Biceps femoris long head architecture: the association with hamstring injury and response to training.

Submitted by

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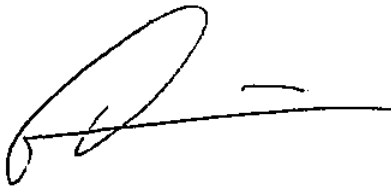*Graduate Research Office:*

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No other person's work has been used without due acknowledgment in the main text of the thesis. All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees.

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Ryan Gregory Timmins   Date

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List of publications related to thesis

1. **Timmins RG,** Shield AJ, Williams MD, Lorenzen C, & Opar, DA (2015). Biceps femoris long head architecture: a reliability and retrospective injury study. *Medicine & Science in Sports & Exercise*, *47*(5), 905-913.

2. **Timmins RG,** Shield AJ, Williams MD, Lorenzen C, & Opar DA (2015). The assessment and adaptability of muscle architecture: Implications for muscle strain injury. *British Journal Sports Medicine*, Accepted August 2015.

3. **Timmins RG** Bourne MN, Shield AJ, Williams MD, Lorenzen C, & Opar DA (2015). Biceps femoris long head architecture, eccentric knee flexor strength and hamstring injury risk in professional football (soccer): a prospective cohort study. *British Journal Sports Medicine*, Accepted September 2015.

Manuscripts related to the thesis currently under review

1. **Timmins RG,** Ruddy J, Presland J, Maniar N, Shield AJ, Williams MD, & Opar DA (2015). Architectural adaptations of the biceps femoris after concentric or eccentric training. *Medicine & Science in Sports & Exercise*, Under Review – Major revisions received – Accepted with minor revisions September 2015.

Other relevant manuscripts

1. **Timmins RG,** Shield AJ, Williams MD, & Opar DA (2015). Is there evidence to support the use of the angle of peak torque as a marker of hamstring injury and re-injury risk? *Sports Medicine*, Accepted August 2015.

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List of conference presentations

1. **Timmins, RG**; Porter, KP; Williams, MD; Shield, AJ; Opar, DA. Biceps femoris muscle architecture – the influence of previous injury. *International Olympic Committee Conference on Injury and Illness in Sport* – Monaco, April 2014.

2. **Timmins, RG,** Shield, AJ, Williams, MD, Lorenzen, C, & Opar, DA. Differences exist in the architectural characteristics of the biceps femoris long head in previously injured individuals. *Australian Conference of Science and Medicine in Sport (Sports Medicine Australia)* – Canberra, October 2014.

3. **Timmins, RG,** Bourne, MN, Shield, AJ, Williams, MD, & Opar, DA. Biceps femoris long head architecture and hamstring injuries in elite soccer. *Football Medicine Strategies for Player Care* – London, April 2015.

4. **Timmins, RG,** Bourne, MN, Shield, AJ, Williams, MD, & Opar, DA. Eccentric hamstring strength and injuries in elite soccer: a prospective study. *Football Medicine Strategies for Player Care* – London, April 2015.

5. **Timmins, RG,** Bourne, MN, Shield, AJ, Williams, MD, & Opar, DA. Biceps femoris long head architecture and hamstring injuries in elite soccer. *World Congress on Science and Football* – Copenhagen, May 2015.

6. **Timmins, RG,** Bourne, MN, Shield, AJ, Williams, MD, & Opar, DA. Eccentric hamstring strength and injuries in elite soccer: a prospective study. *World Congress on Science and Football* – Copenhagen, May 2015.

7. **Timmins, RG,** Bourne, MN, Shield, AJ, Williams, MD, & Opar, DA. Strength and architectural risk factors for hamstring strain injury in elite Australian soccer: A prospective cohort study. *Australian Conference of Science and Medicine in Sport (Sports Medicine Australia)* – Sanctuary Cove, October 2015.

8. **Timmins, RG,** Ruddy, J, Presland, J, Maniar, N, Shield, AJ, Williams, MD, & Opar, DA. Architectural adaptations of the biceps femoris long head following concentric and eccentric resistance training: A randomised control trial: *Australian Conference of Science and Medicine in Sport (Sports Medicine Australia)* – Sanctuary Cove, October 2015.

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Abstract

Hamstring strain injuries (HSIs) are the most common injury in many running based sports, and following the initial insult the risk of recurrence remains high. In order to reduce the risk of a hamstring strain re-injury, developing a greater understanding of the maladaptations associated with a previous insult is important. Despite continual research over the past decade, recurrence rates have not declined suggesting the aetiology of re-injury is still not well understood. Whilst non-modifiable risk factors (e.g. increasing age and previous injury history) have been implicated in the incidence of first time and recurrent injuries, there are still a number of modifiable risk factors that are yet to be examined in detail in the scientific literature. One such factor hypothesized to influence the risk of HSI is the architectural characteristics of the biceps femoris long head (BFlh). The primary aim of this thesis was to explore the association between BFlh architecture and the aetiology of HSI and re-injury. If BFlh architecture was found to be associated with the occurrence and recurrence of HSI, the secondary aim of this thesis was to then determine the malleability of these architectural characteristics in response to a resistance training intervention.

The aim of study 1 was to determine the test-retest reliability of two-dimensional ultrasonography to assess the architectural characteristics of the BFlh at rest and during graded isometric contractions. It also aimed to determine the differences in muscle architecture between a previously strain injured BFlh, in comparison to an uninjured BFlh, at rest and during graded isometric contractions. Two-dimensional ultrasonography was found to be reliable for assessing BFlh architecture at rest and during graded isometric contractions. Additionally, in athletes with a unilateral HSI history, the previously injured limb had significantly shorter BFlh fascicle

lengths when compared to the contralateral uninjured limb (12.9%; mean difference=1.54cm; 95%CI=0.95 to 2.10; p<0.001; d=1.34).

Due to the retrospective nature of study 1, the aim of study 2 was to investigate the role that BFlh fascicle length had in the aetiology of a HSI in elite Australian soccer players. Using a prospective study design, 152 athletes had their BFlh architecture assessed at the beginning of pre-season training. The occurrence of HSIs during the pre-season and in-season period was also monitored. The findings from study 2 showed that possessing short BFlh fascicle lengths resulted in a fold 4 increase in the risk of a future HSI in elite soccer players (relative risk=4.1; 95%CI=1.9 to 8.7).

The aim of study 3 was to determine the adaptations of the BFlh architectural characteristics to an eccentric or concentric training intervention. Recreationally active males completed 17 training sessions in a 6-week period on an isokinetic dynamometer. The findings from study 3 showed that architectural alterations of the BFlh occur within two weeks of the implementation of a resistance training intervention. Of note concentric resistance training resulted in a 13.3% reduction in BFlh fascicle length, whereas eccentric resistance training caused a 14.1% increase following the 6-week intervention.

This program of research has contributed new knowledge to the evidence base relating to HSIs, namely 1) the identification of shorter BFlh fascicle lengths in previously injured limbs; 2) the determination of BFlh fascicle length as a novel, modifiable risk factor for a future HSI in elite soccer players and 3) that the contraction mode employed during resistance training interventions influences the adaptive response of BFlh fascicle length. This new knowledge will inform HSI rehabilitation and injury prevention practices which should consider muscle architecture in the

aetiology of a HSI, the maladaptive responses following injury and how these characteristics can be altered following a training intervention.

List of abbreviations and nomenclature

ACL	anterior cruciate ligament
BFlh	biceps femoris long head
cm	centimetres
d	Cohen's *d* (effect size)
deg	degrees
FL	fascicle length
HSI	hamstring strain injury
ICC	intraclass correlation
kg	kilograms of body mass
MDC_{95}	minimum detectable change at a 95% confidence interval
MVIC	maximal voluntary isometric contraction
MT	muscle thickness
N	newtons of force
N/kg	newtons of force relative to body mass
Nm	newton metres of torque
Nm/kg	newton metres of torque relative to body mass
OR	odds ratio
PA	pennation angle
RFL	fascicle length relative to muscle thickness
RR	relative risk
SD	standard deviation
TE	typical error
%TE	typical error as a % coefficient of variation
95% CI	95% confidence interval

Chapter 1 – Introduction and overview

Hamstring strain injuries (HSIs) are the most prevalent non-contact injury in a number of sports involving high-speed running (Ekstrand, Hagglund, Kristenson, Magnusson, & Walden, 2013; Opar et al., 2014; Orchard, James, Alcott, Carter, & Farhart, 2002; Orchard, Seward, & Orchard, 2013). Over the past decade in the Australian Football League, the incidence of new HSIs is 6.0 per club per season, resulting in 21.1 player games missed per club per season (Orchard et al., 2013). In elite European soccer, the incidence of HSIs has also not declined over the past decade, despite the ongoing scientific endeavour (Ekstrand et al., 2013). The high incidence of HSIs places a significant burden, not only medically, but also financially on organisations, with recent estimations in the Australian Football League placing the yearly average cost of games missed at approximately \$245,842 per club (Hickey, Shield, Williams, & Opar, 2014). In addition to the high prevalence of first time injuries, HSIs have a significant risk of recurrence, with reported re-injury rates of 16%-54% (Arnason, Andersen, Holme, Engebretsen, & Bahr, 2008; Ekstrand et al., 2012; Hagglund, Walden, & Ekstrand, 2013; Orchard et al., 2013). Furthermore, of the hamstring muscles, the biceps femoris long head (BFlh) is the most commonly injured (Askling, Tengvar, Saartok, & Thorstensson, 2007; Koulouris & Connell, 2003; Koulouris, Connell, Brukner, & Schneider-Kolsky, 2007; Opar et al., 2015).

Recently research has focused on developing a greater understanding of the maladaptation's associated with a prior HSI. Retrospective evidence has shown prolonged deficits in BFlh muscle volume (Silder, Heiderscheit, Thelen, Enright, & Tuite, 2008), biceps femoris activation (Opar, Williams, Timmins, Dear, & Shield, 2013a; Sole, Milosavljevic, Nicholson, & Sullivan, 2011), knee flexor rate of torque development (Opar, Williams, Timmins, Dear, & Shield, 2013b), eccentric strength (Opar, Piatkowski, Williams, & Shield, 2013; Opar, Williams, et al., 2013a; Sole et al., 2011; Timmins, Shield, Williams, Lorenzen, & Opar, 2015) as well as differences in

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the angle of peak knee flexor torque (Brockett, Morgan, & Proske, 2004) in previously injured limbs. The shift in the angle of peak torque to shorter muscle lengths was proposed to be as a result of a reduction of in-series sarcomeres and shorter muscle fascicle lengths in the previously injured hamstrings (Brockett et al., 2004). Possessing shorter fascicles, with fewer in-series sarcomeres, has been hypothesized to increase the susceptibility of a muscle to eccentrically induced muscle damage and potentially increasing the risk of injury (Brockett et al., 2004; Morgan, 1990). Despite this indirect evidence, it is still unknown if a previously strain injured BFlh displays shorter fascicles compared to an uninjured BFlh.

Research efforts have also been concentrated on identifying potential risk factors for a future HSI. A number of non-modifiable factors, such as increasing age and previous injury history, have been shown to increase the risk of a future HSI (Arnason et al., 2008; Engebretsen, Myklebust, Holme, Engebretsen, & Bahr, 2010; Hagglund, Walden, & Ekstrand, 2006). However a greater emphasis has been directed towards modifiable risk factors, which can be altered via interventions. These include muscle imbalances (between-limb and hamstring:quadriceps ratios) (Croisier, Ganteaume, Binet, Genty, & Ferret, 2008; Fousekis, Tsepis, Poulmedis, Athanasopoulos, & Vagenas, 2011), knee flexor weakness (Croisier et al., 2008; Opar, Piatkowski, et al., 2013; Orchard, Marsden, Lord, & Garlick, 1997) and flexibility (Witvrouw, Danneels, Asselman, D'Have, & Cambier, 2003). Despite this evidence, there is still no consensus regarding the role that eccentric knee flexor strength plays in the aetiology of a HSI in elite soccer. Furthermore it is also unknown what effect muscle fascicle length has on the risk of a future HSI occurring.

Muscle architecture has been shown to be easily altered when exposed to various stimuli, such as resistance training interventions (Blazevich, 2006; Blazevich, Cannavan, Coleman, & Horne, 2007; Narici et al., 2011; Potier, Alexander, & Seynnes, 2009; Seynnes, Maganaris, de Boer, di

Prampero, & Narici, 2008). The majority of resistance training interventions focussing on architectural adaptations have been undertaken in the triceps surae and quadriceps muscle groups (Blazevich et al., 2007; Narici et al., 2011; Seynnes, de Boer, & Narici, 2007). There is a paucity of literature which has investigated alterations in the BFlh following resistance training interventions. If the hypothesis is true that possessing shorter muscle fascicles increases the susceptibility of a muscle to strain injury, interventions which increase BFlh fascicle length are of interest.

Given the gaps in the literature relating to hamstring muscle architecture, specifically fascicle length, the aims of this program of research were 1) to determine whether a previously strained BFlh displays different architecture compared to an uninjured BFlh, 2) determine the role that BFlh architecture plays in the aetiology of HSI and 3) the time course of adaptation of the BFlh following an eccentric or concentric training intervention as well as a period of de-training. A thorough review of the current literature can be found in Chapter 2, after which Chapters 4 through 6 contain the experimental studies which address the aims of the thesis. The findings and limitations from all studies are then discussed in Chapter 7.

Chapter 2 – Literature review

Publication statement:

This chapter is comprised of the following paper accepted for publication in the *British Journal Sports Medicine*:

2.1 Abstract

Skeletal muscle and the characteristics which form its architecture are crucial in day to day function. These characteristics (fascicle length, pennation angle, muscle thickness) respond to varying forms of stimuli which impose their respective adaptive or maladaptive responses. The beneficial alterations to muscle architecture following training interventions are well understood, as is their response to forced immobilisation or limb suspension (casting etc.). However the maladaptive architectural alterations following a strain injury in response to the associated inhibition, restricted range of motion, and the resultant flow-on conditions are unknown. Or is there a potential, sub-optimal architectural formation which may increase strain injury risk, or are these structures altered in response to the injurious event? Does the neuromuscular inhibition induced from the injury restrict architectural adaptations? If so, what potential flow-on effects does this have to muscle function (such as the force-length and force-velocity relationships)? This review will provide an introduction to the methods used for assessing muscle architecture. It will then introduce how architecture impacts everyday life and the variable responses to training and immobilisation interventions. This review will then present a hypothesis regarding the maladaptive responses to strain injury, linking in the role that inhibition associated with the injury may have. It will then conclude with how this inhibition, and the resultant maladaptive responses, may alter function and potentially increase the risk of future injury.

2.2. Introduction

Skeletal muscle forms the basis of the primary organ system responsible for movement and force generation, which is accountable for enabling actions ranging from simple, single joint efforts, to more complex tasks such as locomotion (Lieber & Ward, 2011). The ability to increase and preserve the maximal force producing capacity of muscle is important for the maintenance of physical function with aging (Bean et al., 2010) and optimising athletic performance (Faria, Parker, & Faria, 2005). There are a wide range of factors that influence the force producing capabilities of skeletal muscle, including, but not limited to, fibre type distribution (Hakkinen, Komi, & Alen, 1985), neural contributions (e.g. central drive) (Gandevia, 2001; Waugh, Korff, Fath, & Blazevich, 2013) and muscle architecture (Lieber, 1993). Architectural characteristics of muscle not only influence maximal force output, but also the interrelationship between force, muscle length, contraction velocity (Lieber & Friden, 2000) and susceptibility to injury (Brockett et al., 2004). The characteristics of muscle architecture are adaptable and can be altered by a range of stimuli, however there is limited evidence regarding maladaptations following strain injury.

Architectural characteristics of muscle include cross sectional area (CSA) which can be further defined as either anatomical CSA (ASCA) or physiological CSA (PCSA); muscle thickness (the distance between the superficial and deep/intermediate aponeuroses); pennation angle (the angle of the fascicles relative to the tendon); fascicle angle (the angle of the fascicle onto the aponeuroses); fascicle length (the length of fascicles running between the aponeuroses/tendon); and muscle volume (the product of the length and ACSA of the skeletal tissue located within the epimysium) (Lieber & Friden, 2000). The ACSA is the area of tissue assessed perpendicular to the longitudinal axis of the muscle (Lieber & Ward, 2011), while the PCSA is the sum of the

cross-sectional area of all fascicles within the muscle and is subsequently influenced by pennation angle (Aagaard et al., 2001; Lieber, 2002).

This review will provide an overview of muscle architectural adaptations to training and injury. Whilst a systematic review is often desirable, the body of evidence in this field is limited, particularly as it relates to injury, and as such a narrative approach was preferred. Firstly this review will describe the methodologies used for the measurement of muscle architecture. The aim then will be to detail the impact that architectural alterations following training interventions, immobilisation as well as injury may have on force production. The review will then present a hypothesis on how neuromuscular inhibition could cause maladaptations to muscle architecture following injury.

2.3. Methods used to measure characteristics of muscle architecture

Historically, cadaveric investigations (Wickiewicz, Roy, Powell, & Edgerton, 1983) were the sole means of assessing muscle architecture. Today, with advances in technology such as magnetic resonance imaging (Scott, Engstrom, & Loeb, 1993) (MRI) and ultrasonography (Kawakami, Abe, & Fukunaga, 1993), it is now possible to obtain *in-vivo* assessments of muscle architecture.

2.3.1 Cadaveric observations

Tissue from cadaveric samples have been used to directly study and measure gross characteristics of muscle architecture (Friederich & Brand, 1990; Wickiewicz et al., 1983) as well as sarcomere lengths (Cutts, 1988). However, with cadaveric studies it is difficult to obtain a significant sample size, due to the limited availability of donor tissue (Kellis, Galanis, Natsis, & Kapetanos, 2009). Furthermore, age matching of cadavers is limited, with the majority of donations coming from elderly individuals (65 to 90 years) (Kwah, Pinto, Diong, & Herbert, 2013). There are a limited number of younger samples and, to the authors' knowledge, no reports in the literature of

architectural characteristics of cadaveric muscle under 45 years of age. As such, cadaver-derived measures of muscle architecture are, more often than not, those obtained from sarcopaenic tissue (Morse, Thom, Birch, & Narici, 2005). Inferences based upon these data may be limited in athletic populations where the majority of individuals are aged between 18-35 years (Blazevich, 2006; Opar, Williams, & Shield, 2012).

2.3.2 Magnetic Resonance Imaging (MRI) modes

Over time, MRI has been developed as a tool to measure muscle morphology (Narici, Roi, & Landoni, 1988). This technique is now more accessible, allowing for more investigations to be completed. MRI is valuable in its spatial ability to clearly identify various anatomical components, such as adipose, nerve and bone tissue. It also allows for the clear identification of individual muscles as well as the determination of morphological parameters (e.g. volume and CSA) due to its high resolution.

Initially, the combination of MRI with mathematical modelling was utilised in an attempt to determine muscle architectural variables (Scott et al., 1993). However the use of MRI is limited by a lack of resolution at the fibre level, leading to considerable amounts of mathematical prediction and associated error when attempting to model muscle architecture. Despite these initial limitations, high resolution imaging has continued to advance and imaging at a muscle fascicle level is now feasible. Specifically, diffusion tensor imaging is an MRI method which has been used to measure fascicle length and pennation angle of skeletal muscle at rest (Damon, Ding, Anderson, Freyer, & Gore, 2002; Heemskerk, Sinha, Wilson, Ding, & Damon, 2010; Van Donkelaar et al., 1999). Diffusion tensor imaging is based on the movement of water through cell membranes within biological tissues in six or more non-collinear directions. This allows for the construction of a model showing the muscle fibre orientations (Lansdown, Ding, Wadington, Hornberger, & Damon, 2007; Van Donkelaar et al., 1999). While diffusion tensor imaging is a

significant step forward for imaging *in-vivo* muscle architecture, there are still limitations such as the variability in the noise of the images and having fibre trajectories interrupted by anatomical artefacts (adipose and scar tissue) (Lansdown et al., 2007). Finally, the cost associated with MRI is significant (approximately $600 AUD per scan) and conducting large scale studies can be limited due to available funding, resulting in smaller sample sizes and reduced statistical power.

2.3.3 Ultrasound imaging

Two-dimensional (2-D) ultrasound imaging provides a relatively inexpensive means of non-invasively assessing the characteristics of muscle architecture (Ikai & Fukunaga, 1968, 1970; Narici, 1999). In the literature, it is established as the most common technique for measuring muscle architecture *in-vivo* (Blazevich, 2006; Kwah et al., 2013; Narici, 1999; Narici & Cerretelli, 1998). Utilising 2-D ultrasound images collected along the longitudinal axis of the muscle belly allows for the determination of fascicle length, pennation angle, muscle thickness and the identification of the aponeuroses in the tissue (Figure 2.1) (Blazevich, Gill, & Zhou, 2006).

