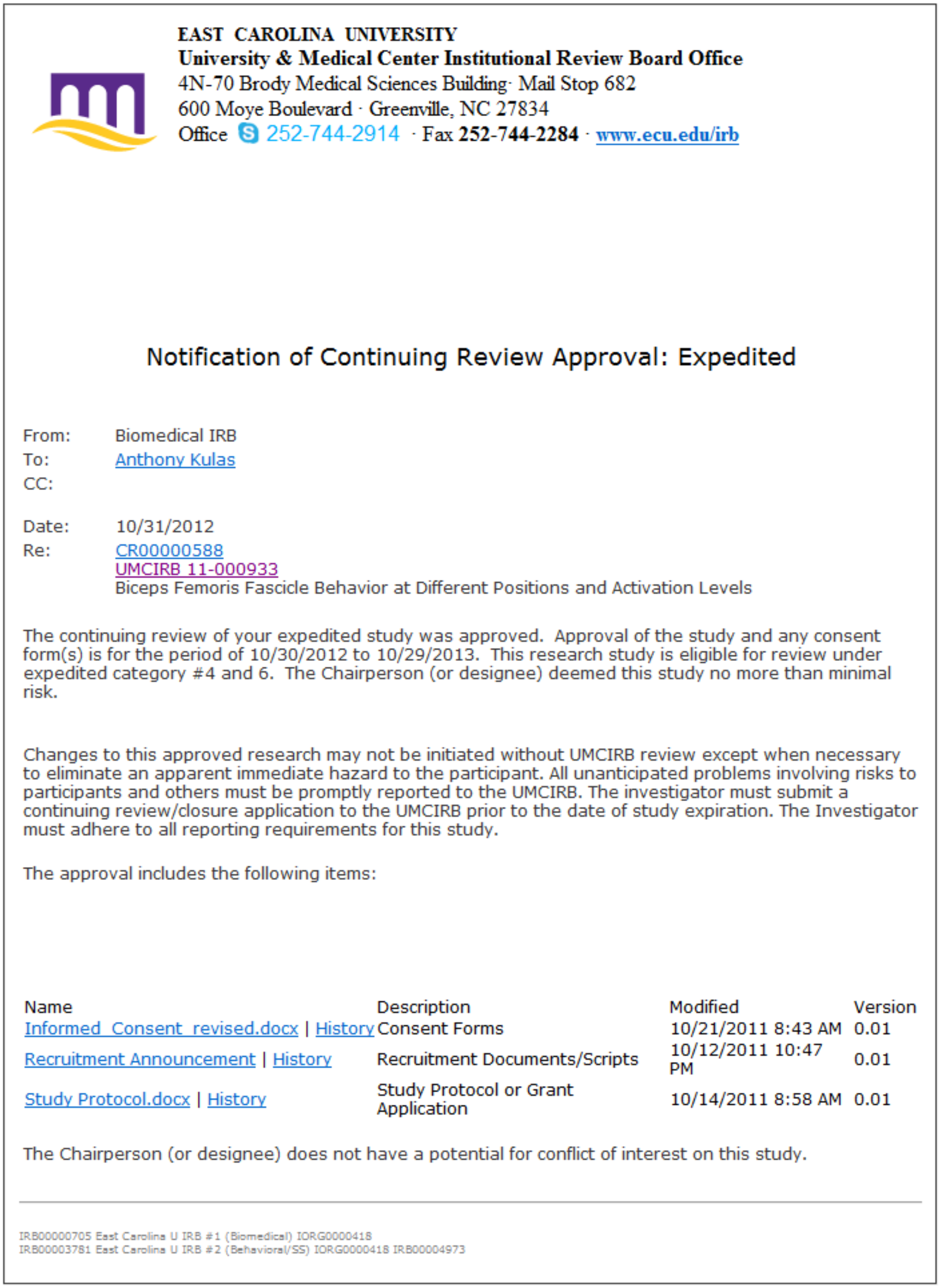
Appendix A: Institutional Review Board Approval



EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office 252-744-2914 · Fax **252-744-2284** · **www.ecu.edu/irb**

Notification of Continuing Review Approval: Expedited

From: Biomedical IRB
To: Anthony Kulas
CC:

Date: 10/31/2012
Re: CR00000588
UMCIRB 11-000933
Biceps Femoris Fascicle Behavior at Different Positions and Activation Levels

The continuing review of your expedited study was approved. Approval of the study and any consent form(s) is for the period of 10/30/2012 to 10/29/2013. This research study is eligible for review under expedited category #4 and 6. The Chairperson (or designee) deemed this study no more than minimal risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The Investigator must adhere to all reporting requirements for this study.

The approval includes the following items:

Name	Description	Modified	Version
Informed_Consent_revised.docx | History	Consent Forms	10/21/2011 8:43 AM	0.01
Recruitment Announcement | History	Recruitment Documents/Scripts	10/12/2011 10:47 PM	0.01
Study Protocol.docx | History	Study Protocol or Grant Application	10/14/2011 8:58 AM	0.01

The Chairperson (or designee) does not have a potential for conflict of interest on this study.

IRB00000705 East Carolina U IRB #1 (Biomedical) IORG0000418
IRB00003781 East Carolina U IRB #2 (Behavioral/SS) IORG0000418 IRB00004973

Appendix B: Subject Consent Form

Study ID: IMCIRB 11-000933 Date Approved: 10/30/2012 Expiration Date: 10/29/2013

1907-2007
CENTENNIAL
East Carolina University

UNIVERSITY HEALTH SYSTEMS
of Eastern Carolina

Informed Consent to Participate in Research

Information to consider before taking part in research that has no more than minimal risk.

