**Study Protocol**

**Version 1**

**Assessment of hamstring muscle architecture using a large field of view ultrasound technique: an intrarater reliability study.**

Authors: Kevin Cronin1, Shane Foley1, Giuseppe De Vito2,3, Sean Cournane⁴, Eamonn Delahunt2,3

¹ Radiography and Diagnostic Imaging, School of Medicine, University College Dublin, Dublin, Ireland.

2 School of Public Health, Physiotherapy and Sports Science, University College Dublin, Ireland.

3 Institute for Sport and Health, University College Dublin, Dublin, Ireland.

⁴ School of Physics, Sciences, University College Dublin, Ireland.

Kevin Cronin is an Assistant Professor in the School of Medicine, University College Dublin, Dublin, Ireland. He is registered as a PhD student in the School of Medicine, University College Dublin, Dublin, Ireland.

Shane Foley is an Assistant Professor in the School of Medicine, University College Dublin, Dublin, Ireland.

Giuseppe De Vito is a Full Professor in the School of Public Health, Physiotherapy and Sports Science, University College Dublin, Ireland.

Sean Cournane is an Assistant Professor in the School of Physics, University College Dublin, Dublin, Ireland

Conor McCarthy is a Consultant Rheumatologist in the Mater Misericordiae University Hospital, Dublin, Ireland.

Eamonn Delahunt is a Professor in the School of Public Health, Physiotherapy and Sports Science, University College Dublin, Ireland.

**Title**

Hamstring muscle architecture assessed sonographically using wide field of view: a reliability study

**Abstract:**

Hamstring injuries are very common in field sports. Muscle architecture has been suggested as a risk factor for hamstring strain injury. Various medical imaging techniques (Magnetic Resonance Imaging and Ultrasound) have been developed to assess muscle architecture. Ultrasound is often used to assess in vivo hamstring muscle architecture. The architecture of the hamstring muscles often extends outside the ultrasounds field of view. Previous ultrasound techniques used often generate questionable results. This study aims to describe the reliability of large field of view ultrasound and test – retest minimum detectable difference of the hamstring muscles.

*Key words:* Diagnostic imaging (MeSH); Ultrasonography (MeSH); Muscles, Skeletal; Hamstring muscles.

Introduction

The hamstring muscles are located in the posterior thigh. They are a complex muscle group consisting of three muscles: semimembranosus (SM), semitendinosus (ST) and biceps femoris (BF). The latter muscle has both a long and short head. All three muscle arise from the ischial tuberosity, which is a large osseous tubercle located at the superior pubic ramus of the ischium bone at the pelvis (Becciolini, Bonacchi and Bianchi, 2019). The ST and the BFlh have a common origin on the posteromedial aspect of the ischial tuberosity (Stepien *et al.*, 2019; Philippon *et al.*, 2015; Sato *et al.*, 2012; van der Made *et al.*, 2015). Some authors (Battermann *et al.*, 2011; Miller, Gill and Webb, 2007) interpret the common origin of the ST and BFlh to be the medial aspect of the ischial tuberosity while (Feucht *et al.*, 2015; Neuschwander, Benke and Gerhardt, 2015) claim the common origin to be located on the lateral aspect of the ischial tuberosity. The SM originates on the anterolateral aspect of the ischial tuberosity with its fibres twisting before forming a proper tendon (Stepien et al., 2019). All hamstring muscles have different insertion/attachment sites. In the proximal section of the hamstring muscle complex two main bursa can be identified (Stepien et al., 2019). However, these bursae are difficult to locate on ultrasound and to our knowledge no author has reported this sonographically. Based on anatomical dissection the more proximal bursa overlies the ischial tuberosity (Dierckman and Guanche, 2012; Guanche, 2015; Martin, 1968). The distal bursa is found next to the proximal BFlh muscle, precisely between the common attachment of the BFlh/ST common tendon and the SM proximal tendon adjacent to their bony attachment (Bejui *et al.*, 1984).

The ST muscle is a superficial muscle that lies in the posteromedial area of the thigh. Proximally from its origin on the ischial tuberosity it runs distally overlying the semimembranosus muscle where it inserts onto the medial condyle of the tibia forming part of the pes anserinus (Stepien et al., 2019). The muscle belly of the ST is fusiform in appearance and is deeper in diameter than it is wide (Woodley and Mercer, 2005). Muscle fascicles run vertically down the belly of the muscle in a parallel fashion. The ST muscle is unique in the hamstring muscle complex in that it contains a mid-muscle belly tendinous inscription. This tendinous inscription is often referred to as “median raphe” or “muscle veil” and represents an inverted “V” on exploration (Kellis *et al.*, 2012b; Kellis *et al.*, 2010; Lee *et al.*, 1988; van der Made et al., 2015; Woodley and Mercer, 2005).  Hamstring Strain Injury (HSI) is infrequent in the ST muscle (Askling *et al.*, 2007; Askling *et al.*, 2008; Cohen *et al.*, 2011; Connell *et al.*, 2004; De Smet *et al.*, 2012; Hallén *et al.*, 2014; Koulouris and Connell, 2003; Slavotinek, Verrall and Fon, 2002). Some authors have hypothesised that the presence of this tendinous inscription within the ST muscle belly acts as a protective property against HSI (Kumazaki, Ehara and Sakai, 2012; van der Made et al., 2015)

The SM muscle also lies posterior- medially and is quite superficial. From its origin proximally it descends over the medial condyle of the proximal tibia to the pes anserinus and descends under the ST (Stepien et al., 2019; Bejui et al., 1984). Muscle fascicles at the mid belly of the muscle have a connection to both proximal and distal tendons (van der Made et al., 2015). The muscle architecture distribution throughout the SM is heterogenous in appearance. Proximally the fascicles originate from the proximal tendon which originates from the ischial tuberosity. These fascicles orientate themselves distally where they attach to the lateral surface of the distal tendon (Woodley and Mercer, 2005). At the mid muscle section of the SM muscle the fascicles are orientated differently. Here the fascicles arise from the thin medial surface of the proximal tendon and travel inferiorly and posteriorly to insert into the lateral aspect of the distal tendon (Woodley and Mercer, 2005). The architecture arrangement in the distal segment of the SM is heterogenous in nature and difficult to observe the trajectory of a true fascicle. The fascicles here are bipennate in arrangement (Woodley and Mercer, 2005). The heterogenous arrangement of the muscle fascicles contribute to the bulky nature of the muscle distally.