Ultrasound imaging is undertaken using transducers with fields of view ranging from 3.8 to 10cm (Narici, 1999). These fields of view are typically shorter than the fascicles under investigation, especially in large muscles such as the major knee flexors and extensors (Blazevich et al., 2006). In these cases fascicle length is estimated with various linear approximations using the measured muscle thickness and pennation angle values (Blazevich et al., 2006; Kellis et al., 2009). 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). These varying levels of error in ultrasound derived architectural assessments range from 0% to 6.6% (Muramatsu, Muraoka, Kawakami, Shibayama, & Fukunaga, 2002). Additionally, extended-field-of-view ultrasonography has also been utilised to assess *in-vivo* vastus lateralis

fascicle lengths (Noorkoiv, Stavnsbo, Aagaard, & Blazevich, 2010). This method displays high levels of reliability (intraclass correlation (ICC) = 0.99 in animal dissection), but is limited since it cannot be utilised during active muscle contraction (Noorkoiv, Nosaka, & Blazevich, 2010), where other 2-D ultrasonography methods can (Timmins et al., 2015).

The skill of the sonographer and the orientation of the transducer contribute to the error and subsequently limit the reproducibility of the method (Kurihara et al., 2005). For example, a change in the orientation and rotation of the ultrasound probe can result in a 12% difference (13.6^{o} to 15.5^{o}) in the pennation angle reported (Klimstra, Dowling, Durkin, & MacDonald, 2007). A recent systematic review (Kwah et al., 2013) reported the reliability and validity of 2-D ultrasound in measuring fascicle length and pennation angle in various muscles. The authors concluded that it is reliable across a number of muscle groups and valid in comparison to cadaveric samples. Despite these conclusions they also stated that the reliability of the measure is mostly dependent upon the assessor's aptitude and using a single assessor will aide in limiting the error in the sample (Klimstra et al., 2007; Kwah et al., 2013). Various studies have used different methods for standardising the transducer orientation and location, however no general consensus has been found regarding the best process to limit measurement error (Blazevich et al., 2006; Kellis et al., 2009; Muramatsu et al., 2002).

Ultrasound imaging studies have examined architecture with the muscle in a passive state (Alegre, Jimenez, Gonzalo-Orden, Martin-Acero, & Aguado, 2006; Blazevich et al., 2006; Bleakney & Maffulli, 2002; Brancaccio, Limongelli, D'Aponte, Narici, & Maffulli, 2008; Chleboun, France, Crill, Braddock, & Howell, 2001) and during isometric contractions (Cronin et al., 2008; Duclay, Martin, Duclay, Cometti, & Pousson, 2009; Fukunaga, Ichinose, Ito, Kawakami, & Fukashiro, 1997; Timmins et al., 2015). The ability of ultrasound to capture these architectural characteristics during contraction is one of its major strengths compared with other

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methodologies such as extended-field-of-view (Noorkoiv, Nosaka, et al., 2010). The assessment of muscle architecture during contraction allows for a greater insight into function than measures taken at rest. For example, pronounced changes in vastus lateralis fascicle length (shortening from 126 to 67mm) and pennation angle (increasing from 16° to 21°) occur as knee extensor forces rise from 0 to 10% of maximal isometric contraction (Fukunaga et al., 1997). Furthermore, the reliability of muscle architecture appears not to be influenced by contraction state, with a level of variance for fascicle length and pennation angle ranging from 0% to 6.3% when passive and 0% to 8.3% when active (Brancaccio et al., 2008; Chleboun et al., 2001; Cronin et al., 2008; Duclay et al., 2009; Fukunaga et al., 1997; Kwah et al., 2013). Additionally passive and active assessments of fascicle length and pennation angle display similar ICC's (passive: 0.74-0.99, active: 0.62-0.99) (Kwah et al., 2013). There are some inconsistencies in the reliability of fascicle length and pennation angle assessments in different muscle groups with the vastus lateralis (ICC = 0.93-0.99) being the most reproducible and the supraspinatus being the least (ICC = 0.74 – 0.93) (Kwah et al., 2013). Additionally, muscle architecture can vary along the length of the muscle. For example, the biceps femoris long head possesses proximal fascicles which are on average 2.8cm longer compared to distal fascicles (Bennett, Rider, Domire, DeVita, & Kulas, 2014). Therefore standardising the assessment location is an important consideration to minimise any variations due to inconsistencies in the scanning site.
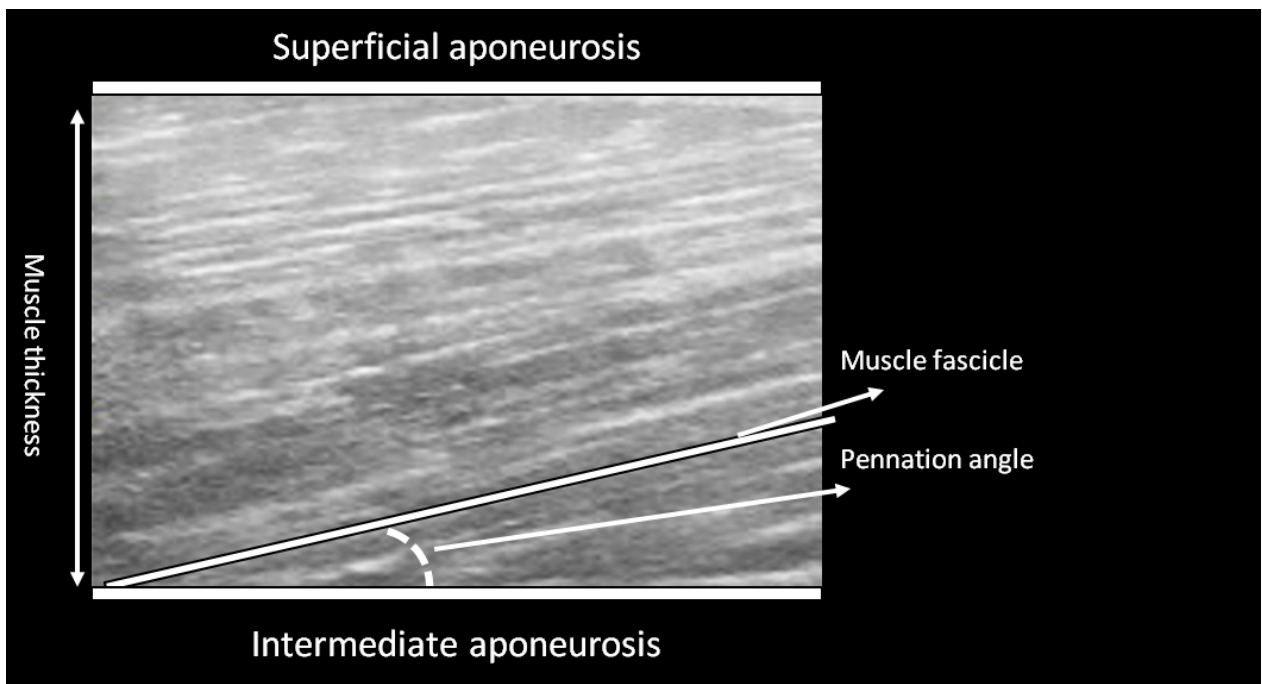
Figure 2-1. *A two dimensional ultrasound image of the biceps femoris long head. This image of the biceps femoris long head was taken along the longitudinal axis of the posterior thigh. From these images it is possible to determine the superficial and intermediate aponeuroses, muscle thickness, angle of the fascicle in relation to the aponeurosis. Estimates of fascicle length can then be made via trigonometry using muscle thickness and pennation angle.*

2.4. Adaptability of muscle architecture

Significant alterations in muscle architecture, torque producing capabilities and neuromuscular activation are evident following various resistance training interventions (Aagaard et al., 2001; Blazevich et al., 2007; Claflin et al., 2011; Narici, Hoppeler, et al., 1996). Skeletal muscle is also significantly altered following immobilisation (Campbell et al., 2013), with increased age (Narici, Maganaris, Reeves, & Capodaglio, 2003; Raj, Bird, & Shield, 2010) and following injury (Timmins et al., 2015). The level of force produced during a contraction and the speed at which contraction occurs, are both influenced by muscle architecture (Lieber & Friden, 2000). Unsurprisingly in response to stimuli which alter muscle architecture, functional changes also arise.

2.4.1 Effect of training interventions on muscle architecture

It is routinely reported that ACSA (6%-9%), PCSA (6%-8%), muscle thickness (6%-14%) and volume (7%-11%) are increased in the vastus lateralis and the lateral and medial gastrocnemius following various resistance training interventions ranging from 3 to 18 weeks (Aagaard et al., 2001; Alegre et al., 2006; Blazevich et al., 2007; Campbell et al., 2013; Duclay et al., 2009; Potier et al., 2009; Seynnes et al., 2007). The range of training interventions reported are a combination of conventional resistance training exercises (squats, leg press, bench press etc.), or exercises with an emphasis on the concentric or eccentric portion of the movement (e.g. overloading the specific contraction mode), or purely eccentric or concentric interventions (mostly done via isokinetic dynamometry).

2.4.1.1 Concentric training

Concentric training of the knee extensors has been shown to produce non-significant reductions of ~6% (isokinetic dynamometry) (Blazevich et al., 2007) and ~5% (leg press) (Franchi et al., 2014) in vastus lateralis fascicle length following two different 10 week training interventions.

Additionally, 8 weeks of concentric shoulder abduction training reduced fascicle length of the supraspinatus by ~10% (Kim, Ko, Farthing, & Butcher, 2015). Reductions in vastus lateralis fascicle length of ~11% have also been found in rats following 10 days of uphill and therefore concentrically-biased walking (Butterfield, Leonard, & Herzog, 2005).

Muscle pennation angle has also been altered following concentric training interventions. Franchi and colleagues found a ~30% increase in pennation angle of the vastus lateralis after 10 weeks of concentric leg press training (Franchi et al., 2014). Additionally, following 8 weeks of concentric shoulder abduction training, the pennation angle of the supraspinatus has been shown to increase by ~20% (Kim et al., 2015). However no significant alterations in the pennation angle of the vastus lateralis and vastus medialis were found following 10 weeks of concentric knee extensor training on an isokinetic dynamometer (Blazevich et al., 2007).

2.4.1.2 Eccentric training

Eccentric training of the plantar flexors resulted in no significant increases in fascicle length (medial gastrocnemius = ~5%, lateral gastrocnemius = ~10% and soleus = ~0%) following a 14-week training intervention (Foure, Nordez, & Cornu, 2013). Additionally non-significant increases of ~3% and ~4% were found in the vastus lateralis after 9 and 10 weeks of eccentric resistance training, respectively (Blazevich et al., 2007; Guilhem, Cornu, Maffiuletti, & Guevel, 2013). In contrast, other studies have reported significant increases in fascicle length following eccentric or eccentrically-biased training (Baroni et al., 2013; Franchi et al., 2014; Potier et al., 2009; Reeves, Maganaris, Longo, & Narici, 2009; Seynnes et al., 2007). These increases range from ~10% in the vastus lateralis to ~34% in the biceps femoris long head (Potier et al., 2009; Seynnes et al., 2007).

Muscle pennation angle has also been shown to be altered following eccentric training interventions. Guilhem and colleagues found an 11% increase in pennation angle in the vastus

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lateralis following an eccentric intervention performed on an isokinetic dynamometer (Guilhem et al., 2013). However, no significant alterations in the pennation angle of the biceps femoris long head (Potier et al., 2009) and triceps surae (Foure et al., 2013) have been reported following 8 and 14 weeks of eccentric resistance training. It is possible that increases in pennation angle are reliant on the extent of fibre hypertrophy that occurs and that concurrent increases in fascicle length may counter the tendency for pennation angle to increase (Foure et al., 2013; Potier et al., 2009).

2.4.1.3 Conventional resistance training

Conventional resistance training (consisting of a concentric and eccentric phase) has also been shown to alter muscle fascicle length. Following 13 weeks of general lower body strength training, fascicle length of the vastus lateralis significantly increased by 10% (Alegre et al., 2006). Additionally, 12 weeks of conventional upper body resistance training increased fascicle length of the triceps brachii lateralis by 16% (Blazevich & Giorgi, 2001). In contrast, following 16 weeks of elbow extension training no changes in fascicle length of the triceps brachii long head were found (Kawakami, Abe, Kuno, & Fukunaga, 1995).

Muscle pennation angle has also been shown to be altered following conventional resistance training interventions. Increases of 30% to 33% in the pennation angle of the vastus lateralis have been reported following 10 and 14-weeks of conventional resistance training (Aagaard et al., 2001; Franchi et al., 2014). Triceps brachii long head pennation angle has also been shown to increase by 29% following 16 weeks of elbow extension training (Kawakami et al., 1995). Similar increases in pennation angle of the triceps brachii lateralis have been found following 13 weeks of conventional upper body resistance training (Blazevich & Giorgi, 2001). In contrast, non-significant reductions of 2.4% in vastus lateralis pennation angle have been found following 13 weeks of lower body strength training (Alegre et al., 2006). Comparable non-significant

reductions in vastus lateralis pennation angle have also been found following 12 weeks of leg extension training (Rutherford & Jones, 1992). Differences in exercise selection, training volume and intensity may account for the divergent responses in studies involving the vastus lateralis. However it is also possible that the individuals in the studies which saw no significant changes in vastus lateralis pennation angle (Alegre et al., 2006; Rutherford & Jones, 1992) were not exposed to a range of motion and excursion greater than their current levels of habitual physical activity. As a result this may have limited the extent of their architectural adaptations. In comparison, large alterations in triceps brachii pennation angle are consistently reported following training interventions. This divergence between muscle groups might be explained by their function during habitual physical activity with the quadriceps being a strong, anti-gravity muscle, whereas the triceps brachii do not have the same requirements. Therefore any interventions aimed at altering the architecture of the triceps brachii, doesn't need to have as large an excursion as those required to change the vastus lateralis.

2.4.1.4 Other exercise modalities

Changes in the characteristics of muscle architecture are potentially reliant on the exercise being undertaken. A training study involving well-trained athletes used three different training interventions in addition to their current training regime (two sprint and jump session/week) (Blazevich, Gill, Bronks, & Newton, 2003). One intervention group undertook additional squat training and one intervention group undertook hack-squat training, while the final group completed two additional sprint and jump training sessions/week. Distal vastus lateralis fascicle lengths increased significantly (~52%) and pennation angles decreased ~3% in the participants who completed extra sprint and jump training. By contrast, there were no significant changes in fascicle length and pennation angle in those who undertook additional squat and hack squat training. The authors concluded that the velocity requirements of exercises may influence the

extent of fascicle length change more so than the type of movement pattern. It is also possible that the range of motion and excursion experienced by the vastus lateralis during eccentric contractions was greater during sprint and jump training than during the squat and front hack-squat. This might presumably influence changes to the number of sarcomeres in-series within a muscle. Furthermore, the results showed that adaptations to architectural characteristics are possible in a well-trained population.

2.4.1.5 Further variables to consider

2.4.1.5.1 Range of motion/muscle length

It is possible that there is an intricate relationship between the range of motion a muscle group routinely undertakes and its adaptations following resistance training interventions. Taking a muscle through a range of motion that is greater than what it is exposed to on a daily basis while adding resistance, may increase muscle fascicle length independent of contraction mode. This may explain different responses between young and elderly adults to eccentric resistance training, as elderly individuals appear to exhibit greater increases in fascicle length than their younger counterparts (Reeves et al., 2009; Reeves, Narici, & Maganaris, 2004). As elderly persons have, on average, a habitually reduced range of motion, it is thought that increasing the excursion their fascicles are familiar with, beyond that of their normal daily living, would result in longer fascicles, more so than interventions that work within their current range of motion. This may also potentially explain why some resistance interventions have elicited no fascicle length adaptations in younger adults who may already experience excursions and ranges of motion similar to those employed in training studies (Blazevich et al., 2003).

2.4.1.5.2 Velocity

One study has compared how a fast (240 deg/s) or slow (90 deg/s) eccentric knee extension training intervention (utilising isokinetic dynamometry) may alter vastus lateralis fascicle length

(Sharifnezhad, Marzilger, & Arampatzis, 2014). Following 10 weeks of fast eccentric knee extension training, fascicle length of the vastus lateralis increased by 14%, with no significant changes in the slow training group. However the slow training group completed their training through a reduced range of motion (35 degrees less than the fast training group) so it is not possible to determine the effect of contraction velocity on changes in muscle fascicle lengths.

2.4.2 Immobilisation

Alterations in muscle CSA, volume, fascicle length, pennation angle and muscle thickness are found following periods of bed rest or immobilisation (limb suspension) (Berg, Dudley, Haggmark, Ohlsen, & Tesch, 1991; Bleakney & Maffulli, 2002; Campbell et al., 2013; Hather, Adams, Tesch, & Dudley, 1992; Narici & Cerretelli, 1998; Seynnes et al., 2008). Fascicle length of the vastus lateralis were reported to decline by ~6% after 14 days of limb suspension, with a ~8% reduction after 23 days (de Boer, Maganaris, Seynnes, Rennie, & Narici, 2007). Similar reductions have been observed in the lateral gastrocnemius, with ~9% decrements in fascicle length after 23 days of lower limb suspension (Seynnes et al., 2008). Not all studies involving bed rest or immobilisation in weight-bearing and non-weight bearing muscles have shown changes in architecture. For example, fascicle lengths in the tibialis anterior and biceps brachii were not significantly altered following 5-weeks of bed rest (de Boer et al., 2008).

It is thought that the muscle length when immobilised may influence the extent of change, with fascicle lengths expected to reduce if immobilisation occurs at lengths which are shorter than those experienced during the activities of daily living (Williams & Goldspink, 1978). If immobilisation occurs at a 'normal' length, it is expected that there may be little change in fascicle lengths (Williams & Goldspink, 1978). Conversely immobilising a muscle at longer lengths may increase fascicle lengths (Williams & Goldspink, 1978).