Title of Research Study: The Effect of Joint Position and Muscle Activation on Proximal vs. Distal Biceps Femoris Fascicle Behavior.

Principal Investigator: Anthony Kulas
Institution/Department or Division: East Carolina University Department of Health and Human Performance
Address: 249 Ward Sports Medicine Building
Telephone #: (252) 737-2884

Researchers at East Carolina University (ECU) study problems in society, health problems, environmental problems, behavior problems and the human condition. Our goal is to try to find ways to improve the lives of you and others. To do this, we need the help of volunteers who are willing to take part in research.

Why is this research being done?The purpose of this research is to investigate proximal and distal biceps femoris fascicle behavior under various contraction levels and in two different hip positions. Understanding this proximal versus distal fascicle behavior will inform us on how the biceps femoris functions overall and ultimately may also give us a better understanding of 1) why this muscle commonly injured in the proximal versus distal portion and 2) how we might be able to better treat hamstring strains in general. The decision to take part in this research is yours to make.

Why am I being invited to take part in this research?

You are being invited to take part in this research because you: 1) are recreationally active, 2) are currently performing resistance training exercises in the gym, 3) and you have no known history of hamstring injuries. If you volunteer to take part in this research, you will be one of twenty (20) people to do so.

Are there reasons I should not take part in this research?

I understand I should not volunteer to be in this study if I am under 18 years of age, if I have a history of hamstring injuries or any known allergies to hypoallergenic gel commonly used with ultrasound imaging.

What other choices do I have if I do not take part in this research?

Because enrollment in this research study is voluntary, you may simply choose not to participate.

Where is the research going to take place and how long will it last?

The research study will be conducted in the Ward Sports Medicine Building at East Carolina University. You will need to come to the Biomechanics Lab located in room 332 in the Ward Sports Medicine Building once during the study. The total amount of time you will be asked to volunteer for this study is approximately 1.5-2 hours.

What will I be asked to do?

You are being asked to do the following:

- You will be asked to lie on your stomach on a table so that we can image your biceps femoris muscle with ultrasound while you are relaxed and while you perform several knee flexion contractions of varying magnitudes against an immovable dynamometer arm. This process will be performed by you in two different positions – with your hip in a neutral position and flexed to 45 degrees.
- We will also place several electrodes on your thigh to monitor your level of muscle activity during these contraction efforts.

What possible harms or discomforts might I experience if I take part in the research?

It has been determined that the risks associated with this research are minimal. However, we will use straps to secure you to the table during testing to ensure you do not slip off the table. In addition, the ultrasound is used for imaging purposes only, and you should not experience any symptoms throughout the testing period. Lastly, the electrodes on your thigh only monitor your muscle activity and do not produce electrical activity themselves and so you will not experience any pain, discomfort, or other feelings from the electrodes.

What are the possible benefits I may experience from taking part in this research?

This research will help us learn more about hamstring muscle behavior when it is actively contracting. This will ultimately allow the scientific and clinical communities to gain a better understanding of hamstring injuries in general. Although you will not directly benefit from this experience, the research community at large will. At your request, we will be glad to share more information related to this study to enrich your educational experience.

Will I be paid for taking part in this research?

No, we will not pay you for the time you volunteer while being in this study

What will it cost me to take part in this research?

It will not cost you any money to be part of the research.

Who will know that I took part in this research and learn personal information about me?

To do this research, ECU and the people and organizations listed below may know that you took part in this research and may see information about you that is normally kept private. With your permission, these people may use your private information to do this research.

- The University & Medical Center Institutional Review Board (UMCIRB) and its staff, who have responsibility for overseeing your welfare during this research, and other ECU staff who oversee this research.

- Additionally, the following people and/or organizations may be given access to your personal health information and they are:
  - Anthony S. Kulas, PhD, LAT, ATC
  - Hunter Bennett, BS

How will you keep the information you collect about me secure? How long will you keep it?

If you elect to enroll in this study by signing this informed consent document, you be assigned an alphanumeric code. Only this alphanumeric code, not your name, will appear on the saved ultrasound images, data files with containing your muscle activation and dynamometer data, or any other electronically saved measurements. All data collected from you will only have this alphanumeric code associated with it and this data will be backed up on a network server in this lab. The only person to have access to the master list of names which link your name to your alphanumeric code will be the two researchers identified above, Mr. Hunter Bennett and/or Dr. Anthony S. Kulas. All paperwork and forms linking you to the study will be kept in Ward Sports Medicine Building, Room 249, Dr. Kulas's office, which remains locked except when in use. Your ultrasound images and/or muscle activation data collected in this study may be used for manuscript/presentation purposes. If used for these reasons, no information identifying you (your name or alphanumeric code) will be on any images/figures used for research purposes.

What if I decide I do not want to continue in this research?

If you decide you no longer want to be in this research after it has already started, you may stop at any time. You will not be penalized or criticized for stopping. You will not lose any benefits that you should normally receive.

Who should I contact if I have questions?

The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Principal Investigator at (252) 737-2884 (days, between 8am-5pm).

If you have questions about your rights as someone taking part in research, you may call the UMCIRB Office at phone number 252-744-2914 (days, 8:00 am-5:00 pm). If you would like to report a complaint or concern about this research study, you may call the Director of UMCIRB Office, at 252-744-1971.

I have decided I want to take part in this research. What should I do now?

The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I know that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

Participant's Name (PRINT) Signature Date

Person Obtaining Informed Consent: I have conducted the initial informed consent process. I have orally reviewed the contents of the consent document with the person who has signed above, and answered all of the person's questions about the research.

Person Obtaining Consent (PRINT) Signature Date

UMCIRB Number:11-000933
Consent Version # or Date:10/30/2012 – 10/29/2013
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Participant's Initials