The biceps femoris is comprised of two heads: the long (BFlh) and the short head (BFsh). The BFlh is a hamstring muscle prone to injury especially to athletes competing that involves high speed running. The proximal tendon of the BFlh forms a conjoint tendon with the ST muscle. The BFlh is a bipennate muscle (Wickiewicz *et al.*, 1983; Woodley and Mercer, 2005; Klein Horsman *et al.*, 2006; Ward *et al.*, 2009). The BFlh contains a non-uniform muscle architecture and a distinct mid muscle aponeurosis (Freitas *et al.*, 2018). It is a biarticular muscle that crosses both knee and hip joints, similar to the SM and ST muscles (Freitas et al., 2018). Distally the BFlh displays shorter fascicles with increased pennation fascicles than those seen proximally within the muscle which exhibit longer fascicle lengths but less pennate fascicles (Tosovic *et al.*, 2016). The BFlh fuses with the BFsh at the distal thigh laterally forming an aponeurotic structure (Schache *et al.*, 2012). Continuing distally this forms a conjoint tendon that attaches to the head of the fibula (Kellis *et al.*, 2012a; van der Made et al., 2015; Klein Horsman et al., 2006) (Wangensteen *et al.*, 2016). The BFsh muscle has much longer muscle fascicles, similar in length to the ST muscle but longer in length than the BFlh muscle (Woodley and Mercer, 2005). The muscle architecture of one hamstring muscle does not represent the muscle architecture of all remaining hamstring muscles (Kellis *et al.*, 2012a).

The sciatic nerve innervates all hamstring muscles (Stępień *et al.*, 2019). The sciatic nerve (also known as the ischiatic nerve) is the longest and largest single nerve in the body that starts in the lower back and runs down the lower limb via the buttocks (Ripani *et al.*, 2006). The ST, SM and BFlh are innervated by the tibial division of the sciatic nerve. The BFsh is innervated by the fibular division of the sciatic nerve. Both heads of the Biceps Femoris muscle and the SM are supplied by one motor branch whereas the ST muscle is supplied by two branches of the sciatic nerve. The sciatic nerve enters the lower limb by exiting the pelvis via the greater sciatic notch underneath the overlying piriformis muscle (Rha *et al.*, 2016). It travels distally towards the popliteal fossa, dividing into its two branches approximately in the lower third of the posterior thigh (Adibatti and Sangeetha, 2014; An *et al.*, 2010; Rab *et al.*, 1997; Rha et al., 2016; Seidel *et al.*, 1996; Woodley and Mercer, 2005).

Hamstring Strain Injury (HSI) is a negative disruption to the architecture of the BFlh, BFsh, ST and SM muscles (Bourne *et al.*, 2017). A HSI most often occurs in a single hamstring muscle, rarely occurring in two or all three muscles at one time (Heiderscheit *et al.*, 2010). HSI are frustrating for all parties involved; for coaches and staff who find themselves without their athlete for selection, for medical staff due to the difficult nature to diagnose and treat effectively but particularly for the athlete as inactivity bears financial difficulties and selection obstacles in team based sports (Hickey *et al.*, 2014). They tend to reoccur and a return to play is capricious (Ernlund and Vieira, 2017; Carlson, 2008). HSI’s are most common in sport and are frequent in field sports but often present in other sports also (Askling *et al.*, 2008). Soccer – the most popular sport in the world, attributes 37 percent of all muscular injuries to HSI’s (Askling *et al.*, 2013; van der Horst *et al.*, 2015). HSI’s are the most frequent injury in soccer (Askling et al., 2013; van der Horst et al., 2015). Incidence and trends are changing relative to HSI’s. There is a mean increase of HSI’s of 4 percent per year reported; with the rate of injuries occurring in training sessions increased more than those occurring in competitive activities (Lempainen *et al.*, 2015; Ekstrand *et al.*, 2016). Hamstring injuries are typically classified into three main categories; grade I (mild), grade II (moderate) or III (severe) (Mason, Dickens and Vail, 2007; Schneider-Kolsky *et al.*, 2006). Depending on the grade of strain and the type of sport involved, return to play variations exist. Where surgery is not needed runners can take as long as 16 weeks, on average, to return to sport without restrictions, whereas dancers can take as long as 50 weeks to return to full activity (Ernlund and Vieira, 2017). Furthermore in professional soccer the average time an athlete is absent from competition due to an HSI is typically 14 days (Ernlund and Vieira, 2017). HSI’s are the primary cause for athlete absence from competitive action (Carlson, 2008; Lempainen et al., 2015; Kerkhoffs *et al.*, 2013; van Dyk *et al.*, 2016). Hamstring injuries have been consistently identified as one of the most common injuries sustained by professional rugby union players (Fuller et al, 2013; Fuller et al, 2016; England Professional Rugby Injury Surveillance Project). They have a high injury burden (i.e., total days injured and unavailable for selection) and a high recurrence rate.

High reinjury rates after an acute hamstring injury are common (Wangensteen *et al.*, 2016). Reinjury rates are reported to range from 14 percent to 63 percent within the same competitive season (Visser *et al.*, 2012; Vos *et al.*, 2014; Ekstrand *et al.*, 2011; Ekstrand *et al.*, 2012; Hallén *et al.*, 2014; Silder *et al.*, 2013). It is apparent that HSI’s appear to be on an upward curve despite the best efforts of coaches and medical personnel to reduce their incidence. (Silder et al., 2013) noted that HSI’s occur at the exact same location as the initial injury site while (Koulouris *et al.*, 2007) noted that the reinjury site was the musculotendinous junction located within the muscle tendon unit. A recurrent HSI is often more severe than the initial HSI (John *et al.*, 2006; Ekstrand et al., 2011). In elite rugby union in Australia recurrent HSI’s have traditionally always been a dilemma with recurrent HSI’s rates as high as 34 percent per season (Seward et al., 1993). However more recent data in elite ruby union conveys approximately 22 percent off all HSI’s were recurrent HSI’s (John et al., 2006). The high recurrence rates perhaps suggest that current rehabilitation practices are not fully addressing the underlying risks factors associated with recurrent HSI’s (Bourne et al., 2017).