2.4.3 Impact of fascicle length on muscle function

Fascicle length has a significant influence on the force-velocity and force-length relationships and, by extension, may alter muscle function. The impact of fascicle length on the force-velocity relationship has been investigated previously in the feline semitendinosus (Bodine et al., 1982). This muscle consists of a proximal and distal head, separated by a thick tendinous inscription. Both portions of the muscle display similar architectural characteristics, differing only in the length of their fascicles, with the distal head containing significantly longer fascicles (3.93 ± 0.1cm) than the proximal head (2.12 ± 0.1cm). An *in-vivo* comparison of the maximal shortening velocities for both of the heads shows that the distal head is able to shorten approximately twice as fast (424 mm/s) as the proximal head (224 mm/s) (Bodine et al., 1982). As muscle fascicle length is shorter in humans with a previous strain injury (Timmins et al., 2015), this could lead to a reduced maximal shortening velocity of the injured muscle (Figure 2.2, Figure 2.3).
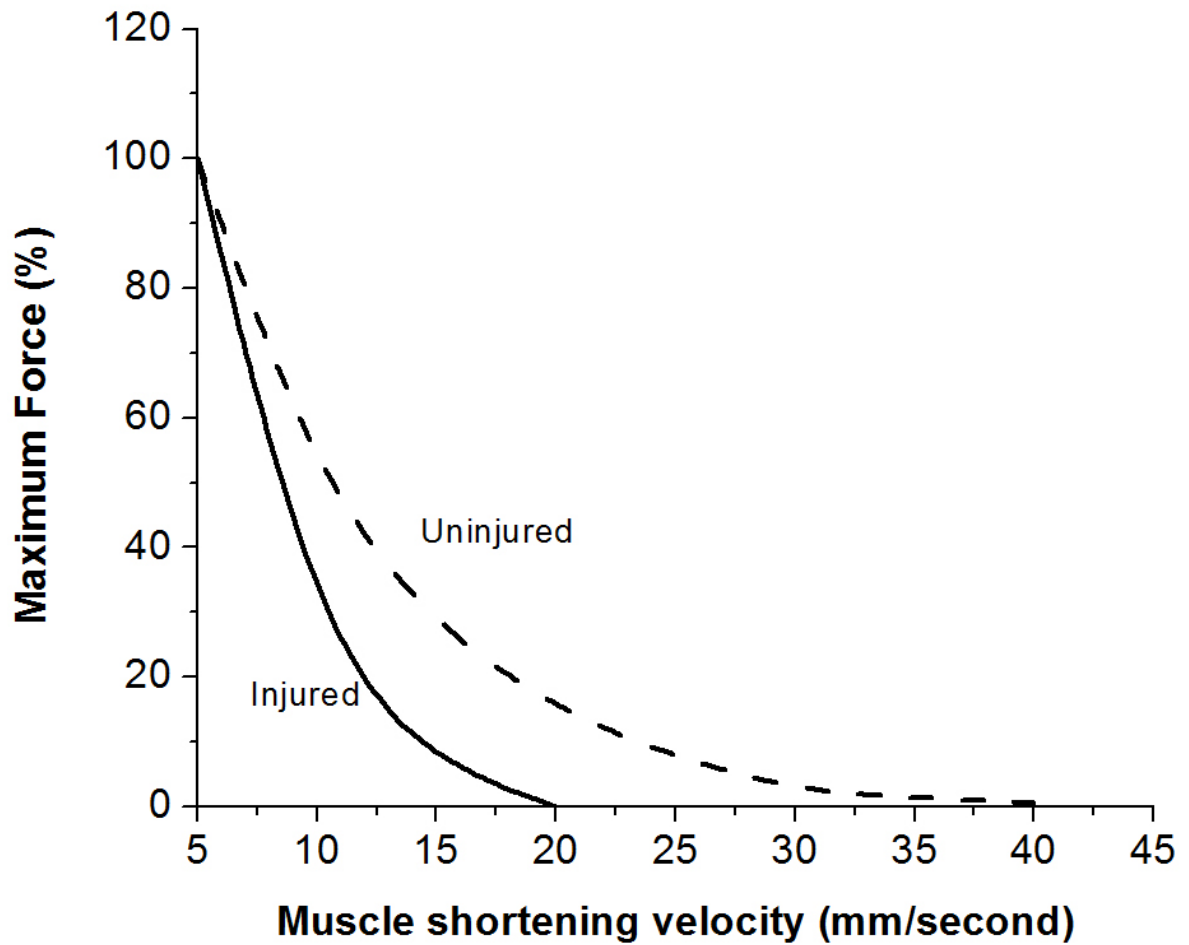
Figure 2-2. *A comparison of two different muscles with identical architectural characteristics, however one contains longer fascicles (uninjured) than the other (injured). Shorter muscles fascicles have been reported in previously injured biceps femoris long head (R. Timmins et al., 2015). Less sarcomeres in-series (shorter fascicles) will result in a slower maximal shortening velocity*

It is also hypothesized that muscle fascicle lengths have some bearing on the force-length relationship, however evidence in humans is limited (Blazevich, 2006; Lieber & Friden, 2000; Lieber & Ward, 2011). It is hypothesized that a previously injured muscle identical to an uninjured muscle, yet with shorter fascicle lengths, will have a reduced working range of the overall muscle as a result of fewer sarcomeres in-series (Brockett, Morgan, & Proske, 2001; Timmins et al., 2015). This may increase the amount of work being completed on the descending limb of the force-length relationship, where a reduced force generating capacity may result in an increased potential for muscle damage (Lieber & Friden, 2000; Lieber & Ward, 2011). This concept is supported in the literature in studies utilising animal models, where an increase of in-series sarcomeres in the vasti of rats and toads resulted in maximal force being produced at longer muscle lengths when compared to the vasti with fewer in-series sarcomeres (Butterfield et al., 2005; Jones, Allen, Talbot, Morgan, & Proske, 1997; Lynn & Morgan, 1994; Lynn, Talbot, & Morgan, 1998). Muscle architecture is also shown to play a role in the active portion of the force-length relationship in animal models (Lieber & Friden, 2000; Lieber & Ward, 2011; Noorkoiv, Nosaka, & Blazevich, 2014). It may also have a role in the generation of passive force that is produced at longer muscle lengths, yet this requires further investigation.
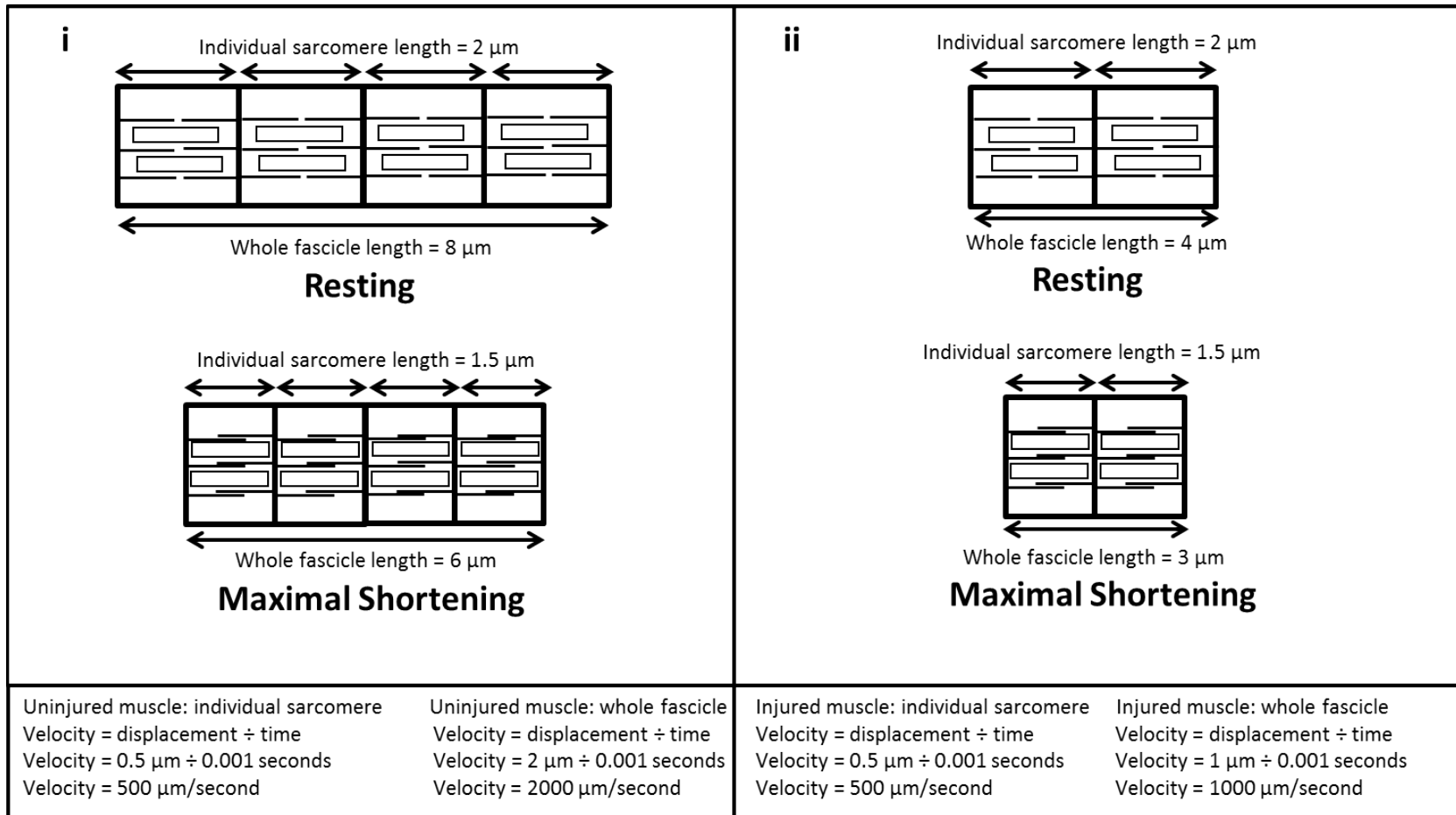
Figure 2-3. *The maximal shortening velocity of a muscle is influenced by the length of the muscle fascicle. An uninjured muscle (i) has twice the number of in-series sarcomeres that a previously injured muscle (ii) does. At any shortening velocity, the individual sarcomeres will shorten across identical distances. However, as an uninjured muscle contains more in-series sarcomeres, the entire muscle shortens over a greater distance than one with a history of injury. As velocity is the quotient of displacement and time, if these muscles shortened over the same time epoch, an uninjured muscle will possess a greater shortening velocity*

2.4.4 Impact of muscle strain injury on architecture

Limited evidence exists to characterise the effect of injury on muscle architecture. From the available literature, the isokinetic dynamometry derived torque-joint angle relationships has been used to postulate the effects of prior hamstring strain injury on fascicle length (Brockett et al., 2004; Brughelli, Nosaka, & Cronin, 2009; Fyfe, Opar, Williams, & Shield, 2013; Sole et al., 2011). These studies suggest that a shift in the angle of peak torque of the knee flexors towards shorter lengths, in individuals with a previously injured hamstring, is the result of a reduction in the number of in-series sarcomeres and a decrease in the optimum length for force production (Brockett et al., 2004; Fyfe et al., 2013; Opar et al., 2012).

Evidence for shorter fascicles in individuals with a history of strain injury has recently been provided through the use of 2-D ultrasound (Timmins et al., 2015). Athletes who had experienced a unilateral biceps femoris long head strain injury within the preceding 18 months had the biceps femoris long head architecture of both limbs assessed at rest and during graded isometric contractions (25%, 50% and 75% of maximal voluntary isometric contraction). The previously injured muscles had shorter fascicles and greater pennation angles at rest and during all isometric contractions when compared to the contralateral, uninjured biceps femoris long head (Timmins et al., 2015). If it is assumed that these architectural changes occur as a consequence of injury (a view that could be disputed), their persistence long after these athletes had returned to full training and competition schedules is perplexing. It must also be acknowledged that factors such as changes in connective tissue content/fibrosis of the scar tissue (Kaariainen, Jarvinen, Jarvinen, Rantanen, & Kalimo, 2000) and damage to the intramuscular nerve branches at the site of injury (Lehto & Jarvinen, 1991) may influence these architectural differences in individuals with a history of strain injury.

Neuromuscular inhibition after strain injury has been proposed to account for fascicular shortening following a strain injury (Fyfe et al., 2013; Opar, Williams, et al., 2013a). The previously injured biceps femoris long head has a reduced level of activation during eccentric contractions at long muscle lengths when compared to the contralateral uninjured biceps femoris long head (Opar, Williams, et al., 2013a; Sole et al., 2011). This reduced activation, as well as the avoidance of long muscle lengths during the early stages of rehabilitation, could result in structural changes (e.g. reduced muscle volume, altered architecture) that would ultimately lead to adverse alterations in function (Fyfe et al., 2013). Despite the best efforts during rehabilitation to include heavily loaded eccentric exercise in an attempt to restore muscle structure and function to pre-injured levels (Goldman & Jones, 2011; Heiderscheit, Sherry, Silder, Chumanov, & Thelen, 2010; Schache, 2012), the altered neural drive and difficulty in isolating the injured muscle may limit the potency of this stimulus and thus limit fascicle length changes. Reductions in eccentric activation at long muscle lengths (Opar, Williams, et al., 2013a; Sole et al., 2011), eccentric weakness (Opar, Piatkowski, et al., 2013; Opar, Williams, et al., 2013a; Sole et al., 2011; Timmins et al., 2015) and the avoidance of long muscle lengths early in rehabilitation could all contribute to the shorter fascicle lengths (Timmins et al., 2015).

Possessing shorter fascicles has been suggested to increase the likelihood of microscopic muscle damage as a consequence of repetitive eccentric actions (e.g. high speed running) and, when coupled with a high frequency of training sessions, may result in an accumulation of damage. This accumulation of eccentrically induced muscle damage would leave the muscle more vulnerable to strain injury when it encounters a potentially injurious situation, thus, increasing the probability of re-injury (Fyfe et al., 2013). It should be noted however, that a number of factors are likely to influence the risk of re-injury and that architectural maladaptations are just one such variable. The width of the proximal biceps femoris tendon has been shown to exhibit high levels

of variability within healthy athletes (Evangelidis, Massey, Pain, & Folland, 2015). Possessing a narrow proximal tendon width has been shown to increase the tissue strains within the muscle fibres adjacent to the proximal musculotendinous junction of the biceps femoris long head during active lengthening (Fiorentino, Epstein, & Blemker, 2012) and high speed running (Fiorentino & Blemker, 2014). The combination of these observations suggests that an athlete with a narrow proximal biceps femoris long head tendon may expose the tissue surrounding this tendon to high strains and potentially have an increased risk for injury at this site during active lengthening or high speed running. Additionally, eccentric strength and activation deficits might themselves elevate the risk of re-injury, perhaps in conjunction with these various other factors. Much work is still required in this area to confirm this hypothesis, including prospective observations to determine if shorter muscle fascicles (fewer sarcomeres in-series) increase the risk of future injury in human muscles. Despite this, the role of muscle architecture in muscle injury is a promising area to pursue.

2.5. Summary

Architectural characteristics of skeletal muscle have a direct impact on human function. These architectural characteristics can be assessed using multiple methods with 2-D ultrasound the most efficient and cost effective. Moreover architecture displays plasticity in response to different stimuli, which can partly explain changes in function following training and immobilisation. Recent work has shown that previously injured muscles have shorter fascicle lengths than uninjured muscles. This review has presented an argument as to how variations in architecture may impact function. However no research has examined the effect that fascicle lengths have on the risk of injury. The role of architectural characteristics in muscle strain injury aetiology currently remains unknown and should be a focus of future work. The relationship between muscle architecture and strain injury presents a hitherto unconsidered avenue worthy of exploration that could assist in the prevention of muscle strain injury and re-injury and could help to guide future rehabilitation endeavours.

Chapter 3 – Methodology and design

As per university guidelines, the methods utilised within each study of this thesis are described in their entirety below. Subsequently chapters 4, 5 and 6 contain the specific methods used in each study presented according to guidelines provided by the respective journals.

3.1 Study 1 - Biceps femoris long head architecture: a reliability and retrospective injury study.

3.1.1 Participants

Thirty six males were recruited to participate in this case-control study. Twenty recreationally active males (age 26.1 ±7.4 years; height 1.80 ±0.05m; body mass 78.1 ±8.7kg) with no history of hamstring strain injury were recruited to determine the test-retest reliability of the ultrasound measures of BFlh architecture, as well as serving as a control group. Sixteen elite (competing at national or international level) athletes with a unilateral BFlh strain injury history within the last 18 months (age 23.7 ±3.3 years; height 1.85 ±0.07m; body mass 83.6 ±7.9kg) were recruited to participate and form the previously injured group. The athletes (12 Australian Rules Football players, 2 soccer players, 1 hockey player and 1 track and field athlete), who had all returned to pre-injury levels of training and competition, were recruited to assess the differences in architecture between their previously injured and uninjured BFlh. Previously injured participants supplied their clinical notes to the research team and all had their diagnosis confirmed by magnetic resonance imaging. All previously injured athletes reported standard rehabilitation progression (Heiderscheit et al., 2010) and the use of some eccentric conditioning as guided by their physical therapist. All participants provided written informed consent prior to testing which was undertaken at the Australian Catholic University, Fitzroy, Victoria, Australia. Ethical

approval for the study was granted by the Australian Catholic University Human Research Ethics Committee.

3.1.2 Experimental design

The test-retest reliability of real-time two-dimensional ultrasound derived measures of muscle thickness, pennation angle and fascicle length of BFlh at rest and during graded isometric contractions was determined across three separate testing sessions. Sessions were separated by at least 24 hours. On the final visit, eccentric knee flexor strength during the Nordic hamstring exercise was also assessed using a custom made device (Opar, Piatkowski, et al., 2013). Determining the impact of a previous strain injury on the BFlh architectural characteristics and eccentric knee flexor strength was performed during a single assessment session in the previously injured cohort.

3.1.3 BFlh architecture assessment

Muscle thickness, pennation angle and fascicle length of the BFlh were determined from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-*i,* Wauwatosa, U.S.A). The scanning site was determined as the halfway point between the ischial tuberosity and the knee joint fold, along the line of the BFlh. Once the scanning site was determined, the distance of the site from various anatomical landmarks were recorded to ensure reproducibility of the scanning site for future testing sessions. These landmarks included the ischial tuberosity, fibula head and the posterior knee joint fold at the mid-point between BF and semitendinosus tendon. On subsequent visits during the reliability study, the scanning site was determined and marked on the skin and then confirmed by replicated landmark distance measures. All architectural assessments were performed with participants in a prone position and the hip in a neutral position following at least 5 minutes of inactivity.

Assessments at rest were always performed first followed by the graded isometric contraction protocol. Assessment of BFlh architecture at rest was performed with the knee at three different positions; 0º, 30º and 60º of knee flexion; which were determined via manual goniometer. Assessment of BFlh architecture during isometric contractions were always performed with the knee at 0º of knee flexion and preceded by a maximal voluntary isometric contraction, performed in a custom made device (Opar, Piatkowski, et al., 2013). The graded isometric contractions of the knee flexors were performed in the same device at 25, 50 and 75% of maximum voluntary isometric contraction (MVIC) with the participants shown the real-time visual feedback of the force produced to ensure that target contraction intensities were met. Assessment of the MVIC of the knee flexors was undertaken in a prone position, with both the hip and knee fully extended (0deg). Participants were instructed to contract maximally over a five second period, from which the single highest force value recorded was used to determine the MVIC.

To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence the accuracy of the measures (Klimstra et al., 2007). Finally, the orientation of the probe was manipulated slightly by the sonographer (RGT) if the superficial and intermediate aponeuroses were not parallel.

Once the images were collected, analysis was undertaken off-line (MicroDicom, Version 0.7.8, Bulgaria). For each image, six points were digitised (Figure 2.1) as described by Blazevich and colleagues (Blazevich et al., 2006). These points included two each on both the superficial and deep aponeurosis as well as two on the fascicle of interest. Following the digitising process, muscle thickness was defined as the distance between the superficial and intermediate

aponeuroses of BFlh. A fascicle of interest was outlined and marked on the image. The angle between this fascicle and the intermediate aponeurosis was measured and given as the pennation angle. The aponeurosis angle for both aponeuroses was determined as the angle between the line marked as the aponeurosis and an intersecting horizontal line across the captured image (Blazevich et al., 2006; Kellis et al., 2009). Fascicle length was determined as the length of the outlined fascicle between aponeuroses. As the entire fascicle was not visible in the field of view of the probe it was estimated via the following equation from Blazevich and colleagues, which has been validated in a range of muscles, including the hamstrings (Blazevich et al., 2006; Kellis et al., 2009):

FL=sin (AA+90deg) x MT/sin (180deg-(AA+180deg-PA)).

Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation angle.

Fascicle length was reported in absolute terms (cm) and also relative to muscle thickness (fascicle length/muscle thickness). The same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers during the analysis.

3.1.4 Eccentric Nordic hamstring exercise strength

The assessment of eccentric hamstring strength using the Nordic hamstring exercise field testing device (Figure 3.1) has been reported previously (Opar, Piatkowski, et al., 2013). Participants were positioned in a kneeling position over a padded board, with the ankles secured superior to the lateral malleolus by individual ankle braces (Figure 3.2) which were secured atop custom made uniaxial load cells (Delphi Force Measurement, Gold Coast, Australia) fitted with wireless data acquisition capabilities (Mantracourt, Devon, UK). The ankle braces and load cells were secured to a pivot which allowed the force to always be measured through the long axis of the

load cells. Following a warm up set, participants were asked to perform one set of three maximal bilateral repetitions of the Nordic hamstring exercise. Participants were instructed to gradually lean forward at the slowest possible speed while maximally resisting this movement with both lower limbs while keeping the trunk and hips in a neutral position throughout, and the hands held across the chest. The participants were encouraged to maintain a similar contraction speed across all three repetitions. Following each attempt a visual analogue scale was given to assess the level of pain that was experienced. None of the participants reported any pain during testing. Verbal encouragement was given throughout the range of motion to ensure maximal effort. The peak force for each of the three repetitions was averaged for all statistical comparisons.

Figure 3-1. *Eccentric strength assessment during the Nordic hamstring exercise (from top to bottom). The participant is instructed to control themselves as far down as possible via a forceful eccentric contraction of the knee flexors. After completing one effort, the participant returns to the starting position (top picture) via pushing up with both hands. Each ankle is secured independently via custom-made braces with in-series, uniaxial load cells.*

Figure 3-2. *Close up of the ankle brace and in-series, uniaxial load cells which determine eccentric force during the Nordic hamstring exercise.*

3.1.5 Statistical analyses

All statistical analyses were performed using SPSS version 19.0.0.1 (IBM Corporation, Chicago, IL). For the determination of reliability, descriptive statistics for the architectural variables of the control group were determined for the left and right limbs separately. Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and homoscedasticity of the data using Levene's test. Intraclass correlation coefficient (ICC), typical error (TE), and TE as a co-efficient of variation (%TE) were calculated to assess the extent of variation between the first to second and the second to third visit (Weir, 2005). Based on previous quantitative reliability literature, it was subjectively determined that an ICC $\geq$ 0.90 was regarded as high, between 0.80 and 0.89 was moderate, and $\leq$ 0.79 was poor (16, 37). Minimum detectable change at a 95% confidence interval (MDC$_{95}$) was calculated as [TE x 1.96 x $\sqrt{2}$]. Additionally, a %TE of $\leq$10% was considered to represent an acceptable level of reliability (Cormack, Newton, McGuigan, & Doyle, 2008).