The architectural characteristics of a muscle refer to the physical arrangements of muscle fascicles and determine a muscles mechanical function (Salimin et al., 2018). The trajectory of the muscle fascicle between both the superficial and deep aponeurosis is referred to as the pennation angle (Kawakami *et al.*, 2000; Baechle *et al.*, 2008). Greater pennation angles allow a greater amount of muscle fascicles to attach to an aponeurosis thus increasing its physiological cross-sectional area of the muscle (Albracht, Arampatzis and Baltzopoulos, 2008; Bamman *et al.*, 2000; Blazevich *et al.*, 2009). In vivo ultrasound assessment of the architectural characteristics of the hamstring muscle measure the pennation angle by observing where the muscle fascicle inserts into the deep aponeurosis of the muscle (Becciolini, Bonacchi and Bianchi, 2019; Freitas *et al.*, 2018a; Pimenta, Blazevich and Freitas, 2018; Kellis *et al.*, 2010; Kellis *et al.*, 2012b; Franchi *et al.*, 2014; Brennan *et al.*, 2017). An increase in pennation angle will increase the cross-sectional area of the muscle, thus increasing the number of muscle fascicles within the muscle (Salimin et al., 2018). Greater fascicle lengths represent longer and more sarcomeres in series (Blazevich and Sharp, 2006; Earp *et al.*, 2014; Albracht et al., 2008).Architectural characteristics of muscle influence the maximal force output, muscle length, contraction velocity and susceptibility to injury (Brockett, Morgan and Proske, 2004). Muscle architecture is adaptable and responds to a range of stimuli (Bourne et al., 2017). Longer muscle fascicles exhibit less shortening over a given amount of total muscle shortening than shorter fascicles would (Blazevich et al., 2009; Blazevich and Sharp, 2006) (Kumagai *et al.*, 2000) (Abe, Kumagai and Brechue, 2000). According to this hypothesis greater fascicle lengths are beneficial for force production at fast movements and reduces the athlete’s susceptibility to HSI.

Often when an athlete suffers an initial HSI the immediate pain halts their progress in the game or competition (Ernlund and Vieira, 2017). When the muscle is functioning beyond its natural boundaries microscopically the muscle fibers become damaged gradually leading to a tear. Retrospective reports have identified numerous factors of maladaptation’s associated with previous HSI that contribute to reinjury; deficits in the rate of torque development (Opar *et al.*, 2013) and an reduction of BFlh muscle volume (Silder *et al.*, 2008). A reduction in fascicle lengths exhibit weaker muscles (Brockett et al., 2004; Timmins *et al.*, 2015). It is understood that there is a reduction in fascicle lengths post HSI (Brockett et al., 2004; Fyfe *et al.*, 2013). Timmins et al. (2005) explains that this is “troublesome” for reinjury because shorter fascicles would increase the muscles susceptibility to eccentrically induced microscopic damage, which could most likely be a precursor to macroscopic damage in the form of strain injury. However, it is still not understood if a previously strained hamstring muscle contains shorter fascicles prior to the injury happening. Evidence suggests that the BFlh muscle displays shorter fascicles in individuals with a history of strain in injury (Timmins et al., 2015). Possessing shorter fascicles has been suggested to increase the likelihood of microscopic muscle damage as a consequence of repetitive eccentric training such as running (Fyfe et al., 2013). Thus, prospective studies will hope to determine if shorter muscle fascicles increase the risk of future injury in human hamstring muscles.

Over the past thirty years various medical imaging techniques have been used to assess fascicle geometric disposition within skeletal muscle. Up to the early 1990’s this was studied in cadaver specimens (Huijing et al., 1985;Wickiewicz *et al.*, 1983). However, with cadaveric specimens bears many limitations primarily difficulties associated with sample size, due to the limited availability of donor tissues (Kellis *et al.*, 2009). Furthermore, all these studies examined muscle architecture in specimens from elderly individuals (Kwah *et al.*, 2013). Tissue from cadaveric specimens is generally sarcopenic tissue and as such the muscle architecture would vary from the athletic population where the majority of individuals are ages between 18-35 years of age (Blazevich and Sharp, 2006; Opar, Williams and Shield, 2012).

To overcome these limitations medical techniques have been applied to assess in vivo architectural characteristics of skeletal muscles. Brightness mode (B-mode) ultrasound imaging is deemed most popular, because it is relatively inexpensive, it is well tolerated by the athlete and yields accurate results with improving techniques and advancing imaging applications (Becciolini et al., 2019; Bolsterlee, Gandevia and Herbert, 2016b; Bourne et al., 2017; Brennan et al., 2017; Connell *et al.*, 2004; e Lima, da Matta and de Oliveira, 2012; Franchi et al., 2014; Franchi *et al.*, 2018; Gabison *et al.*, 2018; Adibatti and Sangeetha, 2014; Kellis et al., 2012a; Kellis et al., 2010; Kellis et al., 2012b; Kellis et al., 2009; Kumagai et al., 2000; Kwah et al., 2013; Legerlotz, Smith and Hing, 2010; Narici *et al.*, 2003; Rab et al., 1997; Timmins et al., 2015; Wickiewicz et al., 1983; Woodley and Mercer, 2005).

Skeletal muscle has a high-water content therefore on ultrasound imaging will appear mostly hypoechoic (i.e., dark) (Ihnatsenka and Boezaart, 2010). Perimysial connective tissue gives muscle a speckled appearance and will appear hyperechoic (white) within the muscle belly (Franchi et al., 2018). Ultrasound can image skeletal muscle in two planes: transverse and longitudinally. It is in the longitudinal plane we can extract most information about a muscle’s architectural characteristics (fascicle length, muscle thickness and pennation angle).

Different muscles have distinct structural arrangements (Friederich and Brand 1990; (Wickiewicz et al., 1983). The human gastrocnemius muscle, frequently assessed with ultrasound (Mathevon *et al.*, 2015; Hauraix *et al.*, 2015; Bolsterlee et al., 2016b; Bolsterlee, Gandevia and Herbert, 2016a; Bolsterlee *et al.*, 2015; Rosenberg *et al.*, 2014) and vastus lateralis (Varanoske *et al.*, 2017; Ticinesi *et al.*, 2018; Hauraix *et al.*, 2017; Khoshkhoo *et al.*, 2016; Raj, Bird and Shield, 2012; Arroyo *et al.*, 2018) present with muscle fascicle lengths ranging from 3cm to 5cm for the gastrocnemius and 6cm to 8cm for the vastus lateralis muscle. Additionally, the human sartorius muscle displays muscle fascicle lengths that exceed 20 cm (Heron and Richmond, 1993).

Ultrasound assessment of the hamstring muscle group is often favorable for ultrasound assessment and analysis as it is quite a superficial muscle and is easily penetrated with the ultrasound beam. The BFlh muscle is the most common hamstring muscle assessed via ultrasound (Kwah et al., 2013; Potier, Alexander and Seynnes, 2009; Bourne et al., 2017; Timmins *et al.*, 2014; Timmins *et al.*, 2016b; Timmins *et al.*, 2016a; e Lima et al., 2012; Tosovic *et al.*, 2016; Kellis et al., 2012a; Kellis et al., 2010; Kellis et al., 2012b; Kellis et al., 2009). Few studies (Kellis et al., 2010) have assessed the architecture of the semitendinosus muscle via ultrasound and to the authors knowledge no studies (in vivo) have assessed the architectural characteristics of the semimembranosus muscles. Perhaps some reasons for this include; they are infrequently injured in comparison to the BFlh muscle (Bourne et al., 2017; Askling *et al.*, 2007; Ekstrand et al., 2011; Ekstrand et al., 2016; Hägglund *et al.*, 2016; Ernlund and Vieira, 2017) therefore may not be a great clinical concern. Additionally, the architecture is heterogenous in nature, and may prove difficult to track architectural characteristics on ultrasound (Balius *et al.*, 2019).

There are many inconsistencies in technique and analysis across studies to assess the BFlh muscle to pursue a similar pathway that is valid, reliable, reproducible, repeatable and transparent (Potier, Alexander and Seynnes, 2009; Bourne et al., 2017; Timmins *et al.*, 2014; Timmins *et al.*, 2016b; Timmins *et al.*, 2016a; e Lima et al., 2012; Tosovic *et al.*, 2016; Kellis et al., 2012a; Kellis et al., 2010; Kellis et al., 2012b; Kellis et al., 2009). Various studies (Kellis et al., 2010; Kellis et al., 2012b; Kellis et al., 2009; Bourne et al., 2017; Timmins et al., 2016b; Timmins et al., 2015; Freitas et al., 2018a; Pimenta et al., 2018) have used different methods to standardise the transducer orientation and location, however no general consensus is established regarding the best process to limit measurement error. Futhermore these studies lack detail on optimal technique to capture reproducible images and often fail to consider and mention how they tracked the same fascicle over time. However a recent systematic review (Kwah et al., 2013) reported the reliability and validity of B-mode ultrasound in measuring fascicle length and pennation angle across many muscles. This review informs us that B-mode ultrasound is a reliable and valid in vivo method to quantify muscle architecture in comparison to cadaveric samples. The study conducted by (Freitas et al., 2018a) overcomes some limitations (same data acquisition site) associated with previous studies (Kellis et al., 2010; Timmins et al., 2015), identifying a reliable method (ICC >0.9) to assess midmuscle architecture of the BFlh when the same rater performs the image acquisition and digitisation process. This study identified the myotendinosus junction (proximal and distal) of the muscle in question (BFlh) and a mark was drawn on the skin. The distance between both marks was measured and accepted as the BFlh muscle length. This is in contrast to other studies (Timmins et al., 2014; Timmins et al., 2016b; Timmins et al., 2016a; Timmins et al., 2015; Kellis et al., 2012a; Kellis et al., 2012b; Kellis et al., 2009; Kellis et al., 2010; Potier et al., 2009) who use bony landmarks to calculate muscle length and decide on a same data acquisition site. This technique is difficult to reproduce as bony landmarks are not constant if the athlete is not set up in an identical position as the previous time. To identify the specific region within the BFlh muscle (Freitas *et al.*, 2018b) scanned the muscle belly in transverse and longitudinal planes until the resultant image cleary identified both the superficial and deep aponeurosis in a parallel orientation and where a muscle fascicle was clearly identified. The ultrasound transducer location on the BFlh muscle was noted and the distance from the distal myotendinosus junction was recorded. Identifying this location again differs from previous studies (Kellis et al., 2012a; Kellis et al., 2010; Kellis et al., 2012b; Kellis et al., 2009; Potier et al., 2009; Timmins et al., 2014; Timmins et al., 2016b; Timmins et al., 2016a). Furthermore, it is necessary to clearly identify the exact location site to acquire muscle architecture data as the architecture within a muscle can vary considerably (Timmins et al., 2015). For example the BFlh posesses proximal fascicle which are longer in length compared to distal fascicles (Bennett, Rider, Domire, DeVita, & Kulas, 2014). Therefore standardising the assessment location is paramount to minimise any variations due to inconsistencies in the scanning site (Timmins et al., 2015). Clearly this (Freitas et al., 2018b) is the current “gold standard” reproducible method to identify the same location site with a proven accurate technique, yet bears associated limitations like similar studies in the small field of view to capture the image and in the image analysis section which assumed the fascicle travel in linear fashion.

One key limitation of ultrasound imaging when assessing the muscle architecture of the hamstring muscles is its relatively small field of view. The field of view is determined by the size of the linear array transducer (Ihnatsenka and Boezaart, 2010). Commercial linear array transducers suitable for assessing the muscle architecture operate with field of views typically between four to six cm (Franchi et al., 2018). These fields of view are typically shorter than the fascicles under investigation (Blazevich, Gill and Zhou, 2006). In this instance fascicle length is estimated with various linear approximations using measured muscle thickness and pennation angle values (Blazevich et al., 2006; Kellis et al., 2012b; Kellis et al., 2009; Kellis et al., 2010; Timmins et al., 2014; Timmins et al., 2016a; Timmins et al., 2015). These methods fail to consider the variability associated with fascicular curvature and as such are prone to error (Darby, Li, Costen, Loram, & Hodson-Tole, 2013; Rana, Hamarneh, & Wakeling, 2014).