At each contraction intensity, a split-plot design ANOVA with the within-subject variable being limb (left/right or uninjured/injured, depending on group) and the between-subject variable being group (control or previously injured) was used to compare BFlh architecture and Nordic hamstring exercise strength variables. Control group data was used from the third trial. Where significant limb x group interactions were detected, post hoc t-tests with Bonferroni adjustments to the alpha level were used to identify which comparisons differed.

Further between group analyses were undertaken to determine the extent of the between limb asymmetry in BFlh architecture and Nordic hamstring exercise strength, in the control and previously injured groups. The control group between limb asymmetry was determined as the right limb minus the left and then converted to an absolute value (Sole et al., 2011), whereas in the previously injured group asymmetry was determined as the uninjured limb minus the injured

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limb. T-tests were used to assess differences in the extent of the between limb asymmetry in the control compared to the previously injured group. Bonferroni corrections were employed to account for inflated type I error due to the multiple comparisons made for each dependent variable. Significance was set at a $p<0.05$ and where possible Cohen's *d* (Cohen, 1988) was reported for the effect size of the comparisons, with the levels of effect being deemed small ($d = 0.20$), medium ($d = 0.50$) or large ($d = 0.80$) as recommended by Cohen (1988).

3.1.6 Power calculations

Following the reliability study, *a-priori* power analysis for the previously injured group was completed using G-Power (Faul, Erdfelder, Lang, & Buchner, 2007). The analysis was based on the anticipated differences in fascicle length between the injured and uninjured limb. The effect size was estimated based on the only study to date that has reported changes in BF fascicle length following eccentric training. That study reported a very large increase (effect size of approximately 1.9) of 33% in fascicle length following the intervention. Therefore an effect size of 0.8 was deemed reasonable as a starting point. Power was set at 80% with an alpha level of 0.05 returning a calculated sample size of 15. As a cross-reference to confirm this sample size calculation previous studies that have used similar designs have used sample sizes from 13 to 15 (Opar, Williams, et al., 2013a, 2013b; Sole et al., 2011).

3.2 Study 2 - Biceps femoris long head architecture, eccentric knee flexor strength and hamstring injury risk in professional football (soccer): a prospective cohort study.

3.2.1 Participants and study design

This prospective cohort study was completed during pre-season (June 2014 to July 2014) and in-season period (October 2014 to May 2015) of the 2014/2015 elite Australian Football (soccer) competition. Ethical approval for the study was granted by the Australian Catholic University Human Research Ethics Committee (approval number: 2014 26V). Eight of the ten professional teams invited to participate elected to take part in the study. All outfield members of the playing squad (approximately 18-22 athletes per team) were approached and provided written, informed consent. In total, 152 elite male football (soccer) players (age 24.8 ± 5.1 years; height 1.8 ± 0.06m; body mass 76.7 ± 7.4kg) consented to participate. Club medical staff completed a retrospective injury questionnaire which detailed each athlete's history of hamstring, quadriceps, groin and calf strain injuries and chronic groin pain in the past 12 months, as well as the history of anterior cruciate ligament (ACL) injury at any stage throughout the athlete's career. Playing positions were defined as: defender (n=52), midfielder (n=59) and attacker (n=41) as per previous research (Bradley et al., 2011). The athletes had their maximal voluntary isometric contraction strength (n=141) (MVIC), BF$_{lh}$ architecture (with relaxed hamstrings (n=152) and while performing isometric knee flexion at 25% of MVIC (n=141)) and eccentric knee flexor strength (n=131) assessed at the beginning of pre-season.

3.2.2 BFlh architecture assessment

The processes for assessing the BFlh architectural characteristics are similar to those outlined in chapter 3.1.3. However in this study the BFlh architectural characteristics were only assessed during a 25% isometric contraction.

3.2.3 Eccentric hamstring strength

Eccentric knee flexor strength during the Nordic hamstring exercise was assessed using the same processes as outlined in chapter 3.1.4 and figure 3.1.

3.2.4 Prospective hamstring strain injury reporting

A HSI was defined as any acute posterior thigh pain which resulted in the immediate cessation of exercise and was later diagnosed by the club medical staff. Injury reports were not completed for injuries which did not fulfil the criteria (e.g. acute posterior thigh pain, however completed the exercise). A recurrent injury was a HSI that occurred on the same side of the body which had already suffered an injury in the current season. For all recurrent and new HSIs that fit the above criteria, the club medical staff completed a standard injury report form which detailed which limb was injured (dominant/non dominant, left/right), the muscle injured (BFlh/biceps femoris short head/semimembranosus/semitendinosus), location of injury (proximal/distal, muscle belly/muscle-tendon junction), activity type performed at time of injury (e.g. running, kicking etc.), grade of injury (I, II or III) and the number of days taken to return to full participation in training/competition. These reports were forwarded to the investigators throughout the season.

3.2.5 Injury specifics and rates

The determination of playing time missed as a result of a HSI was measured as missed matches per club per season (Orchard et al., 2013). Recurrence rate was defined as the number of recurrent injuries in the same season as a percentage of new injuries (Orchard et al., 2013). Additionally time lost as a result of the injury was defined as the amount of days from when the injury occurred to the resumption of full training participation.

3.2.6 Data analysis

Whilst positioned in the custom made device, shank length (m) was determined as the distance from the lateral tibial condyle to the mid-point of the brace which was placed around the ankle.

This measure of shank length was used to convert the force measurements (collected in N) to torque (Nm). Knee flexor eccentric and MVIC strength force data were transferred to a personal computer at 100Hz through a wireless USB base station (Mantracourt, Devon, UK). The peak force value during the MVIC and the three Nordic hamstring exercise repetitions for each of the limbs (left and right) was analysed using custom made software. Eccentric knee flexor strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the average of the peak forces from the 3 repetitions for each limb, resulting in a left and right limb measure (Opar, Piatkowski, et al., 2013). Knee flexor MVIC strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the peak force produced during a 5 second maximal effort for each limb.

Between limb imbalance of BF$_{lh}$ fascicle length, muscle thickness, eccentric and MVIC knee flexor strength was calculated as a left:right limb ratio for the uninjured players and as an uninjured:injured limb ratio in the injured players. As recommended, between limbs imbalance was converted to a percentage difference using log transformed raw data followed by back transformation (Impellizzeri, Bizzini, Rampinini, Cereda, & Maffiuletti, 2008). Negative percentage imbalances indicate that the variable of the left limb was greater than the right limb in the uninjured players, or that the injured limb variable was greater than the uninjured limb in the injured players. For athletes who did not suffer a HSI, as the limbs did not differ for any variables ($p > 0.05$) the left and right limb were averaged to give a single control 'score'.

3.2.7 Statistical analyses

All statistical analyses were performed using JMP version 11.01 Pro Statistical Discovery Software (SAS Inc., Cary, North Carolina, USA). Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and homoscedasticity using Levene's test.

Reliability of the assessor (RGT) and processes used for the determination of the BFlh architectural characteristics have been reported (Timmins et al., 2015).

The mean and standard deviation of age, height, weight, BFlh fascicle length (passive and 25% MVIC), BFlh muscle thickness (passive and 25%MVIC), eccentric and MVIC knee flexor strength were determined for all participants. Univariate analyses were performed to compare between limb imbalances for all variables of the injured and uninjured groups, as well as comparing the injured limb to the contralateral uninjured limb and the average of the left and right limbs from the uninjured group. Univariate comparisons were undertaken using two-tailed t-test with Bonferonni corrections to account for multiple comparisons. To determine univariate relative risk (RR) and 95% confidence intervals (95% CI) of future HSI, athletes were grouped according to:

- those with or without prior
    - hamstring (past 12 months)
    - calf (past 12 months)
    - quadriceps (past 12 months)
    - ACL (at any stage in their career)
    - chronic groin injury (past 12 months)
- those with passive fascicle lengths above or below
    - 10.56cm
        - This threshold was determined utilising receiver operator characteristic (ROC) curves based on the fascicle threshold that maximised the difference between sensitivity and 1- specificity.
- those with 25% MVIC fascicle lengths above or below

- o 9.61cm
    - ▪ Threshold determined as above

- those with passive muscle thickness threshold above or below
    - o 2.35cm
        - ▪ Threshold determined as above

- those with 25% MVIC muscle thickness threshold above or below
    - o 2.61cm
        - ▪ Threshold determined as above

- those with average eccentric knee flexor strength threshold above or below
    - o 337N
        - ▪ Threshold determined as above

- those with MVIC knee flexor strength threshold above or below
    - o 400N
        - ▪ Threshold determined as above

- those with limbs above or below arbitrarily selected cut offs of 10%, 15% and 20% between limb imbalance for
    - o passive fascicle length
    - o 25% MVIC fascicle length
    - o average eccentric knee flexor strength
    - o MVIC knee flexor strength

- athletes above these age cut offs (which represent the 10th, 25th, 50th, 75th and 90th percentiles for this sample)
    - o 18.0 years
    - o 20.4 years

- o   23.7 years

- o   28.8 years

- o   32.6 years

- athletes above and below the height (182.3cm) and weight (77.9kg) means as defined previously by Hagglund and colleagues (Hagglund et al., 2013)

HSI rates from these groups were then compared and RR calculated utilising a two-tailed Fisher's exact test to determine significance. Additionally, univariate logistic regressions were conducted with the prospective occurrence of a HSI (yes/no) as the dichotomous dependant variable and eccentric knee flexor strength and BFlh fascicle length as continuous independent variables in separate analyses. These data are reported as odds ratios (OR) and 95% CI per 10-N increase in knee flexor force and 0.5cm increase in fascicle length.

As per a previous investigation in elite Australian Football (Opar et al., 2015), to improve the understanding of the risk from the univariate analysis and remove the possible confounding effects, multivariate logistic regression models were built using risk factors from previously published evidence (Arnason et al., 2004; Ekstrand, Hagglund, & Walden, 2011; Hagglund, Walden, & Ekstrand, 2009; Opar et al., 2015; Woods et al., 2004). The first model included passive fascicle length (average of both limbs) and history of HSI and their interaction. The second model included fascicle length (average of both limbs) and age and their interaction. The third model included mean eccentric strength (average of both limbs) and history of HSI and their interaction. The fourth model included mean eccentric strength (average of both limbs) and age and their interaction. The final model included both fascicle length (average of both limbs) and mean eccentric strength (average of both limbs) and their interaction. Additionally using this final model the Nagelkerke R^2 coefficient was used to display the strength of the association between the two continuous independent variables (eccentric strength and fascicle length) with a

prospective HSI occurrence (Nagelkerke, 1991). Significance was set at a p<0.05 and where possible Cohen's *d* (Cohen, 1988) was reported for the effect size of the comparisons, with the levels of effect being deemed small (*d* = 0.20), medium (*d* = 0.50) or large (*d* = 0.80) as recommended by Cohen (1988).

3.2.8 Power calculations

Power analysis was undertaken *post-hoc* using G-Power (Faul et al., 2007). Using BFlh architecture data, power was calculated as 0.97 for the use of two-tailed independent t-tests to compare groups (input parameters: effect size = 0.80; alpha = 0.05; sample size group 1 = 125; sample size group 2 = 27). Using a similar *post-hoc* comparison for eccentric knee flexor strength, power was calculated as 0.95 (input parameters: effect size = 0.80; alpha = 0.05; sample size group 1 = 105; sample size group 2 = 26).

3.3 Study 3 - Architectural changes of the biceps femoris after concentric or eccentric training.

3.3.1 Participants

Twenty-eight recreationally active males (age 22.3±4.2 years; height 1.81±0.07m; body mass 76.9±8.2kg) with no history of lower limb injury in the past 12 months were recruited to participate in this study. All participants provided written informed consent prior to testing and training which was undertaken at the Australian Catholic University, Fitzroy, Victoria, Australia. Ethical approval for the study was granted by the Australian Catholic University Human Research Ethics Committee.

3.3.2 Study design

After the participants undertook a maximal dynamometry familiarisation session, no less than 7 days later they had their BFlh architectural characteristics assessed on both limbs. Following this

initial testing session (*28 days pre baseline*), the participants were paired according to passive BFlh fascicle length and randomly assigned to one of two training groups (allocation ratio 1:1) to undertake either concentric- or eccentric-only knee flexor training. All participants (n=28) returned to the lab four weeks later (*baseline*) and had the maximal knee flexor strength and BFlh architectural characteristics assessed on both limbs. Following this the participants underwent 6-weeks of either a concentric or eccentric training intervention in a randomly selected limb (the contralateral limb served as a within participant control). Participants also provided an overview of their weekly physical activity to the research team throughout the study. BFlh architecture of both limbs was re-assessed at day 14, 21 and 42 of the intervention, as well as 28 days after the completing of the training intervention to determine any de-training effect. Knee flexor strength of both limbs was re-tested at the end of the training intervention (*day 42*) and 28 days after the completion of the intervention. All tests were performed at the same time of the day for each participant.

3.3.3 Outcome measures

3.3.3.1 Isokinetic dynamometry

All knee flexor strength testing was completed on a Humac Norm® isokinetic dynamometer (CSMI, Massachusetts, U.S.A), on both legs (left or right) in a randomised order. Participants were seated on the dynamometer with their hips flexed at approximately 85deg from neutral and were restrained by straps around the tested/exercised thigh, waist and chest to minimise compensatory movements. Participants were instructed to hold onto hand grips by their side whilst instructed to limit any compensatory movement of their non-tested leg. All seating variables (e.g. seat height, pad position, etc.) were recorded to ensure the replication of the participants' positions. Gravity correction for limb weight was also conducted and range of motion was set between 0deg and 90deg of knee flexion (full extension = 0deg) with the starting

position for each contraction during strength testing being 90deg of knee flexion. The starting position for all training contractions were dependant on training group, with the concentric training group starting from 0deg of knee flexion and the eccentric group beginning from 90deg. Prior to all testing sessions, participants undertook a warm-up consisting of three sets of three concentric knee extension and flexion contractions at an angular velocity of 240deg/sec. The intensity of these contractions increased each set (1st set ~75% and 2nd set ~90% of the participants perceived maximum) until the final set at this velocity was performed at a maximal level. The test protocol began one minute following the final warm-up set and consisted of three sets of three repetitions of concentric and eccentric maximal voluntary contractions of knee flexion at 60deg/sec and 180deg/sec (30s inter-set rest). For all concentric knee flexion efforts, the participants were instructed to 'pull down' against the lever as fast as possible, whereas during eccentric contractions they were told to 'resist' the lever arm from extending their knee as hard as they could. All participants were provided visual feedback of their efforts as well as being verbally encouraged by the investigators to ensure maximal effort for all contractions. The testing order of contraction modes was randomised across the participant pool and the testing protocol has been previously reported to not alter concentric or eccentric knee flexor strength (Timmins et al., 2014). Dynamometer torque and lever position data were transferred to computer at 1 kHz and stored for later analysis where it was fourth-order low pass Butterworth filtered (5Hz). Average peak torques at 240, 180 and 60deg/sec for concentric and 180 and 60deg/sec for eccentric knee flexion were defined as the mean of the six highest torque values for each contraction mode at each velocity.

3.3.3.2 BFlh architectural assessment

The processes for assessing the BFlh architectural characteristics were the same as those outlined in chapter 3.1.3.

3.3.4 Intervention

The participants performed 6 weeks of either maximal eccentric or concentric knee flexion training, two (first week of intervention) to three times a week on an isokinetic dynamometer (Humac Norm, CSMI, Massachusetts, U.S.A) using the same range of motion and seat positions configuration as dynamometry testing sessions. Only one limb received the training stimulus, with the contralateral limb acting as a within subject control limb. Across the training period the volume (number) of contractions was increased following the progression in Table 3.1.

Each training session was separated by at least 48 hours. Contractions were distributed evenly across 60deg/sec and 180deg/sec. All participants started with two sets of three warm up efforts at 60deg/sec, in the contraction mode utilised for their training. For all training repetitions, the concentric training participants were moved to full knee extension (0deg) by the investigator and were instructed to flex their knee as fast as possible through to 90deg of knee flexion. The investigator then returned the lever arm to full knee extension and the subsequent repetition was completed. This was undertaken until all repetitions were completed in their respective set, with a 30 second inter-set rest period. The eccentric training participants began with their knee at 90deg of flexion. They were then instructed to maximally flex against the lever arm until full knee extension was reached (0deg). The participant was then instructed to relax, the lever arm was repositioned to 90deg of knee flexion by the investigators and the subsequent contraction was performed. This was undertaken until all repetitions were completed in each set, with a 30 second inter-set rest period. All participants were provided visual and verbal feedback on the consistency of the torque produced during each repetition. These were compared against personal best performances, which were known by the participant, to aid motivation. During the pre-control (*28 days pre baseline to baseline*), intervention (*baseline to intervention day 42*) and de-training periods (*intervention day 42 to post intervention day 28*), participants continued their habitual

levels of physical activity. The only restriction was to not perform any unilateral lower limb strength exercises. Finally, training compliance was determined as a percentage of sessions that were completed within 24 hours of the intended time.

Table 3-1. *Training volume progression during the 6-week intervention period.*

Training variable	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Frequency (days/week)	2	3	3	3	3	3
Sets	4	4	5	5	6	6
Repetitions	6	6	6	8	6	8
Total repetitions	48	72	90	120	108	144

3.3.5 Statistical analysis

All statistical analyses were performed using SPSS version 22.0.0.1 (IBM Corporation, Chicago, IL). Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and homoscedasticity of the data using Levene's test. Greenhouse-Geisser adjustment was applied when the assumption of sphericity was violated ($p < 0.05$ for Mauchly's test of sphericity). At each contraction intensity, a split-plot design ANOVA with the within-subject variables being limb (trained or untrained) and time point (*28 days pre baseline, baseline, intervention day 14, intervention day 21, intervention day 42, post intervention day 28*) and the between-subject variable being group (eccentric or concentric), was used to compare changes in BFlh architecture throughout the training study. Architectural changes across the 28 day control period (*28 days pre baseline to* baseline) were not significant ($p > 0.05$). Therefore when determining the alterations in BFlh architectural characteristics following a 6 week intervention, all comparisons were made to *baseline*. Knee flexor average peak torque comparisons, at each contraction velocity, used a similar split-plot design ANOVA, however with different time point variables (*baseline, intervention day 42, and post intervention day 28*). Where significant limb x time x group

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interactions for architecture and limb x time for knee flexor average peak torque were detected, post hoc t-tests with Bonferroni adjustments were used to identify which comparisons differed. Significance was set at α p<0.05 and where possible Cohen's *d* (Cohen, 1988) was reported for the effect size of the comparisons, with the levels of effect being deemed small (*d* = 0.20), medium (*d* = 0.50) or large (*d* = 0.80) as recommended by Cohen (1988).

3.3.6 Sample Size

Sample size analysis was completed *a-priori* using G-Power (Faul et al., 2007). The analysis was based on the anticipated differences in fascicle length following the training intervention. The effect size was estimated based on the only intervention study to date that has reported changes in the BFlh architecture (Potier et al., 2009). That study reported a 33% increase in fascicle length following the intervention with an approximate effect size of 1.9. Therefore an effect size of 1.2 was deemed as a reasonable starting point. Power was set at 80% with an alpha level of 0.05 returning a calculated sample size of 12 per group. As a cross-reference to confirm the effect size, fascicle length differences in individuals with a unilateral BFlh strain injury displayed an effect size of 1.34 when comparing between the previously injured and contralateral uninjured limb (Timmins et al., 2015).

Chapter 4 – Study 1: Biceps femoris long head architecture: a reliability and retrospective injury study.

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4.1. Linking paragraph

As outlined in Chapter 2.4.4, there is a paucity of evidence which has investigated the effect that injury has on muscle architecture. The evidence that does exist has been derived from isokinetic dynamometry which suggests that changes in the angle of peak knee flexor torque to shorter muscle lengths in limbs with a previous hamstring injury is due to a reduction in fascicle length. As the angle of peak torque measure lacks muscle specificity, it is still unknown if a previously strain injured muscle possesses shorter fascicle lengths. Chapter 4 focuses on determining if limbs with a history of strain injury in the BFlh have different architectural characteristics when compared to uninjured limbs. It is hypothesised that the previously strain injured BFlh would possess shorter fascicles when compared to the contralateral uninjured limb.