Extended Field of View (EFOV) was introduced by many researchers to overcome this key small field of view limitation (Adkins, Franks and Murray, 2017; Noorkoiv *et al.*, 2010; Seymore *et al.*, 2017; Simpson *et al.*, 2017). This technique impliments texture mapping algorithms to merge sequences of images collected during dynamic scanning to reconstruct large composite images (Franchi et al., 2018). It requires a high skill from the operator however (Noorkoiv et al., 2010) reports high levels of reliability with an ICC of 0.99. It is important to note that EFOV is not suitable for all muscles particulary the hamstring muscles as the architecture distributuion within these muscles is hetergenous in nature (Blazevich and Sharp, 2006). Thefore this is a challenge practically, as it is cumbersome and extremly difficult to satisfy the fundamental requirement of keeping the transducer parallel to the fascicle plane when covering a large area of interest (Franchi et al., 2018). Additionally a limitation of this technique is observed in the use of a linear array transducer over curved surfaces such as the hamstring muscle in the poster thigh – where exerting any pressure on the muscle to ensure proper fascicle alignment mighy depress the underlying muscle and influence measurement results (Franchi et al., 2018). This technique requires extensive operator training and still will not guarantee high reproducible/repeatable values. Not all ultrasound applications support EFOV and this technique is not popular in assessing the architecture of muscles during contraction, which is also quite popular (Franchi et al., 2018).

Furthermore to obtain a field of view without estimating the remaining fascicle length (extrapolation method with a small field of view) or utilising EFOV some authors (Brennan et al., 2017; Herbert *et al.*, 2011) have suggested using a dual transducer system. This system places two 5cm linear array transducers side by side and held together in a small frame. According to (Brennan et al., 2017) this new technique allows more precise estimations of muscle fascicles. However this technique means that there is a 2cm gap between both images taken and could be tedious and difficult to “stitch” images together to calculate muscle architecture properties.

One way to overcome all these difficulties to assess muscle architecture in muscles with long fascicles is to use an ultrasound transducer with a long field of view. Such a transducer would capture the full fascicle in its length and not rely on estimates via linear exploration equations to calculate the correct fascicle length. Therefore by implementing a correct technique, the full fascicle can be identified on the sonogram and be saved for later analysis. A large field of view transducer like this would capture static images unlike EFOV, which requires dynamic scanning, and allows a transparent, valid, repeatible and reproducible technique to persue. Temporal resolution is often limited with transducers with larger field of views however with advancements in technology this issue is minimal to none, and (this transducer) still acquire higher quality images than other studies ('<ECR2014 Ultrasound Anatomy.pdf>,' ; Abe et al., 2000)which used now dated ultrasound applications.

Acquired ultrasound images are often manually analysed identifying muscles fascicles and pennation angles (Abe et al., 2000; Timmins et al., 2014; Timmins et al., 2016b; Timmins et al., 2015; Cronin *et al.*, 2011; Cronin and Lichtwark, 2012). Muscle fascicle length was estimated by placing a minumum of two points that represented the proximal and distal ends of the fascicles inserting into the deep and superficial aponeurosis (Cronin and Lichtwark, 2012). This method is incorrect as we know fascicles do not travel in a straight line, are curved in nature, and the fact that the muscle belly is not homogenous in thickness throughout (Blazevich and Sharp, 2006). Some studies (Af Klint *et al.*, 2010; Cronin *et al.*, 2009) used a three point tracking procedure along the fascicle (proximal, mid and distal fascicle) in an attempt to account for fascicle curvature. This process was either performed by a number of raters or a single rater and was performed a number of times. This procedure was tedious, time consuming and depending on the number of raters was subjective and prone to error (Drazan, Hullfish and Baxter, 2019). To calculate the pennation angle researchers calculate the angle the fascicle inserts into the deep aponeurosis. Many studies track the architectural characterstics of muscles over time in eith a passive or active state. This produces huge amounts of data. In the past decade many authors experimented with automated analysis to track architectural characterstics of skeletal muscles. Several different methods have been developed with the view of efficient and effective ultrasound data analysis of skeletal muscle (Loram, Maganaris and Lakie, 2004; Loram, Maganaris and Lakie, 2006; Herbert et al., 2011; Cronin et al., 2011; Cronin et al., 2009; Cronin and Lichtwark, 2012; Zhao and Zhang, 2011; Miyoshi *et al.*, 2008; Rana, Hamarneh and Wakeling, 2009). Not all these software applications are free access however we experimented with some of these automated analysis that were available to us. None fulfilled our requirements for various reasons; the software did not correctly identify the muscle, and/or more often than not we were unable to analyse the same fascicle on a particular sonogram. Due to the relative novelty of some of these methods, and the specific experimantal conditions under which each of these has been tested it was not possible to identify an optimal method of tracking suitable to our study. What we propose is to use a semi automated image analysis using the Matlab software. We have developed our own algorithm within this application that will encorporate a six point tracking procedure (two points at the proximal, mid and distal fascicle). This will account for the curvature illustrated along the full fascicle captured. A measurement in millimeters will be given. This is termed the numerical method. The full fascicle length is then extracted and placed within a interpolated curve. The numerical method interpolates between each of the selected points, using a cubic spline interpolation. Then the curve is differentiated along the interpolated curve. The trapezoidal rule is then used to sum the length.The percentage difference between the numerically calculated (using points on the digitised sonogram) and the mathematically determined (true) is calculated. The difference between both sets of measurements should be minimal. We tested this semi automated analysis for measuring fascicle length. We found the difference in millimeters between the numerical method (6 point system) and the interpolation method (extracted fascicle onto a interpolated curve) to be a 0.2 percent difference.

**Aims**

The aim of our study is to determine the intrarater reliability of a wide field of view ultrasound technique to assess the architectural characteristics (thickness; fascicle length; fascicle pennation angle) of the hamstring muscles.

**Hypothesis**

We expect to achieve excellent intrarater reliability (ICC values >0.90) when using the large field of view ultrasound technique to assess the architectural characteristics of the hamstring muscles.

**Design**

This study is designed as an intrarater reliability study.

**Technique to calculate muscle length**

An initial scan of the Hamstring Muscle Group should be completed. To calculate the length of each muscle; Locate the proximal myotendinous junction by orientating the transducer transversely along the long axis of the muscle. When the smallest proximal muscle section is observed, the transducer is orientated longitudinally at the intersection of the deep and superficial aponeurosis. Mark on the skin “X” with a washable Crayola skin marker. This “X” represents the proximal MTJ. Next locate the distal MTJ in similar fashion – mark on the skin “X” with a washable Crayola skin marker. The distance between the two “X” marks represents the muscle length. Before a mark (X) is made – confirmation of both proximal MTJ and distal MTJ should be assessed three times. This ensures an accurate marking of the MTJ occurs and thus an accurate measurement of the entire muscle. This technique is identical to the technique implemented by Freitas et al (2017) when assessing the BFlh muscle architecture which yielded high intra rater ICC (0.95).

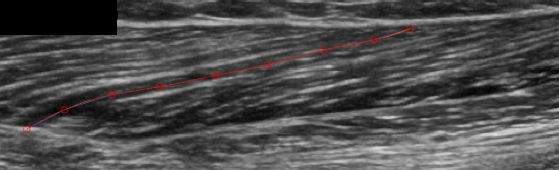
Once both proximal MTJ and distal MTJ are marked; the distance between both “X’s” represents the true length of the muscle. The length is measured with tape in millimetres.

**Standardisation of specific region**

Each muscle is divided into a proximal and distal zone. The proximal zone of the muscle represents the upper half of the muscle length while the distal zone represents the lower half of the muscle length. The specific region for examination of muscle architecture (Fascicle length, pennation angle, muscle thickness) is chosen in each zone by scanning the zone in both transverse and longitudinal planes until the sonogram clearly captures the superficial and deep aponeuroses in parallel orientation, where the fascicles are easily identifiable and attaching to the deep aponeurosis of the muscle. This is somewhat similar to the assessment of BFlh architecture by Freitas et al. (2017) yet differs in that they only acquired data from the mid muscle region of the BFlh. Other studies failed to convey their specific region for data acquisition.

**Standardisation of region on Ultrasound Transducer**

The Ultrasound transducer has a Field of View (FOV) of 92mm. Once the proximal/distal zone is scanned initially to identify a suitable fascicle to image, the transducer will be orientated longitudinally. A mark “X” will be made at the proximal end of the transducer. This mark will then be measured with tape in millimetres, its distance from the proximal MTJ. Marking an “X” at the proximal end of the transducer utilises the full 92mm field of view. This will ensure that the full fascicle is captured on the sonogram. Additionally, the fascicle appearing at the bottom left of the screen (inserting into the deep aponeurosis) ensures that this is the same fascicle being monitored over time.



**Proximal Zone**



Figure 1: Each muscle will be divided into a proximal and distal zone. The proximal end of the transducer will be placed at a fascicle clearly visible. This location will be marked and recorded from the proximal MTJ to ensure the same fascicle is tracked over time.

**Data Acquisition sites**

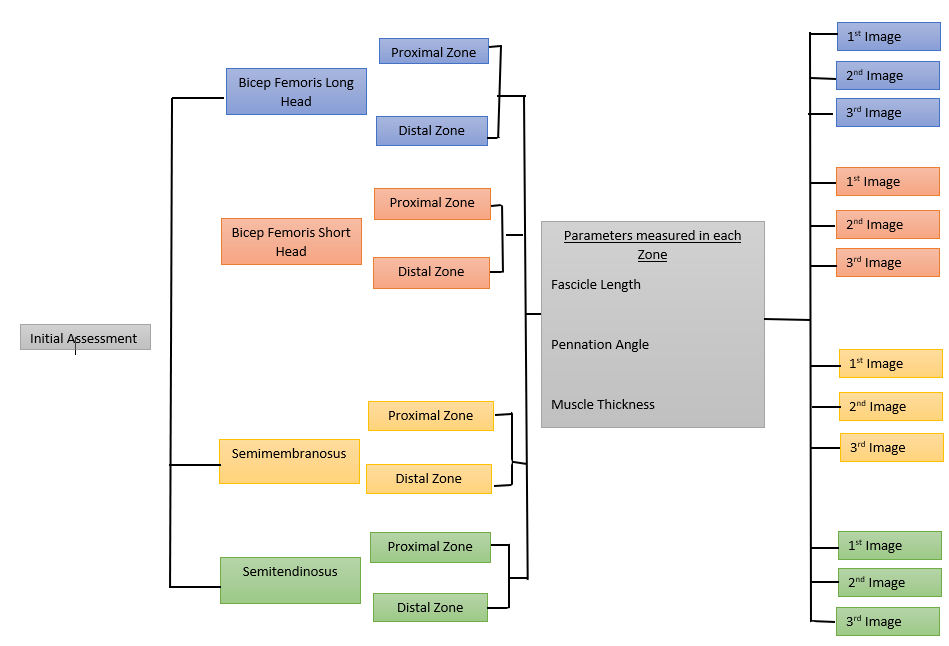
Please also refer to the flowchart seen in figure 2.

Bicep Femoris Long Head: At the proximal and Distal Zones, Fascicle Length, Pennation Angle and Muscle thickness will be recorded three times.

Bicep Femoris Short Head: Only the Muscle thickness will be recorded three times. The BFsh contains fascicles that extended beyond the transducers FOV.

Semitendinosus: Only the Muscle thickness will be recorded three times. The ST contains fascicles that extended beyond the transducers FOV.

Semimembranosus: At the proximal Zone only will fascicle length, pennation angle and muscle thickness be recorded. The distal zone of the semimembranosus muscle displays heterogenous architecture difficult to quantify.