4.2. Abstract

Purpose: To determine i) the reliability of two-dimensional ultrasonography for the assessment of biceps femoris long head (BFlh) architectural characteristics; ii) if limbs with a history of strain injury in the BFlh display different architecture and eccentric strength compared to uninjured limbs. **Methods:** This case-control study (control [n=20], injured group [n=16], males) assessed the BFlh architecture at rest and during graded isometric contractions using two-dimensional ultrasonography. The control group were assessed three times (>24hrs apart) to determine reliability. Previously injured individuals were evaluated once. **Results:** The assessment of BFlh architecture was highly reliable (intraclass correlations >0.90). Fascicle length ($p<0.001$; d range: 0.67 to 1.34) and fascicle length relative to muscle thickness ($p<0.001$; d range: 0.58 to 0.85) of the previously injured BFlh were significantly less than the contralateral uninjured BFlh at all intensities. Pennation angle of the previously injured BFlh was significantly greater ($p<0.001$; d range: 0.62 to 0.88) than the contralateral uninjured BFlh at all intensities. Eccentric strength in the previously injured limb was significantly lower than the contralateral limb (-15.4%; -52.5N; 95% CI=28.45 to 76.23; $p<0.001$, d=0.56). **Conclusion:** These data indicate that two-dimensional ultrasonography is reliable for assessing BFlh architecture at rest and during graded isometric contractions. Fascicle length, fascicle length relative to muscle thickness and pennation angle are significantly different in previously injured BFlh compared to an uninjured contralateral BFlh. Eccentric strength of the previously injured limb is also significantly lower than the uninjured contralateral limb. These findings have implications for rehabilitation and injury prevention practices which should consider altered architectural characteristics.

4.3. Introduction

Hamstring strain injuries (HSIs) are the most prevalent injury type in many running based sports (Brooks, Fuller, Kemp, & Reddin, 2006; Ekstrand et al., 2012; Orchard et al., 2013) and result in athlete unavailability (Brooks et al., 2006; Woods et al., 2004), reduced performance on return to competition (Verrall, Kalairajah, Slavotinek, & Spriggins, 2006) and financial loss for sporting teams/organisations (Hickey, Shield, Williams, & Opar, 2013). An unresolved issue with hamstring strain injury is the elevated risk of recurrence, with reported re-injury rates 16-54% (Arnason et al., 2008; Ekstrand et al., 2012; Hagglund et al., 2013; Orchard et al., 2013).

To minimise the risk of hamstring strain re-injury a greater understanding of the maladaptations associated with a prior insult is required. Retrospective reports have identified prolonged deficits in the rate of torque development (Opar, Williams, et al., 2013b), biceps femoris activation (Opar, Williams, et al., 2013a; Sole et al., 2011), muscle volume (Silder et al., 2008), changes in the angle of peak torque (Brockett et al., 2004), and eccentric knee flexor strength (Opar, Williams, et al., 2013a) when tested via isokinetic dynamometry (Opar, Williams, et al., 2013a) as well as during the performance of the Nordic hamstring exercise (Opar, Piatkowski, et al., 2013) in previously injured hamstrings. Shifts in the angle of peak torque towards shorter muscle lengths have also been reported after hamstring strain and it has been proposed that these are indicative of a reduction of in-series sarcomeres and muscle fascicle length (Brockett et al., 2004). Lesser fascicle lengths following hamstring strain injury, hypothesised previously (Brockett et al., 2004; Fyfe et al., 2013), might be most troublesome for re-injury, as it would increase muscle susceptibility to eccentrically-induced microscopic muscle damage which may be a precursor to macroscopic damage in the form of a muscle strain injury (Morgan, 1990). However it is not yet known if a previously strained BFlh displays shorter fascicles compared to an uninjured BFlh.

Of all the methods available for the *in-vivo* assessment of muscle architecture (Lieber & Friden, 2000), real-time two-dimensional B-mode ultrasound is the most cost-effective and time-efficient. Two-dimensional ultrasonography has been shown to be a valid (Kellis et al., 2009) and reliable (Chleboun et al., 2001; E Lima, Carneiro, de, Peixinho, & de Oliveira, 2014; Potier et al., 2009) measure of BFlh architecture at a number of different hip and knee joint angles (Chleboun et al., 2001) whilst the muscle is at rest (Chleboun et al., 2001; Potier et al., 2009). Yet, muscle architecture during graded isometric contraction is also of interest, as architecture is altered significantly when the muscle is active (Kwah et al., 2013; R. L. Lieber & Friden, 2000) and is likely to have greater implications for understanding function. Currently, however, there is no reported reliability data in the literature for the assessment of BFlh architecture during graded isometric contractions.

The purposes of this study were to: 1) determine the test-retest reliability of real-time two-dimensional ultrasound measures of BFlh architecture (muscle thickness, pennation angle and fascicle length) at rest and during graded isometric contractions and; 2) determine if a previously strained BFlh displays different architecture compared to an uninjured BFlh. It is hypothesised that the previously injured limb will present with a BFlh displaying shorter fascicles and greater pennation angles compared to the contralateral uninjured BFlh.

4.4. Methods

As per university guidelines, the methodologies for this study are also presented in chapter 3.1. These methods are replicated below in the format required for *Medicine and Science in Sports and Exercise*.

4.4.1 Participants

Thirty six males were recruited to participate in this case-control study. Twenty recreationally active males (age 26.1 ±7.4 years; height 1.80 ±0.05m; body mass 78.1 ±8.7kg) with no history of hamstring strain injury were recruited to determine the test-retest reliability of the ultrasound measures of BFlh architecture, as well as serving as a control group. Sixteen elite (competing at national or international level) athletes with a unilateral BFlh strain injury history within the last 18 months (age 23.7 ±3.3 years; height 1.85 ±0.07m; body mass 83.6 ±7.9kg) were recruited to participate and form the previously injured group. The athletes (12 Australian Rules Football players, 2 soccer players, 1 hockey player and 1 track and field athlete), who had all returned to pre-injury levels of training and competition, were recruited to assess the differences in architecture between their previously injured and uninjured BFlh. Previously injured participants supplied their clinical notes to the research team and all had their diagnosis confirmed by magnetic resonance imaging. All previously injured athletes reported standard rehabilitation progression (Heiderscheit et al., 2010) and the use of some eccentric conditioning as guided by their physical therapist. All participants provided written informed consent prior to testing which was undertaken at the Australian Catholic University, Fitzroy, Victoria, Australia. Ethical approval for the study was granted by the Australian Catholic University Human Research Ethics Committee.

4.4.2 Experimental design

The test-retest reliability of real-time two-dimensional ultrasound derived measures of muscle thickness, pennation angle and fascicle length of BFlh at rest and during graded isometric contractions was determined across three separate testing sessions. Sessions were separated by at least 24 hours. On the final visit, eccentric knee flexor strength during the Nordic hamstring exercise was also assessed using a custom made device (Opar, Piatkowski, et al., 2013).

Determining the impact of a previous strain injury on the BFlh architectural characteristics and eccentric knee flexor strength was performed during a single assessment session in the previously injured cohort.

4.4.3 BFlh architecture assessment

Muscle thickness, pennation angle and fascicle length of the BFlh was determined from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-*i*, Wauwatosa, U.S.A). The scanning site was determined as the halfway point between the ischial tuberosity and the knee joint fold, along the line of the BFlh. Once the scanning site was determined, the distance of the site from various anatomical landmarks were recorded to ensure reproducibility of the scanning site for future testing sessions. These landmarks included the ischial tuberosity, fibula head and the posterior knee joint fold at the mid-point between BF and semitendinosus tendon. On subsequent visits during the reliability study, the scanning site was determined and marked on the skin and then confirmed by replicated landmark distance measures. All architectural assessments were performed with participants in a prone position and the hip in a neutral position following at least 5 minutes of inactivity. Assessments at rest were always performed first followed by the graded isometric contraction protocol. Assessment of BFlh architecture at rest was performed with the knee at three different positions; 0º, 30º and 60º of knee flexion; which were determined via manual goniometer. Assessment of BFlh architecture during isometric contractions were always performed with the knee at 0º of knee flexion and preceded by a maximal voluntary isometric contraction, performed in a custom made device (Opar, Piatkowski, et al., 2013). The graded isometric contractions of the knee flexors were performed in the same device at 25, 50 and 75% of maximum voluntary isometric contraction (MVIC) with the participants shown the real-time visual feedback of the

force produced to ensure that target contraction intensities were met. Assessment of the MVIC of the knee flexors was undertaken in a prone position, with both the hip and knee fully extended (0deg). Participants were instructed to contract maximally over a five second period, of which the peak force was used to determine the MVIC.

To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence the accuracy of the measures (Klimstra et al., 2007). Finally, the orientation of the probe was manipulated slightly by the sonographer (RGT) if the superficial and intermediate aponeuroses were not parallel.

Once the images were collected analysis was undertaken off-line (MicroDicom, Version 0.7.8, Bulgaria). For each image, six points were digitised as described by Blazevich and colleagues (Blazevich et al., 2006). These points included two each on both the superficial and deep aponeurosis as well as two on the fascicle of interest. Following the digitising process, muscle thickness was defined as the distance between the superficial and intermediate aponeuroses of BFlh. A fascicle of interest was outlined and marked on the image. The angle between this fascicle and the intermediate aponeurosis was measured and given as the pennation angle. The aponeurosis angle for both aponeuroses was determined as the angle between the line marked as the aponeurosis and an intersecting horizontal line across the captured image (Blazevich et al., 2006; Kellis et al., 2009). Fascicle length was determined as the length of the outlined fascicle between aponeuroses. As the entire fascicle was not visible in the field of view of the probe it was estimated via the following validated equation from Blazevich and colleagues (Blazevich et al., 2006; Kellis et al., 2009):

FL=sin (AA+90deg) x MT/sin (180deg-(AA+180deg-PA)).

Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness, and PA=pennation angle.

Fascicle length was reported in absolute terms (cm) and also relative to muscle thickness (fascicle length/muscle thickness). The same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers during the analysis.

4.4.4 Eccentric Nordic hamstring exercise strength

The assessment of eccentric hamstring strength using the Nordic hamstring exercise field testing device has been reported previously (Opar, Piatkowski, et al., 2013). Participants were positioned in a kneeling position over a padded board, with the ankles secured superior to the lateral malleolus by individual ankle braces which were secured atop custom made uniaxial load cells (Delphi Force Measurement, Gold Coast, Australia) fitted with wireless data acquisition capabilities (Mantracourt, Devon, UK) (Figure 3.1 and 3.2). The ankle braces and load cells were secured to a pivot which allowed the force to always be measured through the long axis of the load cells. Following a warm up set, participants were asked to perform one set of three maximal bilateral repetitions of the Nordic hamstring exercise. Participants were instructed to gradually lean forward at the slowest possible speed while maximally resisting this movement with both lower limbs while keeping the trunk and hips in a neutral position throughout, and the hands held across the chest. Following each attempt a visual analogue scale was given to assess the level of pain that was experienced. None of the participants reported any pain during testing. Verbal encouragement was given throughout the range of motion to ensure maximal effort. The peak force for each of the three repetitions was averaged for all statistical comparisons.

4.4.5 Statistical analyses

All statistical analyses were performed using SPSS version 19.0.0.1 (IBM Corporation, Chicago, IL). For the determination of reliability, descriptive statistics for the architectural variables of the control group were determined for the left and right limbs separately. Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and homoscedasticity of the data using Levene's test. Intraclass correlation coefficient (ICC), typical error (TE), and TE as a co-efficient of variation (%TE) were calculated to assess the extent of variation between the first to second and the second to third visit (Weir, 2005). Based on previous quantitative reliability literature, it was subjectively determined that an ICC $\geq$ 0.90 was regarded as high, between 0.80 and 0.89 was moderate, and $\leq$ 0.79 was poor (16, 37). Minimum detectable change at a 95% confidence interval (MDC$_{95}$) was calculated as [TE x 1.96 x $\sqrt{2}$]. Additionally, a %TE of $\leq$10% was considered to represent an acceptable level of reliability (Cormack et al., 2008).

At each contraction intensity, a split-plot design ANOVA with the within-subject variable being limb (left/right or uninjured/injured, depending on group) and the between-subject variable being group (control or previously injured) was used to compare BFlh architecture and Nordic hamstring exercise strength variables. Control group data was used from the third trial. Where significant limb x group interactions were detected, post hoc t-tests with Bonferroni adjustments to the alpha level were used to identify which comparisons differed.

Further between group analyses were undertaken to determine the extent of the between limb asymmetry in BFlh architecture and Nordic hamstring exercise strength, in the control and previously injured groups. The control group between limb asymmetry was determined as the right limb minus the left and then converted to an absolute value (Sole et al., 2011), whereas in the previously injured group asymmetry was determined as the uninjured limb minus the injured limb. T-tests were used to assess differences in the extent of the between limb asymmetry in the

control compared to the previously injured group. Bonferroni corrections were employed to account for inflated type I error due to the multiple comparisons made for each dependent variable. Significance was set at a p<0.05 and where possible Cohen's *d* (Cohen, 1988) was reported for the effect size of the comparisons, with the levels of effect being deemed small (*d* = 0.20), medium (*d* = 0.50) or large (*d* = 0.80) as recommended by Cohen (1988).

4.4.6 Power calculations

Following the reliability study, *a-priori* power analysis for the previously injured group was completed using G-Power (Faul et al., 2007). The analysis was based on the anticipated differences in fascicle length between the injured and uninjured limb. The effect size was estimated based on the only study to date that has reported changes in to show changes in BF fascicle length following eccentric training. That study reported a very large increase (effect size of approximately 1.9) of 33% in fascicle following the intervention. Therefore an effect size of 0.8 was deemed reasonable as a starting point. Power was set at 80% with an alpha level of 0.05 returning a calculated sample size of 15. As a cross-reference to confirm this sample size calculation previous studies that have used similar designs have used sample sizes from 13 to 15 (Opar, Williams, et al., 2013a, 2013b; Sole et al., 2011).

4.5 Results

4.5.1 Reliability study

All architectural variables examined displayed high reliability at rest (Table 3.1) and during graded isometric contractions (Table 3.2). For fascicle length, all ICCs were above 0.93 at rest and during isometric contractions and all %TE were below 4.9% at rest and 3.7% during isometric contractions. For muscle thickness, all ICCs were above 0.95 at rest and above 0.96 during isometric contractions. All muscle thickness %TE were below 4.2% at rest and 3.8% during isometric contraction. For pennation angle, at rest all ICCs were above 0.95, and during

isometric contractions all ICCs were above 0.93 with all %TE below 3.2% and 2.6% at rest and during isometric contractions respectively.

Table 4-1. *Descriptive statistics and test-retest reliability data from the control group for the architectural characteristics of the biceps femoris long head assessed at rest.*

(n=20)	Mean (±SD)			ICC (95% CI)	TE (95% CI)	%TE (95% CI)	MDC$_{95}$
Left Leg: 0°	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.34±0.26	2.37±0.33	2.39±0.28	0.97 (0.95 – 0.99)	0.08 (0.06-0.11)	3.4 (2.8-4.7)	0.22
PA (degrees)	12.81±1.13	12.93±1.20	12.94±1.11	0.96 (0.92 – 0.98)	0.32 (0.26-0.44)	2.5 (2.0-3.4)	0.88
FL (cm)	10.70±1.40	10.72±1.50	10.72±1.50	0.97 (0.95 – 0.99)	0.32 (0.26-0.44)	3.0 (2.4-4.1)	0.88
Right Leg: 0°	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.43±0.28	2.45±0.29	2.42±0.30	0.98 (0.96 – 0.99)	0.06 (0.05-0.08)	2.6 (2.0-3.5)	0.16
PA (degrees)	12.72±1.11	12.91±1.16	12.77±1.09	0.96 (0.92 – 0.98)	0.37 (0.30-0.50	3.0 (2.4-4.2)	1.02
FL (cm)	11.10±1.44	11.09±1.41	11.01±1.48	0.98 (0.97 – 0.99)	0.22 (0.18-0.30)	2.1 (1.7-2.9)	0.61
Left Leg: 30°	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.43±0.28	2.43±0.32	2.41±0.34	0.95 (0.90 – 0.98)	0.10 (0.08-0.14)	4.2 (3.4-5.8)	0.27
PA (degrees)	14.93±1.17	14.80±1.15	14.79±1.10	0.95 (0.90 – 0.98)	0.43 (0.35-0.58)	3.0 (2.4-4.1)	1.19
FL (cm)	9.47±1.24	9.60±1.32	9.46±1.31	0.97 (0.94 – 0.98)	0.32 (0.26-0.44)	3.4 (2.7-4.6)	0.88
Right Leg: 30°	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.49±0.34	2.50±0.34	2.51±0.33	0.98 (0.95 – 0.99)	0.08 (0.07-0.12)	3.5 (2.8-4.8)	0.22
PA (degrees)	15.09±1.09	14.96±1.35	14.95±1.22	0.95 (0.90 – 0.98)	0.45 (0.36-0.61)	3.2 (2.6-4.4)	1.24
FL (cm)	9.60±1.37	9.69±1.21	9.74±1.28	0.97 (0.95 – 0.99)	0.34 (0.27-0.46)	3.6 (2.9-4.9)	0.94
Left Leg: 60°	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.45±0.30	2.43±0.30	2.41±0.30	0.95 (0.90 – 0.98)	0.10 (0.08-0.13)	4.1 (3.3-5.6)	0.27
PA (degrees)	17.31±1.56	16.95±1.36	17.09±1.40	0.95 (0.90 – 0.98)	0.43 (0.35-0.59)	2.5 (2.0-3.3)	1.19
FL (cm)	8.29±1.10	8.35±1.07	8.25±1.11	0.96 (0.91 – 0.97)	0.36 (0.29-0.49)	4.2 (3.4-5.7)	0.99
Right Leg: 60°	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.51±0.32	2.54±0.26	2.51±0.28	0.96 (0.92 – 0.98)	0.10 (0.08-0.13)	4.0 (3.2-5.4)	0.27
PA (degrees)	16.97±1.68	17.07±1.55	17.28±1.45	0.95 (0.91 – 0.98)	0.52 (0.42-0.70)	3.0 (2.4-4.1)	1.44
FL (cm)	8.63±1.12	8.69±1.08	8.45±0.77	0.93 (0.86 – 0.97)	0.42 (0.34-0.57)	4.9 (3.9-6.7)	1.16

SD = standard deviation, ICC = intraclass correlation, TE = typical error, %TE = typical error as a % co-efficient of variation, 95% CI = 95% confidence interval, MDC$_{95}$ = minimum detectable change at a 95% confidence interval, MT = muscle thickness, cm = centimetres, PA = pennation angle, FL = fascicle length.

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Table 4-2. *Descriptive statistics and test-retest reliability data from the control group for the architectural characteristics of the biceps femoris long head assessed during graded isometric contractions*

Active (n=20)	Mean (±SD)			ICC (95% CI)	TE (95% CI)	%TE (95% CI)	MDC$_{95}$
Left Leg: 25% MVIC	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.55±0.29	2.58±0.35	2.61±0.32	0.97 (0.95-0.99)	0.07 (0.06-0.10)	2.9 (2.3-3.9)	0.19
PA (degrees)	14.59±0.98	14.71±0.99	14.84±0.97	0.95 (0.90-0.98)	0.34 (0.28-0.47)	2.3 (1.8-3.1)	0.94
FL (cm)	10.22±1.44	10.20±1.42	10.22±1.36	0.93 (0.86-0.97)	0.28 (0.23-0.38)	2.9 (2.3-4.0)	0.77
Right Leg: 25% MVIC	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.64±0.30	2.65±0.34	2.59±0.33	0.97 (0.95-0.99)	0.09 (0.07-0.12)	3.6 (2.9-4.9)	0.24
PA (degrees)	14.69±1.01	14.70±0.95	14.70±1.05	0.95 (0.91-0.98)	0.39 (0.31-0.53)	2.6 (2.1-3.6)	1.08
FL (cm)	10.44±1.05	10.43±1.13	10.21±1.12	0.97 (0.94-0.98)	0.30 (0.24-0.41)	3.1 (2.5-4.2)	0.83
Left Leg: 50% MVIC	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.61±0.33	2.60±0.35	2.67±0.34	0.97 (0.93-0.98)	0.10 (0.08-0.13)	3.8 (3.1-5.2)	0.27
PA (degrees)	16.33±1.16	16.39±1.03	16.61±1.09	0.93 (0.87-0.97)	0.41 (0.33-0.56)	2.5 (2.0-3.4)	1.13
FL (cm)	9.35±1.48	9.27±1.37	9.39±1.28	0.97 (0.95-0.99)	0.33 (0.27-0.45)	3.7 (2.9-5.0)	0.91
Right Leg: 50% MVIC	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.72±0.29	2.76±0.32	2.69±0.29	0.97 (0.95-0.99)	0.08 (0.07-0.11)	2.8 (2.3-3.9)	0.22
PA (degrees)	16.67±1.09	16.84±1.20	16.62±1.13	0.97 (0.93-0.98)	0.32 (0.26-0.44)	1.9 (1.6-2.6)	0.86
FL (cm)	9.51±1.19	9.56±1.18	9.46±1.08	0.97 (0.95-0.99)	0.30 (0.24-0.41)	3.2 (2.6-4.4)	0.83
Left Leg: 75% MVIC	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.65±0.35	2.63±0.36	2.67±0.34	0.97 (0.95-0.99)	0.08 (0.07-0.11)	3.3 (2.6-4.5)	0.22
PA (degrees)	17.98±1.16	17.78±1.06	17.72±1.06	0.97 (0.94-0.98)	0.28 (0.22-0.38)	1.6 (1.3-2.3)	0.77
FL (cm)	8.64±1.23	8.64±1.18	8.81±1.11	0.97 (0.93-0.98)	0.31 (0.25-0.42)	3.7 (3.0-5.1)	0.85
Right Leg: 75% MVIC	*Test 1*	*Test 2*	*Test 3*	*Mean*	*Mean*	*Mean*	
MT (cm)	2.73±0.30	2.73±0.29	2.69±0.28	0.96 (0.92-0.98)	0.09 (0.07-0.12)	3.2 (2.6-4.3)	0.24
PA (degrees)	17.99±1.41	17.97±1.20	17.73±1.24	0.95 (0.90-0.98)	0.43 (0.35-0.59)	2.4 (1.9-3.3)	1.19
FL (cm)	8.87±0.98	8.86±0.96	8.88±0.97	0.95 (0.90-0.98)	0.32 (0.26-0.43)	3.5 (2.8-4.8)	0.88

SD = standard deviation, ICC = intraclass correlation, TE = typical error, %TE = typical error as a % co-efficient of variation, 95% CI = 95% confidence interval, MDC$_{95}$ = minimum detectable change at a 95% confidence interval, *MVIC* = maximum voluntary isometric contraction, MT = muscle thickness, cm = centimetres, PA = pennation angle, FL = fascicle length.