**Figure 2:** A flowchart illustration proposed data acquisition sites of the hamstring muscle group.

**Image Quality**

**Quality Assurance**

The benefits of implementing regular quality control tests are widely recognised in terms of image quality (Sipila, Mannila and Vartiainen, 2010). Quality control tests may be tedious to perform but are worthwhile because it may be difficult or even impossible to detect calibration and measurement distortion from examination of images alone (Sanders and Winters, 2006). A series of tests (listed below) will be carried out on a multi-purpose, multi-tissue ultrasound phantom (CIRS phantom, certified to ISO 9001:2008 standards). Tests will be carried out at least two times per year, for the duration of the project. Baselines will be established from a series of tests conducted. Allowable deviations from baselines measurements will be established. If the baseline is exceeded (established cut off point), the operator will run the tests again and where baselines again exceed the cut-off point, the equipment will be removed from practice and the equipment manufacturer will be contacted to rectify the issue.

**Quality Control Tests**

**Uniformity Testing**

Uniformity is defined as the ability of the ultrasound application system to display echoes of the same magnitude and depth with equal brightness on the display monitor. This is a good test to ensure all crystals within the transducer are functioning.

**Depth of Penetration**

This is the greatest distance in a phantom for which echo signals caused by scattering in the background material can be detected on the display. Again, this is a good test to ensure all crystals within the transducer are functioning.

**Axial and Lateral Resolution Testing**

Axial resolution is the ability of an ultrasound system to resolve objects in close proximity along the axis of the beam. It determines how close two objects can be along the axis of the beam can be detected as two distinct objects. Lateral resolution, similar to axial resolution, however this determines the resolution perpendicular to the beam axis.

Ultrasound examination of the hamstring muscles requires a demanding technique from the operator and an in-depth anatomical knowledge of the posterior thigh (Balius *et al.*, 2019). The skill of the operator is of utmost importance when assessing the complex architecture of the hamstring muscle complex (Timmins *et al.*, 2015). In all instances an initial scan of the ST, SM, BFlh and BFsh will ensue as per (Balius et al., 2019) identifying landmarks and common structures before any measurements will ensue. A single assessor aids in the reliability of measurements both at acquisition and at data analysis (Klimstra et al., 2007; Kwah et al., 2013).

**B-mode**

Brightness mode (B-mode) ultrasound relies on the transmission of echo pulses from an ultrasound transducer (Franchi et al., 2018). The ability of the ultrasound transducer to penetrate skeletal is proportional to its wavelength whereas the spatial resolution is inversely proportional to it (ibid et al., 2018). Skeletal muscle is superficial therefore is easily penetrated and requires only a high frequency wavelength (Zagzebski, 1996). Frequencies of between 5-10MHz are often used to analyse skeletal muscle (Abe, Kumagai and Brechue, 2000; Aeles *et al.*, 2017; Arroyo *et al.*, 2018; Balius et al., 2019; Becciolini, Bonacchi and Bianchi, 2019; Blazevich *et al.*, 2009; Blazevich, Gill and Zhou, 2006; Bolsterlee, Gandevia and Herbert, 2016b; Bourne *et al.*, 2017; Brennan *et al.*, 2017; Cho and Kim, 2016; Cronin *et al.*, 2011; e Lima, da Matta and de Oliveira, 2012; Farris and Lichtwark, 2016; Franchi *et al.*, 2014; Freitas *et al.*, 2018; Hauraix *et al.*, 2017; Kellis *et al.*, 2010; Kellis *et al.*, 2012b; Kellis *et al.*, 2009; Klimstra *et al.*, 2007; Koulouris and Connell, 2003; Kumagai *et al.*, 2000; Kwah *et al.*, 2013; Mairet, Maïsetti and Portero, 2006; Mathevon *et al.*, 2015; Miyoshi *et al.*, 2008; Narici, 1999; Pimenta, Blazevich and Freitas, 2018; Rab *et al.*, 1997; Raj, Bird and Shield, 2012; Rana, Hamarneh and Wakeling, 2009; Seymore *et al.*, 2017; Simpson *et al.*, 2017; Ticinesi *et al.*, 2018; Timmins *et al.*, 2014; Tosovic *et al.*, 2016; Zhao and Zhang, 2011).With reference to muscle architecture (Cronin and Lichtwark, 2012) explain that modern ultrasound devices possess the necessary resolution to optimally analyse ultrasound images efficiently.

**Focus**

The focus must be set at the region of interest (40mm). This is where the ultrasound beam is at its narrowest, and strongest, thus improves the resolution (lateral resolution) of the overall image(Zagzebski, 1996). Thus, it will add clarity to the image, cleansing and sharpening the sonogram enabling a vivid image (Ihnatsenka and Boezaart, 2010), which is important when viewing the fascicle after the digitisation process. A single broad focal zone will be implemented – that spans the entire muscle thickness in the longitudinal plane.

**Gain**

The Gain control adjusts the overall amplification of the returning reflections displayed on the ultrasound image.  The gain adjustment can be likened to the brightness adjustment on a television. There seems to be a vast difference towards gain preference. Some studies (Kellis *et al.*, 2012a; Kellis et al., 2010; Kellis et al., 2012b; Kellis et al., 2009) often prefer an overall darker sonogram to track fascicles whereas more recent authors choose brighter sonograms to observe the muscle architecture (Adkins, Franks and Murray, 2017; Blazevich et al., 2006; Franchi *et al.*, 2018; Freitas et al., 2018; Pimenta et al., 2018). A reason for this could be newer ultrasound technology possesses many applications to improve and enhance the resolution of the sonogram (Cronin and Lichtwark, 2012; Ihnatsenka and Boezaart, 2010). An optimal gain was set based on analysis of the literature by the primary author of images provided in all electronic and hard copy literature. Additionally, a “Hamstring parameter setting” was developed by an application specialist in the company of the primary researcher, a medical physicist and a sonographer with over ten years clinical experience. This selected gain will remain constant throughout the duration of the study. Unit of measurement is in decibels (Db) (Zagzebski, 1996).

**Depth**

The depth simply determines “how far” into the body one wishes to image. Depth is in increments of centimetres (Zagzebski, 1996). Initially it was assumed the same depth will remain throughout each examination. However, it is wise to optimise your field of view to enable the full fascicle to be captured on the sonogram and analysed later. This will often require the operator to alter the depth parameter specific to muscle location and per athlete. At the analysis section once all images are digitised, the depth at each location will be recorded on an Excel spreadsheet and will be reviewed and referred to next time the athlete is scanned. This ensures similar parameters (in this case depth) is implemented each time. It is important to note that as body habitus differs between individuals, some hamstring muscles appear deeper on ultrasound than others. Thus, it would be incorrect to standardise a depth increment into this protocol. Depth will be set to 80mm.