4.5.2 BFlh architectural comparisons

Fascicle length, pennation angle, muscle thickness and relative fascicle length in the injured and uninjured limb of the previously injured group were not significantly different from the control group data at any contraction intensity (p>0.05). A significant limb-by-group interaction effect was found for fascicle length and fascicle length relative to muscle thickness at all contraction intensities (p<0.011). Post hoc testing showed that fascicle length and fascicle length relative to muscle thickness were significantly shorter in the injured BFlh compared to the contralateral uninjured BFlh in the previously injured group at all contraction intensities (p<0.05, d range: 0.58 to 1.34, Table 4.3, Figure 4.1). A significant interaction effect for limb-by-group was detected at all contraction intensities (p<0.004) for pennation angle. Post hoc comparisons showed that pennation angle was significantly greater in the injured BFlh compared to the contralateral uninjured BFlh in the previously injured group (p<0.05, d range: 0.62 to 0.88, Table 4.3, Figure 4.1). There were no significant main effects detected for comparisons of muscle thickness between the injured and uninjured BFlh in the previously injured group at any contraction intensity (p>0.05, $d = 0.18$ to 0.43, Table 4.3, Figure 4.1). Furthermore, the control group showed no (p>0.05, d=0.01 to 0.19, Table 4.3) between limb differences in any BFlh architectural characteristics at any contraction intensity.

When comparing the extent of between limb asymmetry in BFlh architecture of the control group to the previously injured group, the asymmetry in fascicle length, fascicle length relative to muscle thickness and pennation angle was significantly greater in the previously injured group at all contraction intensities (p<0.05, $d = 0.75$ to 1.19, Table 4.4). There were no differences between groups in the extent of between limb asymmetry in muscle thickness at any contraction intensity (p>0.359, d <0.31, Table 4.4, Figure 4.2).

Table 4-3. *Within group comparisons of the biceps femoris long head architectural characteristics for the control group (right vs left) and the previously injured group (uninjured vs injured) assessed at all contraction intensities.*

0% of MVIC	Control group (3rd visit)					Injured group				
	Right Leg (Mean±SD)	Left leg (Mean±SD)	Right minus left (95% CI)	p	Effect Size (*d*)	Uninjured (Mean±SD)	Injured (Mean±SD)	Uninjured minus Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	11.01±1.48	10.72±1.50	0.29 (-0.38 to 0.95)	0.383	0.19	11.94±1.25	10.40±1.12	1.54 (0.95 to 2.10)	0.001**	1.34
RFL	4.55±0.39	4.50±0.40	0.05 (-0.13 to 0.24)	0.543	0.14	4.97±0.56	4.57±0.69	0.40 (0.22 to 0.60)	0.001**	0.65
PA (deg)	12.77±1.09	12.94±1.11	0.16 (-0.36 to 0.69)	0.527	0.15	11.74±1.38	12.94±1.80	-1.20 (0.71 to 1.70)	0.001**	-0.74
MT (cm)	2.42±0.30	2.39±0.28	0.03 (-0.15 to 0.09)	0.613	0.11	2.41±0.23	2.30±0.26	0.11 (-0.04 to 0.25)	0.141	0.43

25% of MVIC	Control group (3rd visit)					Injured group				
	Right Leg (Mean±SD)	Left leg (Mean±SD)	Right minus left (95% CI)	p	Effect Size (*d*)	Uninjured (Mean±SD)	Injured (Mean±SD)	Uninjured minus Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	10.21±1.12	10.22±1.36	0.01 (-0.51 to 0.49)	0.974	0.14	10.67±1.16	9.50±1.10	1.17 (0.69 to 1.64)	0.001**	1.02
RFL	3.96±0.30	3.92±0.28	0.04 (-0.09 to 0.18)	0.513	0.13	4.25±0.51	3.86±0.42	0.39 0.20 to 0.58)	0.001*	0.85
PA (deg)	14.70±1.05	14.84±0.97	0.14 (-0.43 to 0.70)	0.619	0.12	13.82±1.75	15.18±1.69	-1.36 (0.68 to 2.04)	0.001*	-0.79
MT (cm)	2.59±0.33	2.61±0.32	0.02 (-0.11 to 0.16)	0.715	0.06	2.53±0.34	2.47±0.26	0.06 (-0.06 to 0.18)	0.319	0.20

50% of MVIC	Control group (3rd visit)					Injured group				
	Right Leg (Mean±SD)	Left leg (Mean±SD)	Right minus left (95% CI)	p	Effect Size (*d*)	Uninjured (Mean±SD)	Injured (Mean±SD)	Uninjured minus Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	9.46±1.08	9.39±1.28	0.07 (-0.40 to 0.54)	0.771	0.05	9.65±1.45	8.83±1.07	0.82 (0.36 to 1.28)	0.002*	0.67
RFL	3.51±0.25	3.52±0.24	0.01 (-0.10 to 0.10)	0.930	0.05	3.72±0.41	3.49±0.37	0.23 0.11 to 0.33)	0.001*	0.58
PA (deg)	16.62±1.13	16.61±1.09	0.01 (-0.48 to 0.46)	0.962	0.01	15.76±1.67	16.78±1.75	-1.02 (0.53 to 1.50)	0.001**	-0.62
MT (cm)	2.69±0.29	2.67±0.34	0.02 (-0.15 to 0.11)	0.750	0.06	2.60±0.31	2.53±0.26	0.07 (-0.05 to 0.18)	0.260	0.23

75% of MVIC	Control group (3rd visit)					Injured group				
	Right Leg (Mean±SD)	Left leg (Mean±SD)	Right minus left (95% CI)	p	Effect Size (*d*)	Uninjured (Mean±SD)	Injured (Mean±SD)	Uninjured minus Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	8.88±0.97	8.81±1.11	0.07 (-0.38 to 0.51)	0.757	0.05	8.95±1.02	8.22±0.70	0.73 (0.32 to 1.14)	0.002*	0.84
RFL	3.29±0.21	3.29±0.18	0.00 (-0.05 to 0.06)	0.870	0.04	3.42±0.28	3.23±0.23	0.19 0.08 to 0.30)	0.002*	0.76
PA (deg)	17.73±1.24	17.72±1.06	0.01 (-0.35 to 0.34)	0.988	0.01	16.95±1.41	18.10±1.27	-1.15 (0.55 to 1.74)	0.001*	-0.88
MT (cm)	2.69±0.28	2.67±0.34	0.02 (-0.16 to 0.12)	0.777	0.06	2.60±0.29	2.55±0.25	0.05 (-0.07 to 0.17)	0.404	0.18

MVIC = maximum voluntary isometric contraction, SD = standard deviation, 95% CI = 95% confidence interval , FL = fascicle length, cm = centimetres, RFL = fascicle length relative to muscle thickness, PA = pennation angle, deg = degrees, MT = muscle thickness. *=p<0.05, **=p<0.001.

Table 4-4. *Between limb asymmetry of the biceps femoris long head architectural characteristics comparing the control group (absolute differences) and the previously injured group (uninjured minus injured) assessed at all contraction intensities.*

0% of MVIC	Control vs Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	1.25 (0.36 to 2.12)	0.007*	0.98
RFL	0.35 (0.09 to 0.61)	0.009*	0.93
PA (deg)	1.04 (0.34 to 1.74)	0.005*	1.00
MT (cm)	0.07 (-0.11 to 0.26)	0.410	0.27

25% of MVIC	Control vs Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	1.16 (0.49 to 1.86)	0.001*	1.19
RFL	0.35 (0.12 to 0.57)	0.003*	1.05
PA (deg)	1.22 (0.37 to 2.07)	0.006*	0.98
MT (cm)	0.04 (-0.10 to 0.27)	0.359	0.31

50% of MVIC	Control vs Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	0.75 (0.10 to 1.41)	0.024*	0.80
RFL	0.22 (0.08 to 0.37)	0.004*	1.04
PA (deg)	1.01 (0.37 to 1.68)	0.003*	1.04
MT (cm)	0.05 (-0.13 to 0.22)	0.602	0.18

75% of MVIC	Control vs Injured (95% CI)	p	Effect Size (*d*)
FL (cm)	0.67 (0.05 to 1.26)	0.032*	0.75
RFL	0.19 (0.07 to 0.30)	0.002*	1.08
PA (deg)	1.14 (0.51 to 1.78)	0.001*	1.19
MT (cm)	0.03 (-0.15 to 0.21)	0.755	0.10

MVIC = maximum voluntary isometric contraction, SD = standard deviation, 95% CI = 95% confidence interval , FL = fascicle length, cm = centimetres, RFL = fascicle length relative to muscle thickness, PA = pennation angle, deg = degrees, MT = muscle thickness, MT = muscle thickness. *=p<0.05.

4.5.3 Eccentric Nordic hamstring exercise strength

The control group showed no statistically significant difference between the right (295.1N±74.5) and left limb (281.4N±78.1) in average peak force during the Nordic hamstring exercise (between limb difference = 4.8%; 13.7N; 95% CI = -0.17 to 27.67; p = 0.053; d = 0.18In contrast, the injured limb (288.6N±84.8) was weaker than the contralateral uninjured (341.1N±100.2) limb in the previously injured group (between limb difference = -15.4%; 52.5N; 95% CI = 28.45 to 76.23; p < 0.001, d = 0.56). The previously injured group also displayed a larger between limb asymmetry compared to the control group (p = 0.007, d = 1.01).

4.5.4 Maximal isometric knee flexor strength

The knee flexor MVIC forces did not differ between the right (273.8N±33.4) and left limbs (263.2N±33.5) of the control participants (between limb difference = 3.8%; 10.6N; 95% CI = -2.4 to 23.8; p = 0.106; d=0.31) or between the injured (236.6N±53.1) and contralateral uninjured limbs (262.6N±51.4) in the previously injured group (between limb difference = 9.9%; 26N; 95% CI = -15.8 to 67.7; p = 0.205; d=0.49). Furthermore the extent of between limb asymmetry during the knee flexor MVIC was not significantly different when comparing the control group and previously injured group (p=0.467, d=0.25).
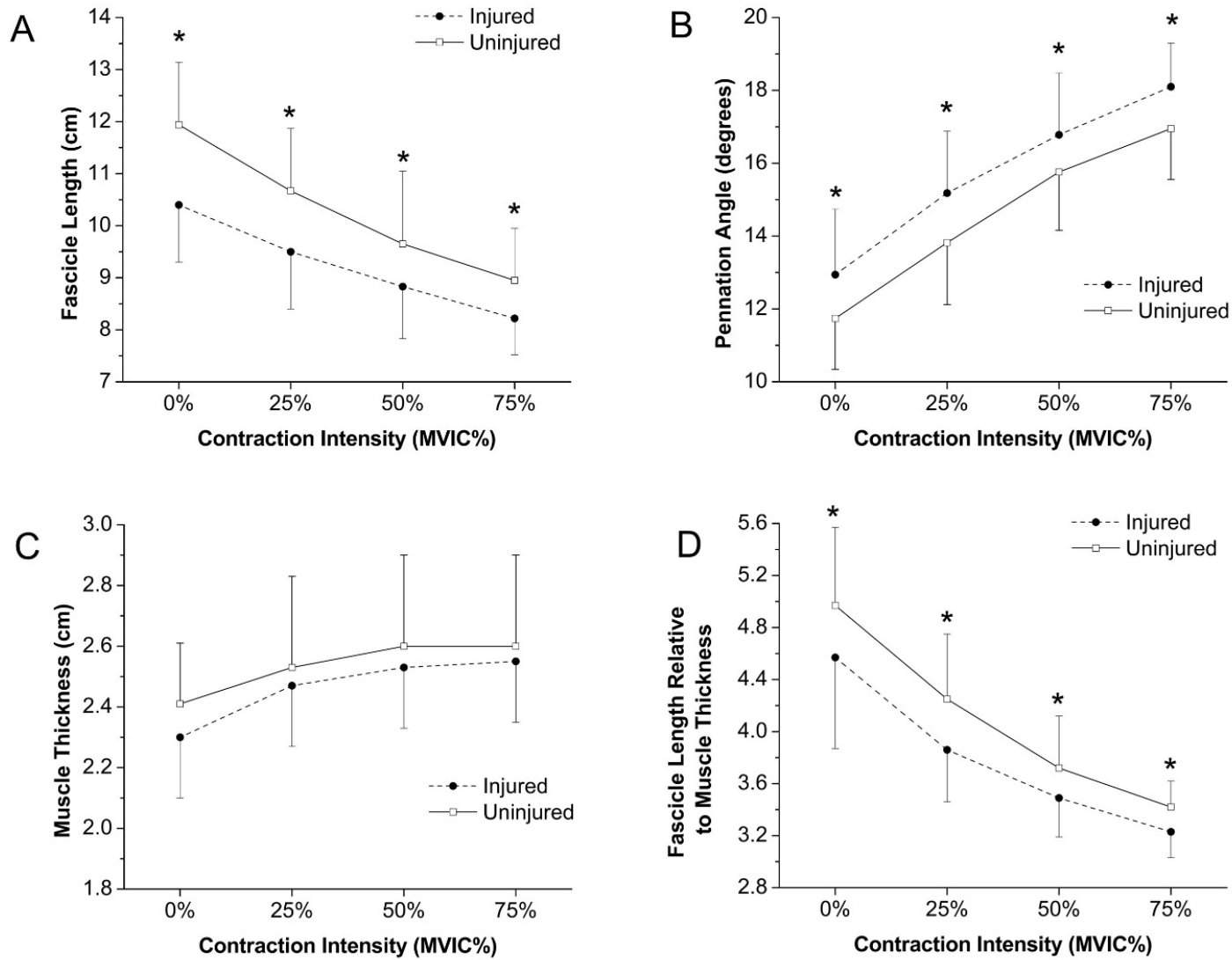
Figure 4-1. *Architectural characteristics of the injured BFlh and the contralateral uninjured BFlh in the previously injured group at all contraction intensities: fascicle length (A), pennation angle (B), muscle thickness (C), fascicle length relative to muscle thickness (D). Error bars illustrate SD. *P<0.05 injured versus uninjured, cm=centimetres, MVIC=maximum voluntary isometric contraction.*
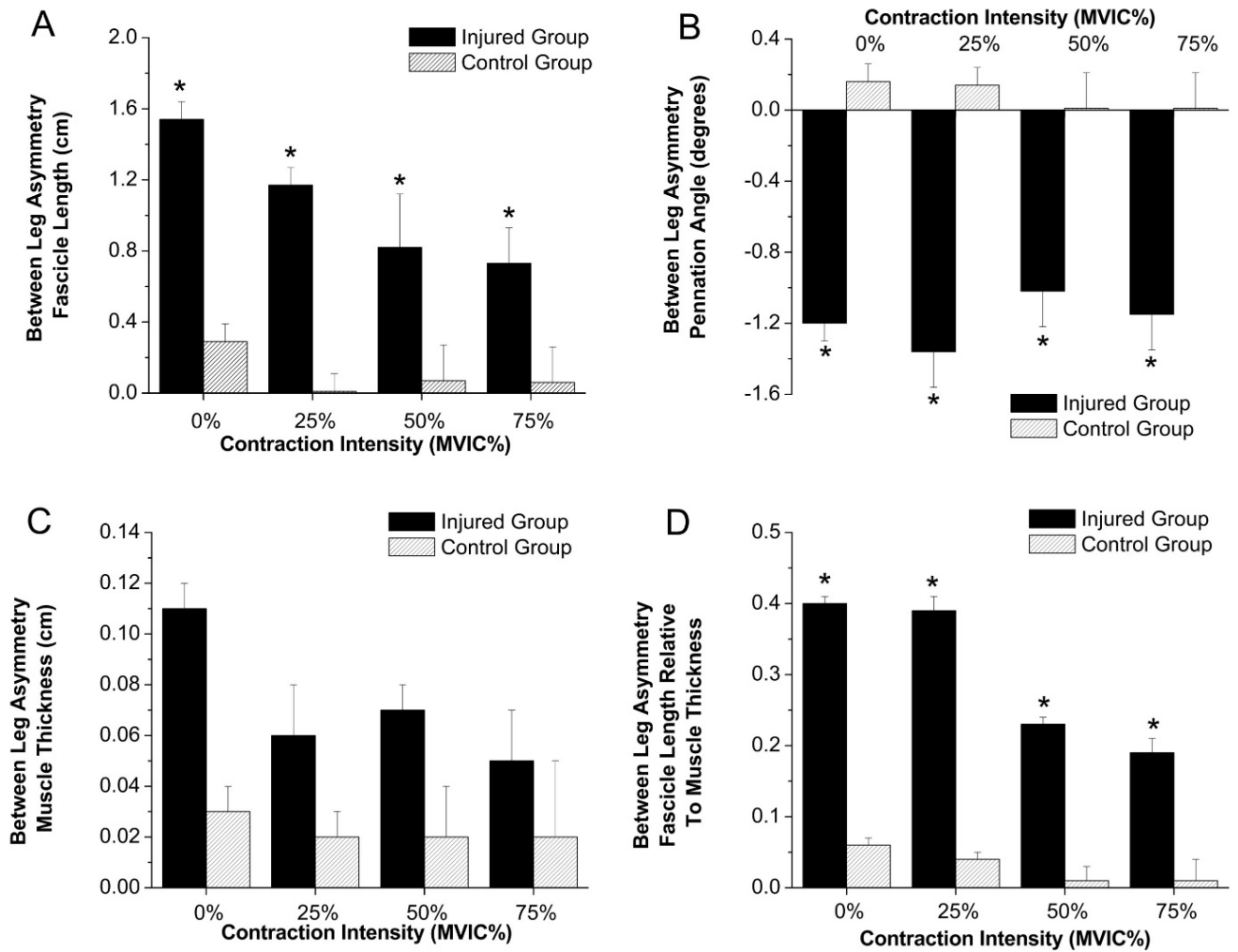
Figure 4-2. *Comparisons of between-limb asymmetry for the architectural characteristics of the previously injured BFlh group (uninjured mins injured) to the absolute between-limb differences of the control group at all contraction intensities: fascicle length (A), pennation angle (B), muscle thickness (C), and fascicle length relative to muscle thickness (D). Error bars illustrate the SD. *P<0.05 injured versus control, cm=centimetres, MVIC=maximum voluntary isometric contraction*

4.6 Discussion

To the authors' knowledge this is the first study that describes the reliability of assessing the architecture of the BFlh muscle *in-vivo* during graded isometric contractions. Additionally no prior work has examined architectural differences between previously injured and uninjured muscles. The major findings were that the use of two-dimensional ultrasound to assess *in-vivo* BFlh architecture is highly reliable at rest and during isometric knee flexion, when performed by a skilled operator. Additionally, in elite athletes with a unilateral history of BFlh strain injury, fascicles were shorter and pennation angles greater in the previously injured limb compared to the contralateral uninjured limb. Moreover, between limb asymmetry in fascicle length and pennation angle was greater in the previously injured group, compared to the control group. Eccentric knee flexor force during the Nordic hamstring exercise was also significantly reduced in the previously injured limbs compared to the contralateral uninjured limb in the previously injured group. By contrast, isometric strength was not statistically different in previously injured limbs compared to the contralateral uninjured limb.