**Transducer orientation**

The most accurate measurements are made when the transducer are orientated so that the image plane aligns with the muscle fascicles, and for measurements of pennation angle when the image plane intersects the aponeurosis (i.e. fascicle is seen inserting into the deep aponeurosis) (Bolsterlee, Gandevia and Herbert, 2016a). However, this can be practically difficult as the smallest mal manoeuvre of the transducer can distort the image. To limit error the primary researcher will implement recommendations on transducer orientation as per (Bolsterlee et al., 2016a)

* ensure the transducer is always perpendicular to the skin surface. If this is not implemented pennation angles will be overestimated.
* ensure the transducer is aligned to the image plane of the muscle fascicles. A tilt error of 5.5⁰ induces a >2mm error in fascicle length, tilt error of 10⁰ induces a >4mm error while a tilt error of 20⁰ induces an error of almost 7mm.
* ensure the fascicle in question is seen inserting into the deep aponeurosis of the muscle. The author acknowledges that this was conducted on a gastrocnemius muscle and might prove challenging in larger and deeper muscles such as the hamstring muscles.

**Room Lightening**

To replicate the clinical setting, light will be powered off. The researcher scans with the light off clinically.

**Setting**

A single room in the clubhouse of each volunteers. All windows will be blacked out to represent the clinical setting of the researcher who clinically scans with ultrasound in total darkness. All clubhouses are within Dublin, Ireland. Volunteers will not receive any payment for participation in this study.

**Subjects**

We will test 20 consecutive male volunteers. The volunteers were recruited through the dissemination of flyers (see appendix) throughout numerous sporting organisations clubhouses (amateur GAA teams) in Dublin.

Each volunteer will need an Ultrasound Examination Entry to be created on the ultrasound application system. Participant Examination Entry on the ultrasound application system should not include the participants name but should be coded according to the participant e.g. “ParHamRelX” (Par = Participant, Ham = Hamstring, Rel = Reliability, X = Participant Number).

**Inclusion criteria**

* Male gender
* Athletes

**Exclusion criteria**

* Age less than 18 or more than 65 years.
* Not in a good general health

**Storage**

The ultrasound application system used in scanning the volunteers (Hitatchi Noblus) is a portable ultrasound machine. It facilitates extraction of data from the machine via a USB port. All images will be saved on an USB key and uploaded and digitised for image analysis on the Image J software. All material stored/assessed on either the USB key or Image J will be password protected. The USB key will be stored in a locked filing cabinet in the primary researcher office in University College Dublin. This office is a locked shared office only accessible with a key.

**Sample size considerations**

Sample size estimation is an important initial step when researchers are planning the design and conduct of their study (Walter et al, 1998). The sample size for a test-retest reliability study depends on the minimally acceptance level of reliability ᵨₒ, the target level of reliability ᵨ (measured as interclass coefficient), and the number of repetitions of the measurement n. The sample size was calculated by use of G\*Power software. Considering an effect size of 0.9, significance level of 0.05 and a statistical power (*p*) of 0.8, the minimal sample size was 16. In summary, 20 subjects participated in this study, *p* = 0.87.

**Statistical Analysis**

Reliability refers to the consistency of a test or a measurement (Weir, 2005). This study will assess the reliability of a new ultrasound measurement technique used to quantify muscle architecture (fascicle length, pennation angle and muscle thickness). The calculation of reliability starts with the performance of repeated measures ANOVA (test retest). Each volunteer will have both limbs assessed over two sessions. Each session will be approximately one week apart.

*Intraclass correlation coefficient (ICC):* it measures the relative homogeneity within sessions in relation to the total observer variation between sessions. For this analysis an absolute agreement will be selected, and ICC for grouped measurements (three measurements will be acquired per zone in each muscle for each muscle) (fascicle length, pennation angle and muscle thickness). An ICC value >0.9 indicates excellent reliability.

**Relevance**

To the knowledge of the authors, there has not been any study on the reliability of a large FOV (>6cm) linear array ultrasound assessment of the muscle architecture of the hamstring muscles to date. Most studies on quantifying muscle architecture of skeletal muscle used a transducer with a narrow field of view which was typically shorter than the muscle fascicles. Thus, muscle fascicle length was estimated with various linear approximations and failed to consider the variability associated with fascicular curvature and as such are prone to error.

**Risks**

No risks related to the ultrasound assessment of the hamstring muscles are known.

**Financing and Insurance**

The purchase of an ultrasound transducer (€6000) was supported by Professor Eamonn Delahunt and Professor Giuseppe De Vito. Additionally, the 1st author will have 75% of his PhD registration fees covered for this first three years of the overall project.

**Ethics**

Ethical exemption was granted by the university’s ethics committee, HREC Ref no: LS-E-19-83.

**Authorship**

Authorship for the project will be based on the Uniform Requirements for Manuscripts of the International Committee of Medical Journal Editors. According to these requirements, authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3. Changes in the tasks of the authors listed below may lead to loss of authorship for some contributors or addition of new authors during the course of the project.

Tasks of Authors

Study design: all the authors

Draft of the study protocol: Mr Kevin Cronin, Professor Eamonn Delahunt, Dr Shane Foley

Logistic organisations: Mr Kevin Cronin, Professor Eamonn Delahunt, Dr Shane Foley

Performance of the experiment: Mr Kevin Cronin

Analysis of data: Mr Kevin Cronin, Professor Eamonn Delahunt, Dr Shane Foley, Dr Sean Cournane

Interpretation of findings: all authors

Draft of article: Mr Kevin Cronin, Professor Eamonn Delahunt, Dr Shane Foley, Giuseppe de Vito, Dr Sean Cournane

Final version of article: all authors

Senior responsibility of all processes: Professor Eamonn Delahunt and Dr Shane Foley

The study will be part of the dissertation of Mr Kevin Cronin

**Time plan**

Volunteer recruitment: from April 2019 to June 2019

Ultrasound assessment of each volunteer: from September 2019 to October 2019

Data analysis: November 2019

First draft of paper: January 2020

Submission to peer – reviewed journal: February 2020.

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