Observations of shorter muscle fascicles in the previously injured BFlh (Figure 4.1.a) supports preceding literature which inferred altered architectural characteristics through the use of isokinetic dynamometry (Brockett et al., 2004). Brockett and colleagues proposed that differences in the torque-joint angle relationship in those with a history of hamstring strain injury were mediated by a reduction in the number of in-series sarcomeres (Brockett et al., 2004).

The current study supports their hypothesis; however, the extent to which injury and fascicle shortening in one of many knee flexor muscles can influence the knee flexor torque-joint angle relationship is not clear. Future work should examine whether architectural changes extend beyond the injured muscle into the neighbouring knee flexors.

Changes to muscle activation patterns across the range of knee motion may also contribute to shifts in the torque-joint angle relationship. Inhibition of the previously injured hamstrings appears to be greatest at long muscle lengths (Sole et al., 2011) and this would be expected to shift torque-joint angle relationships to shorter lengths. It has also been argued that neuromuscular inhibition and its preferential effect on eccentric strength after hamstring injury (Opar, Piatkowski, et al., 2013; Opar, Williams, et al., 2013a) may also contribute to the persistence of architectural deficits (Fyfe et al., 2013). Fascicles may shorten in response to the reduced excursions experienced in the early stages of recuperation. As rehabilitation continues and the athlete returns to more intense training it might be thought that progressively stronger eccentric contractions would act as a stimulus for sarcomerogenesis (Potier et al., 2009). However, inhibited muscles may fail to lengthen back to normal if they are minimally activated during active lengthening (Opar, Williams, et al., 2013a; Sole et al., 2011). The results of this study are consistent with a theoretical model proposed by Fyfe and colleagues who proposed that reductions in fascicle lengths persist in those with a history of HSI, even after they return to full training and match play (Fyfe et al., 2013). Such maladaptations may contribute to the re-injury risk that is evident in sport and it has been argued that fascicles with fewer in-series sarcomeres are more prone to damage caused by the powerful eccentric contractions (Brockett et al., 2004; Morgan, 1990).

In the current study, individuals with a unilateral history of HSI displayed no significant differences in muscle thickness when comparing the injured BFlh to the contralateral uninjured BFlh at any of the contraction intensities (Figure 4.1.c). However, previous investigations utilising magnetic resonance imaging have shown a significant reduction in muscle volume in those with a history of BFlh strain injury (Silder et al., 2008), although there are a number of potential explanations for this apparent discrepancy. Firstly, the greater pennation angle may

counter any tendency for muscle thickness to be lower in the injured BFlh in comparison to the contralateral uninjured BFlh. As a consequence, some atrophy may have occurred and been effectively masked when muscle thickness was assessed. Secondly, atrophy, if it has occurred, may not be uniform along the length of the injured BFlh. It is well known that changes in muscle thickness and anatomical cross-sectional area, following resistance training interventions, are variable along the length of a muscle (Blazevich et al., 2007) and there is no reason to suppose that atrophy is somehow more uniform. It is therefore possible that the assessment of muscle thickness in the current study may have occurred at a point of the BFlh where the muscle displays limited atrophy. Future research should examine potential BFlh architectural differences at multiple sites along the muscle.

Differences in the slopes of the fascicle length-contraction intensity relationships for previously injured and uninjured BFlh (Figure 4.1.a) suggest differences in tendon-aponeurosis compliance; specifically that the previously injured BFlh may have stiffer tendons and/or aponeurosis than the homonymous muscle in the uninjured limbs. This may be consequent to scar tissue accumulation at the site of the original injury, although it is impossible to rule out the possibility that discrepancies in stiffness may have predated the injuries. Regardless, elevated knee flexor muscle stiffness has been previously associated with an increased risk of HSI (Watsford et al., 2010). Future work should focus on how tendon mechanics are altered following HSI.

The current data indicates that BFlh architectural characteristics are significantly altered as a function of isometric force production. From rest to 75% of MVIC, pennation angle increased from 12.8deg to 17.8deg with a concomitant reduction in FL from 10.8cm to 8.7cm in the control group. Using cadaveric data of mean muscle lengths (van der Made et al., 2013; Woodley & Mercer, 2005) the alterations in muscle architecture reported in this study equate to an

approximate 14% decrease in muscle length (Narici, Binzoni, et al., 1996). These results suggest that the aponeurosis and tendon of the BFlh exhibit significant compliance and this supports the concept that they act as mechanical buffers to reduce the extent of damage caused within the myofibrils during high intensity eccentric contractions (Roberts & Konow, 2013). The current data may serve an additional purpose, as previous computational models of the human BFlh have utilised the geometrical data obtained from cadaveric samples to determine such variables as force-length and force-velocity relationships and fibre force production (Schache, Dorn, Blanch, Brown, & Pandy, 2012; Thelen, Chumanov, Best, Swanson, & Heiderscheit, 2005).

Previous investigations have examined the reliability of ultrasonography to assess BFlh architecture at rest. These studies report a range of ICCs from 0.78 to 0.97 (Chleboun et al., 2001; E Lima et al., 2014; Potier et al., 2009), which, at the upper end, is similar to values reported in the present study. In two of these studies, %TE for the assessment of the BFlh architecture is also reported, with values ranging from 2.15% to 9.7%, with the current study reporting values ranging from 1.6% to 4.9% (E Lima et al., 2014; Kellis et al., 2009). Only one study has investigated the reliability of ultrasonography of the BFlh during an MVIC (100% of maximum), however the ICC reported was poor (0.78) (E Lima et al., 2014). The current study examined the reliability of the BFlh architecture at 25, 50 and 75% of MVIC and found high reliability of these measures (Table 4.2). Imaging maximal contractions is difficult due to the inability of participants to maintain a smooth contraction long enough to allow the images to be captured. The combined findings from this paper and previous work (E Lima et al., 2014), suggests that the assessment of BFlh architecture during active contractions is reproducible but not for maximal contractions.

There are limitations in the current study. The validation of the ultrasound assessment technique through the use of cadaveric samples was not undertaken. However, all data obtained at rest is comparable to that in the existing *in-vivo* literature, which show valid comparisons to cadaveric data (Blackburn, Bell, Norcross, Hudson, & Kimsey, 2009; Blackburn & Pamukoff, 2013; Chleboun et al., 2001; Kellis et al., 2009; Kellis, Galanis, Natsis, & Kapetanos, 2010). Additionally, the assessment of the architectural alterations which occur at 100% of MVIC was not completed. Initial pilot studies were undertaken to assess the BFlh architecture during 100% MVIC, however the participants were unable to maintain the required contraction intensity long enough to ensure a high quality of images was obtained. The comparison of two different cohorts, with elite athletes in the previously injured group and recreationally active participants in the control group, might appear to be a limitation. However all architectural comparisons show no difference between the control group average and the uninjured limb in the previously injured group, suggesting homogeneity between the groups. Finally, the retrospective nature of the study limits any determinations of whether the reported differences in muscle architecture and eccentric strength are the cause or the result of injury. Prospective investigations are required to determine if these variables are associated with the risk of sustaining a hamstring strain injury.

In summary, the current study reported the use of ultrasonography to be highly reliable for the assessment of the BFlh architectural characteristics, at rest and during graded isometric contractions. Furthermore, BFlh absolute fascicle length, pennation angle and fascicle length relative to muscle thickness as well as knee flexor eccentric force during the Nordic hamstring exercise are all significantly different in those with a previous BFlh strain injury. Whether or not these differences exist before an injury or are a result of the incident, the findings of the current study provide significant insight into architectural asymmetries in previously injured individuals.

Much work is required in this area to determine what role, if any, variations in BFlh architecture has in the aetiology of hamstring strain injury.

Chapter 5 - Study 2: Biceps femoris long head architecture, eccentric knee flexor strength and hamstring injury risk in professional football (soccer): a prospective cohort study.

Publication statement:

This chapter is comprised of the following paper accepted for publication at the *British Journal Sports Medicine*:

5.1. Linking paragraph

Chapter 4 identified architectural differences in limbs with a history of BFlh strain injury. Despite being the first study to compare the architectural characteristics of a previously injured muscle to uninjured limbs, it is still unclear what role muscle architecture plays in the aetiology of a HSI. Chapter 5 aims to address this via a prospective cohort study, focussing on investigating the role that BFlh architecture has on the risk of a future HSI in elite soccer players. It is hypothesised that shorter BFlh fascicle lengths will be associated with an increased risk of HSI.

5.2 Abstract

Background/Aim: To investigate the role that eccentric knee flexor strength, between-limb imbalance and biceps femoris long head (BFlh) fascicle length have on the risk of a future hamstring strain injury (HSI). **Methods:** Elite soccer players (n=152) from eight different teams participated. Eccentric knee flexor strength during the Nordic hamstring exercise and BF$_{lh}$ fascicle length were assessed at the beginning of pre-season. The occurrences of a HSI following this were recorded by the team medical staff. Relative risk (RR) was determined for univariate data, and logistic regression was employed for multivariate data. **Results:** Twenty-seven new HSIs were reported. Eccentric knee flexor strength below 337N (RR = 4.4; 95% CI = 1.1 to 17.5) and BFlh fascicles shorter than 10.56cm (RR = 4.1; 95% CI=1.9 to 8.7) significantly increased the risk of a subsequent HSI. Multivariate logistic regression revealed significant effects when combinations of age, previous history of HSI, eccentric knee flexor strength and BFlh fascicle length were explored. From these analyses the likelihood of a future HSI in older athletes or those with a previous HSI history was reduced if high levels of eccentric knee flexor strength and longer BFlh fascicles were present. **Conclusions:** Possessing short BFlh fascicles and low levels of eccentric knee flexor strength increased the risk of a future HSI in elite soccer players. The greater risk of a future HSI in those with a previous HSI or of increasing age can be offset by possessing longer BFlh fascicles and high levels of eccentric strength.

5.3 Introduction

Hamstring strain injuries (HSI) are the most prevalent cause of lost playing and training time in elite soccer and account for approximately 37% of all muscle strain injuries (Ekstrand et al., 2011; Hagglund et al., 2009; Woods et al., 2004). Of these HSIs the majority occur in the biceps femoris long head (BFlh) (Ekstrand et al., 2011; Hagglund et al., 2009; Woods et al., 2004). Despite a concerted scientific effort over the past decade, the incidence of HSIs has not declined in elite soccer (Ekstrand et al., 2013). What is known is that a number of non-modifiable risk factors, including increasing age and previous injury history, have been shown to increase the risk of a future HSI in elite soccer (Arnason et al., 2004; Engebretsen et al., 2010; Hagglund et al., 2006). More recently modifiable risk factors, which are able to be altered via interventions, have received greater attention (Bahr & Holme, 2003; Croisier et al., 2008; Opar et al., 2015). These include isokinetically derived eccentric knee flexor strength (Croisier et al., 2008) and muscle imbalances (between-limb and hamstring:quadriceps ratios) (Croisier et al., 2008; Fousekis et al., 2011). In addition a recent prospective cohort study in elite Australian Rules football identified eccentric weakness during the Nordic hamstring exercise as risk factor for a future HSI (Opar et al., 2015). Furthermore this study showed that the increased risk of sustaining a future HSI in older athletes and those with a prior HSI were able to be mitigated by high levels of eccentric knee flexor strength (Opar et al., 2015). Despite this evidence, there is still no consensus regarding the role that eccentric knee flexor strength plays in the aetiology of a HSI in soccer and this requires further attention (Freckleton & Pizzari, 2013).

It has also been suggested, despite a lack of direct evidence, that hamstring muscle fascicle length may alter the risk for a future HSI (Brockett et al., 2001, 2004). One retrospective study has shown BFlh fascicles are shorter in previously injured muscles than in the contralateral uninjured muscles (Timmins et al., 2015). However, as the available evidence is retrospective in nature

(Timmins et al., 2015), it is not possible to determine if these differences in fascicle length increased the risk of a HSI occurring or were the result of the initial insult.

The purposes of this study were to determine if eccentric knee flexor strength and between-limb imbalances during the Nordic hamstring exercise and BFlh fascicle length influenced the risk of a future HSI in elite Australian soccer players. Additionally, this study aimed to assess the interrelationship between these two modifiable factors (fascicle length and eccentric strength) and the non-modifiable risk factors of increasing age and previous HSI in determining the risk of a future HSI. It is hypothesized that shorter BFlh fascicle lengths, low levels of eccentric knee flexor strength and larger between-limb imbalances will be associated with an increased risk of HSI. The interaction between increasing age and a previous HSI history with eccentric strength and BFlh fascicle length will provide novel information for an athlete's risk profile.

5.4 Methods

As per university guidelines, the methodologies for this study are also presented in chapter 3.2. These methods are replicated below in the format required for *British Journal of Sports Medicine*.

5.4.1 Participants and study design

This prospective cohort study was completed during pre-season (June 2014 to July 2014) and in-season period (October 2014 to May 2015) of the 2014/2015 elite Australian Football (soccer) competition. Ethical approval for the study was granted by the Australian Catholic University Human Research Ethics Committee (approval number: 2014 26V). Eight of the ten professional teams invited to participate elected to take part in the study. All outfield members of the playing squad (approximately 18-22 athletes per team) were approached and provided written, informed consent. In total, 152 elite male football (soccer) players (age 24.8±5.1 years; height 1.8 ±0.06m; body mass 76.7±7.4kg) provided written and informed consent to participate. Club medical staff

completed a retrospective injury questionnaire which detailed each athlete's history of hamstring, quadriceps, groin and calf strain injuries and chronic groin pain in the past 12 months, as well as the history of anterior cruciate ligament (ACL) injury at any stage throughout the athlete's career. Playing positions were defined as: defender (n=52), midfielder (n=59) and attacker (n=41) as per previous research (Bradley et al., 2011). The athletes had their maximal voluntary isometric contraction strength (n=141) (MVIC), BFlh architecture (with relaxed hamstrings (n=152) and while performing isometric knee flexion at 25% of MVIC (n=141)) and eccentric knee flexor strength (n=131) assessed at the beginning of pre-season.

5.4.2 BFlh architecture assessment

Muscle thickness, pennation angle and fascicle length of the BFlh was determined from ultrasound images taken along the longitudinal axis of the muscle belly utilising a two dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm) (GE Healthcare Vivid-*i,* Wauwatosa, U.S.A). The scanning site was determined as the halfway point between the ischial tuberosity and the knee joint fold, along the line of the BFlh. All architectural assessments were performed with participants in a prone position and the hip neutral following at least 5 minutes of inactivity. Assessments at rest were always performed first followed by an isometric contraction protocol. During all assessments of the BFlh architectural characteristics (passive and 25% of MVIC), the knee joint was fully extended. Assessment of the MVIC of the knee flexors was undertaken in the same position and was performed in a custom made device (Opar, Piatkowski, et al., 2013; Opar et al., 2015). Participants were instructed to contract their knee flexors maximally over a five second period. The peak force value during this effort was used to determine their MVIC strength. The assessment of the BFlh architectural characteristics during a 25% isometric contraction then occurred in the same position and device, with the participants shown the real-time visual feedback of the force produced to ensure that target

contraction intensities were met. To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence the accuracy of the measures (Klimstra et al., 2007). Finally, the orientation of the probe was manipulated slightly by the sonographer (RGT) if the superficial and intermediate aponeuroses were not parallel. Ultrasound image analysis was undertaken off-line (MicroDicom, Version 0.7.8, Bulgaria). For each image, six points were digitised as described by Blazevich and colleagues (Blazevich et al., 2006). Following the digitising process, muscle thickness was defined as the distance between the superficial and intermediate aponeuroses of BFlh. A fascicle of interest was outlined and marked on the image. The angle between this fascicle and the intermediate aponeurosis was measured and given as the pennation angle. The aponeurosis angle for both aponeuroses was determined as the angle between the line marked as the aponeurosis and an intersecting horizontal reference line across the captured image (Blazevich et al., 2006; Kellis et al., 2009). Fascicle length was determined as the length of the outlined fascicle between aponeuroses. As the entire fascicle was not visible in the field of view of the probe it was estimated via the following equation from Blazevich and colleagues (Blazevich et al., 2006; Kellis et al., 2009):

FL=sin (AA+90°) x MT/sin (180°-(AA+180°-PA)).

Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation angle. Fascicle length was reported in absolute terms (cm) and relative to BFlh length. The same assessor (RGT) collected and analysed all scans and was blinded to participant identifiers during the analysis.

5.4.3 Eccentric hamstring strength

The assessment of eccentric knee flexor strength using the Nordic hamstring device has been reported previously (Opar et al., 2015; Opar, Piatkowski, et al., 2013; Opar et al., 2015; Timmins et al., 2015). Participants were positioned in a kneeling position over a padded board, with the ankles secured superior to the lateral malleolus by individual ankle braces which were secured atop custom made uniaxial load cells (Delphi Force Measurement, Gold Coast, Australia) fitted with wireless data acquisition capabilities (Mantracourt, Devon, UK). The ankle braces and load cells were secured to a pivot which ensured that force was always measured through the long axis of the load cells. Following a warm up set of three submaximal efforts with a subsequent 1 minute rest period, participants were asked to perform one set of three, maximal bilateral repetitions of the Nordic hamstring exercise. Participants were instructed to gradually lean forward at the slowest possible speed while maximally resisting this movement with both lower limbs while keeping the trunk and hips in a neutral position throughout, and the hands held across the chest. Verbal encouragement was given throughout the range of motion to ensure maximal effort.

5.4.4 Prospective hamstring strain injury reporting

A HSI was defined as any acute posterior thigh pain which resulted in the immediate cessation of exercise and was later diagnosed by the club medical staff. Injury reports were not completed for injuries which did not fulfil the criteria (e.g. acute posterior thigh pain, however completed the exercise). A recurrent injury was a HSI that occurred on the same side of the body which had already suffered an injury in the current season. For all recurrent and new HSIs that fit the above criteria, the club medical staff completed a standard injury report form which detailed which limb was injured (dominant/non dominant, left/right), the muscle injured (BFlh /biceps femoris short head/semimembranosus/semitendinosus), location of injury (proximal/distal, muscle

belly/muscle-tendon junction), activity type performed at time of injury (e.g running, kicking etc.), grade of injury (I, II or III) and the number of days taken to return to full participation in training/competition. These reports were forwarded to the investigators throughout the season.

5.4.5 Injury specifics and rates

The determination of playing time missed as a result of a HSI was measured as missed matches per club per season (Orchard et al., 2013). Recurrence rate was defined as the number of recurrent injuries in the same season as a percentage of new injuries (Orchard et al., 2013). Additionally time lost as a result of the injury was defined as the amount of days from when the injury occurred to the resumption of full training participation.

5.4.6 Data analysis

Whilst positioned in the custom made device, shank length (m) was determined as the distance from the lateral tibial condyle to the mid-point of the brace which was placed around the ankle. This measure of shank length was used to convert the force measurements (collected in N) to torque (Nm). Knee flexor eccentric and MVIC strength force data were transferred to a personal computer at 100Hz through a wireless USB base station (Mantracourt, Devon, UK). The peak force value during the MVIC and the three Nordic hamstring exercise repetitions for each of the limbs (left and right) was analysed using custom made software. Eccentric knee flexor strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the average of the peak forces from the 3 repetitions for each limb, resulting in a left and right limb measure (Opar, Piatkowski, et al., 2013). Knee flexor MVIC strength, reported in absolute terms (N and Nm) and relative to body mass (N/kg and Nm/kg), was determined as the peak force produced during a 5 second maximal effort for each limb.

Between limb imbalance of BFlh fascicle length, muscle thickness, eccentric and MVIC knee flexor strength was calculated as a left:right limb ratio for the uninjured players and as an

uninjured:injured limb ratio in the injured players. As recommended, between limbs imbalance was converted to a percentage difference using log transformed raw data followed by back transformation (Impellizzeri et al., 2008). Negative percentage imbalances indicate that the variable of the left limb was greater than the right limb in the uninjured players, or that the injured limb variable was greater than the uninjured limb in the injured players. For athletes who did not suffer a HSI, as the limbs did not differ for any variables ($p>0.05$) the left and right limb were averaged to give a single control 'score'.

5.4.7 Statistical analyses

All statistical analyses were performed using JMP version 11.01 Pro Statistical Discovery Software (SAS Inc., Cary, North Carolina, USA). Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and homoscedasticity using Levene's test. Reliability of the assessor (RGT) and processes used for the determination of the BFlh architectural characteristics have been reported (Timmins et al., 2015).

The mean and standard deviation of age, height, weight, BFlh fascicle length (passive and 25% MVIC), BFlh muscle thickness (passive and 25% MVIC), eccentric and MVIC knee flexor strength were determined for all participants. Univariate analyses were performed to compare between limb imbalances for all variables of the injured and uninjured groups, as well as comparing the injured limb to the contralateral uninjured limb and the average of the left and right limbs from the uninjured group. Univariate comparisons were undertaken using two-tailed t-test with Bonferonni corrections to account for multiple comparisons. To determine univariate relative risk (RR) and 95% confidence intervals (95% CI) of future HSI, athletes were grouped according to:

- those with or without prior

  - hamstring (past 12 months)

  - calf (past 12 months)

  - quadriceps (past 12 months)

  - ACL (at any stage in their career)

  - chronic groin injury (past 12 months)

- those with passive fascicle lengths above or below

  - 10.56cm

    - This threshold was determined utilising receiver operator characteristic (ROC) curves based on the fascicle threshold that maximised the difference between sensitivity and 1- specificity.

- those with 25% MVIC fascicle lengths above or below

  - 9.61cm

    - Threshold determined as above

- those with passive muscle thickness threshold above or below

  - 2.35cm

    - Threshold determined as above

- those with 25% MVIC muscle thickness threshold above or below

  - 2.61cm

    - Threshold determined as above

- those with average eccentric knee flexor strength threshold above or below

  - 337N

    - Threshold determined as above

- those with MVIC knee flexor strength threshold above or below

- o 400N

    - Threshold determined as above

- those with limbs above or below arbitrarily selected cut offs of 10%, 15% and 20% between limb imbalance for

    - o passive fascicle length

    - o 25% MVIC fascicle length

    - o average eccentric knee flexor strength

    - o MVIC knee flexor strength

- athletes above these age cut offs (which represent the 10th, 25th, 50th, 75th and 90th percentiles for this sample)

    - o 18.0 years

    - o 20.4 years

    - o 23.7 years

    - o 28.8 years

    - o 32.6 years

- athletes above and below the height (182.3cm) and weight (77.9kg) means as defined previously by Hagglund and colleagues (Hagglund et al., 2013)

HSI rates from these groups were then compared and RR calculated utilising a two-tailed Fisher's exact test to determine significance. Additionally, univariate logistic regressions were conducted with the prospective occurrence of a HSI (yes/no) as the dichotomous dependant variable and eccentric knee flexor strength and BFlh fascicle length as continuous independent variables in separate analyses. These data are reported as odds ratios (OR) and 95% CI per 10-N increase in knee flexor force and 0.5cm increase in fascicle length.

As per a previous investigation in elite Australian Football (Opar et al., 2015), to improve the understanding of the risk from the univariate analysis and remove the possible confounding effects, multivariate logistic regression models were built using risk factors from previously published evidence (Arnason et al., 2004; Ekstrand et al., 2011; Hagglund et al., 2009; Opar et al., 2015; Woods et al., 2004). The first model included passive fascicle length (average of both limbs) and history of HSI and their interaction. The second model included fascicle length (average of both limbs) and age and their interaction. The third model included mean eccentric strength (average of both limbs) and history of HSI and their interaction. The fourth model included mean eccentric strength (average of both limbs) and age and their interaction. The final model included both fascicle length (average of both limbs) and mean eccentric strength (average of both limbs) and their interaction. Additionally using this final model the Nagelkerke R^2 coefficient was used to display the strength of the association between the two continuous independent variables (eccentric strength and fascicle length) with a prospective HSI occurrence (Nagelkerke, 1991). Significance was set at a p<0.05 and where possible Cohen's *d* (Cohen, 1988) was reported for the effect size of the comparisons, with the levels of effect being deemed small (*d* = 0.20), medium (*d* = 0.50) or large (*d* = 0.80) as recommended by Cohen (1988).

5.4.8 Power calculations

Power analysis was undertaken *post-hoc* using G-Power (Faul et al., 2007). Using BFlh architecture data, power was calculated as 0.97 for the use of two-tailed independent t-tests to compare groups (input parameters: effect size = 0.80; alpha = 0.05; sample size group 1 = 125; sample size group 2 = 27). Using a similar *post-hoc* comparison for eccentric knee flexor strength, power was calculated as 0.95 (input parameters: effect size = 0.80; alpha = 0.05; sample size group 1 = 105; sample size group 2 = 26).

5.5 Results

5.5.1 Participant and injury details

One-hundred and fifty two athletes were assessed at the beginning of pre-season (age 24.8±5.1 years; height 1.80 ±0.06m; body mass 76.9±7.5kg). One hundred and twenty five did not sustain a HSI (age 24.2±5.1 years; height 1.78 ±0.06m; body mass 75.3±6.6kg) and 27 did (age 27.0±3.8 years; height 1.80±0.07m; body mass 76.4±6.7kg). The athletes who went on to be injured displayed no differences in height and weight, but were significantly older than those who did not suffer an injury (mean difference: 2.8 years; 95% CI=1.1 to 4.5; p=0.002; d=0.62). Twenty-seven initial HSIs were sustained (11 left limb, 16 right limb) and of these, eight went on to reoccur in the same season (recurrence rate=29.6%). Of the initial injuries, ten occurred during the pre-season period, with the remaining seventeen occurring during the competitive season. The total amount of matches missed as a result of a HSI (initial and recurrent) was sixty three, resulting in 7.8 matches missed per club for the competitive season. Of the 27 initial injuries, the average time lost was 17.7 (±9.3) days, with the eight recurrent injuries resulting in an average of 28.4 (±23.7) days.

Of the twenty-seven initial HSIs, 88.8% occurred in the BFlh, with the remaining 11.2% occurring in the semimembranosus (7.5%) and semitendinosus (3.7%), respectively. The primary mechanism for the initial injuries was high speed running (81.5%), followed by stretching for a ball or opponent (11.1%) and then kicking (7.4%). All recurrences occurred during high speed running. No injuries occurred during the Nordic hamstring exercise testing sessions. The distribution of player positions in the injured group (defender: 29.6%, midfielder: 37.1%, attacker: 33.3%) compared to the uninjured group (defender: 36.8%, midfielder: 40.0%, attacker: 23.2%) suggested that defenders were under-represented and attackers over-represented in the subsequently injured group.

5.5.2 Univariate analysis

Eccentric and isometric knee flexor strength, BFlh architectural characteristics and between limb asymmetries of the injured and uninjured limbs from the injured players and the average of both limbs from the uninjured players can be found in Table 5.1.

5.5.2.1 BFlh architectural characteristics

BFlh architectural characteristics of the left and right limbs in the uninjured players were not significantly different when assessed relaxed or during 25% MVICs (p>0.05). The subsequently injured limbs had shorter BFlh fascicle lengths than the two-limb-average of uninjured players when assessed at rest (mean difference: 1.37cm; 95% CI=0.8 to 1.8; $p=<0.001$; $d=1.08$; Table 5.1) and during 25% MVIC (mean difference: 1.02cm; 95% CI=0.5 to 1.5; $p=<0.001$; $d=0.92$; Table 5.1). In comparison to the contralateral uninjured limb, the BFlh fascicle length of the subsequently injured limbs was significantly shorter when assessed at rest (mean difference: 1.05cm; 95% CI=0.6 to 1.5; $p=<0.001$; $d=0.91$; Table 5.1) and during 25% MVIC (mean difference: 0.65cm; 95% CI=0.3 to 1.0; $p=<0.001$; $d=0.57$; Table 5.1). Using univariate logistic regression, BFlh fascicle length (OR = 0.261; 95% CI = 0.10 to 0.57; p=0.002) had a significant inverse relationship with the incidence of prospectively occurring HSIs. As such, for every 0.5cm increase in BFlh fascicle length, the risk of HSI was reduced by 73.9%. Additionally, BFlh muscle thickness measures (at rest and during 25% MVIC) from the subsequently injured limbs were no different from either the contralateral uninjured limbs or the two-limb-average of the uninjured players (p<0.05, d range=0.13 to 0.23; Table 5.1).

The measures of between limb asymmetry in BFlh fascicle length and muscle thickness, assessed at rest and at 25% MVIC, did not differ significantly between the injured and uninjured players (p<0.05, d range=0.03 to 0.48; Table 5.1).

Table 5-1. *Pre-season BFlh architectural characteristics (n=152), eccentric knee flexor strength during the Nordic hamstring exercise (n=131) and MVIC knee flexor strength (n=141) in elite Australian soccer players.*

BFlh architecture	Uninjured group			Injured group		Compared to uninjured group average		
	Two-limb average	Between-limb imbalance (%)	Between-limb imbalance (%)	Uninjured limb	Injured limb	Uninjured group vs injured limb (95% CI)	*p*	Effect size (*d*)
Passive FL (cm)	11.20 (±1.2) (n=125)	11.2 (±8.2)	13.8(±1.3)	10.90 (±0.9) (n=27)	9.85 (±1.3)[##] (n=27)	1.35 (0.8 to 1.8)	0.001**	1.08
25% MVIC FL (cm)	9.53 (±1.2) (n=116)	11.7 (±9.2)	11.3 (±1.0)	9.16 (±1.2) (n=25)	8.51 (±1.0)[##] (n=25)	1.02 (0.5 to 1.5)	0.001**	0.92
Passive MT (cm)	2.54 (±0.3) (n=125)	8.0 (±6.1)	7.8 (±6.2)	2.58 (±0.2) (n=27)	2.47 (±0.3) (n=27)	0.07 (-0.1 to 0.2)	0.357	0.23
25% MVIC MT (cm)	2.66 (±0.3) (n=116)	9.8 (±8.0)	6.4 (±5.8)	2.63 (0.3) (n=25)	2.62 (±0.3) (n=25)	0.04 (-0.1 to 0.2)	0.671	0.13

Knee flexor strength measures	Uninjured group			Injured group		Compared to uninjured group average		
	Two-limb average	Between-limb imbalance (%)	Between-limb imbalance (%)	Uninjured limb	Injured limb	Uninjured group vs injured limb (95% CI)	*P*	Effect size (*d*)
Eccentric force (N)	309.5 (±73.4) (n=105)	10.1 (±8.8)	19.7 (±32.4)	262.6 (±63.2) (n=26)	260.6 (±82.9) (n=26)	48.9 (16.2 to 81.5)	0.004*	0.62
Eccentric torque (Nm)	135.5 (±33.7) (n=105)			116.2 (±28.7) (n=26)	115.2 (±37.1) (n=26)	20.3 (5.3 to 35.1)	0.008*	0.57
Relative eccentric force (N/Kg)	4.11 (±0.9) (n=105)			3.47 (±0.9) (n=26)	3.46 (±1.2) (n=26)	0.65 (0.2 to 1.1)	0.004*	0.61
Relative eccentric torque (Nm/Kg)	1.79 (±0.4) (n=105)			1.54 (±0.4) (n=26)	1.53 (±0.5) (n=26)	0.26 (0.1 to 0.5)	0.007*	0.57
Isometric force (N)	373.7 (±75.6) (n=116)	10.4 (±7.56)	12.1 (±13.7)	365.2 (±73.9) (n=25)	367.9 (±72.7) (n=25)	5.8 (-26.1 to 39.4)	0.690	0.08
Isometric torque (Nm)	163.2 (±34.2) (n=116)			160.2 (±30.1) (n=25)	161.4 (±34.2) (n=25)	1.8 (-13.1 to 16.7)	0.811	0.05
Relative isometric force (N/Kg)	4.99 (±1.0) (n=116)			4.81 (±1.1) (n=25)	4.81 (±1.1) (n=25)	0.18 (-0.3 to 0.6)	0.428	0.17
Relative isometric torque (Nm/Kg)	2.18 (±0.4) (n=116)			2.10 (±0.4) (n=25)	2.11 (±0.4) (n=25)	0.07 (-0.1 to 0.3)	0.495	0.18

All data represented as mean±SD unless otherwise stated. BFlh = biceps femoris long head, FL = fascicle length, cm = centimetres, SD = standard deviation, 95% CI = 95% confidence interval, MVIC = maximum voluntary isometric contraction, MT = muscle thickness, N = newtons, Nm = newton metres, N/Kg = newtons per kilogram of body weight, Nm/kg = newton metres per kilogram of body weight, **=$p<0.01$, *=$p<0.05$ vs average of uninjured group, ##=$p<0.01$ injured vs uninjured limb in the injured group

5.5.2.2 Eccentric and isometric knee flexor strength

Between-limb differences in absolute eccentric knee flexor forces between the left and right limbs of uninjured players and between the subsequently injured and contralateral uninjured limbs of injured players, were not significant (p>0.05, *d* range=0.02 to 0.21; Table 5.1). However, between group comparisons of absolute eccentric knee flexor force showed that subsequently injured limbs were weaker (260.6N±82.9) than the two-limb-average of uninjured players (309.5N±73.4) (mean difference: 48.9N; 15.8%; 95% CI=16.2 to 81.5N; p=0.004; *d*=-0.62; Table 5.1). Additionally, the uninjured limbs of the injured players were also significantly weaker (262.6N±63.2) than the uninjured players' two-limb-average (mean difference: 46.9N; 15.1%; 95% CI=15.9 to 77.9N; p=0.003; *d*=-0.68; Table 5.1). Eccentric strength represented as knee flexor torque showed similar differences, with the subsequently injured limbs (115.2Nm±37.1) being weaker than the two-limb-average (135.5Nm±33.7) of uninjured players (mean difference: 20.3Nm; 14.9%; 95% CI=5.3 to 35.1Nm; p=0.008; *d*=0.57; Table 5.1). Similarly, the uninjured limbs (116.2Nm±28.7) from the injured players were weaker than the two-limb-average of the uninjured players (135.5Nm±33.7; mean difference: 19.3Nm; 14.2%; 95% CI=4.9 to 33.4Nm; p=0.008; *d*=0.62; Table 5.1). Using univariate logistic regression, eccentric knee flexor strength (OR = 0.910; 95% CI = 0.85 to 0.97; p=0.004) had a significant inverse relationship with the incidence of prospectively occurring HSIs. As such, for every 10N increase in eccentric knee flexor strength, the risk of HSI was reduced by 8.9%. Additionally, comparisons of between-limb imbalance in eccentric knee flexor strength did not differ between the subsequently injured and uninjured players (mean difference: 9.6%; 95% CI=-3.6 to 22.7; p=0.147; *d*=0.40; Table 5.1).

Regardless of how knee flexor MVIC strength was expressed (as force or torque) there were no significant differences between the left and right limbs of the uninjured players or between the

subsequently injured and contralateral uninjured limbs, in the injured players (p>0.05; *d* range=0.11 to 0.24). Additionally, there were no significant differences in knee flexor MVIC strength between either the subsequently injured limbs or the contralateral uninjured limbs of the injured players and the two-limb-averages of uninjured players (p>0.05; *d* range=0.07 to 0.22; Table 5.1).

5.5.2.3 Relative Risk

The univariate relative risks of a future HSI associated with all variables examined can be found in Table 5.2. Athletes with a relaxed BFlh fascicle length shorter than that of the ROC-curve-determined threshold of 10.56cm (area under the curve = 0.71; sensitivity = 0.70; 1-specificity = 0.29) were 4.1 times more likely to suffer a subsequent HSI than those with longer fascicles (RR = 4.1; 95% CI=1.9 to 8.7; p<0.001). Similar RR values were seen for BFlh fascicle length assessed during 25% MVIC (Table 5.2). Furthermore, athletes with average eccentric knee flexor forces below the ROC-curve determined threshold of 337N (area under the curve = 0.65; sensitivity = 0.96; 1-specificity = 0.68) had 4.4 times greater risk of a subsequent HSI than stronger players (RR = 4.4; 95% CI=1.1 to 17.6; p=0.013). Similar RR values were seen for the other measures of knee flexor strength (torque, force/kg body mass and torque/kg body mass) (Table 5.2). No measure of MVIC strength or between-limb imbalance in this measure led to a statistically significant increase in RR (Table 5.2).

Table 5-2. *Univariate relative risk (RR) to sustain a future HSI using BFlh fascicle length, muscle thickness, eccentric strength, MVIC strength, between-limb imbalances of these variables, previous injury history and demographic data as risk factors.*

Risk Factor	*n*	Percentage from each group that sustained a HSI	RR (95% CI)	*p*
Passive fascicle length	152			
<10.56 cm	56	33.9	4.1 (1.9-8.7)	0.001**
≥10.56 cm	96	8.3		
25% MVIC fascicle length	141			
<9.61 cm	79	25.3	3.2 (1.2-7.9)	0.008*
≥9.61 cm	62	8.0		
Passive fascicle length imbalance	152			
<10% imbalance	80	16.3	1.2 (0.6-2.4)	0.673
≥10% imbalance	72	19.4		
<15% imbalance	99	15.1	1.5 (0.7-3.0)	0.271
≥15% imbalance	53	22.6		
<20% imbalance	125	16.8	1.3 (0.6-3.0)	0.579
≥20% imbalance	27	22.2		
25% MVIC fascicle length imbalance	141			
<10% imbalance	78	20.0	1.3 (0.6-2.6)	0.512
≥10% imbalance	63	20.6		
<15% imbalance	100	22.7	0.7 (0.3-1.7)	0.630
≥15% imbalance	41	14.6		
<20% imbalance	118	22.0	0.4 (0.1-1.7)	0.249
≥20% imbalance	23	8.7		
Passive muscle thickness	152			
<2.35 cm	36	11.1	0.56 (0.2-1.5)	0.320
≥2.35 cm	116	19.8		
25% MVIC muscle thickness	141			
<2.61 cm	58	20.6	1.3 (0.6-2.7)	0.504
≥2.61 cm	83	15.6		
Eccentric strength	131			
<337 N	96	25.0	4.4 (1.1-17.5)	0.013*
≥337 N	35	5.7		
<145 Nm	89	25.8	3.6 (1.2-11.4)	0.017*
≥145 Nm	42	7.1		
<4.35 N/kg	82	25.6	2.5 (1.1-6.2)	0.041*
≥4.35 N/kg	49	10.0		
<1.86 Nm/kg	78	26.9	2.9 (1.1-7.1)	0.011*
≥1.86 Nm/kg	53	9.4		
Eccentric strength imbalance	131			
<10% imbalance	76	19.7	1.0 (0.5-2.0)	1.000
≥10% imbalance	55	20.0		
<15% imbalance	98	18.3	1.3 (0.6-2.7)	0.459
≥15% imbalance	33	24.2		
<20% imbalance	117	18.8	1.5 (0.6-3.8)	0.476
≥20% imbalance	14	28.5		
MVIC strength	141			
<400 N	93	21.5	2.0 (0.8-5.2)	0.161
≥400 N	48	10.4		
<172 Nm	88	20.4	1.5 (0.7-3.5)	0.364
≥172 Nm	53	13.2		
<4.60 N/kg	52	23.1	1.5 (0.7-3.2)	0.254
≥4.60 N/kg	89	14.6		
<2.07 Nm/kg	62	22.6	1.6 (0.8-3.3)	0.192
≥2.07 Nm/kg	79	13.9		

	N	%	RR (95% CI)	p
MVIC strength imbalance	141			
<10% imbalance	81	19.7	0.8 (0.4-1.8)	0.826
≥10% imbalance	60	16.6		
<15% imbalance	110	16.3	1.3 (0.6-2.9)	0.434
≥15% imbalance	31	22.6		
<20% imbalance	126	17.4	1.1 (0.4-3.3)	0.732
≥20% imbalance	15	20.0		
Prior injury	152			
HSI	30	30.0	2.0 (1.0-4.0)	0.063
No HSI	122	14.7		
ACL	16	31.1	1.9 (0.8-4.4)	0.164
No ACL	136	16.1		
Calf strain	13	23.1	1.3 (0.4-3.8)	0.713
No calf strain	139	17.3		
Quadriceps strain	21	28.6	1.8 (0.8-3.9)	0.215
No Quadriceps strain	131	16.0		
Chronic groin pain	13	23.1	1.3 (0.5-3.8)	0.703
No chronic groin pain	139	17.3		
Age (yr)	152			
≤18.0	8	12.5	1.5 (0.2-9.7)	1.000
>18.0	144	18.7		
≤20.4	37	2.7	8.4 (1.1-59.5)	0.005*
>20.4	115	22.6		
≤23.7	74	6.7	4.2 (1.6-10.4)	0.001*
>23.7	78	28.2		
≤28.8	116	13.8	2.2 (1.1-4.3)	0.043*
>28.8	36	30.5		
≤32.6	136	12.5	0.7 (0.2-2.6)	0.739
>32.6	16	18.4		
Height (cm)				
≤182.3	111	15.3	1.6 (0.8-3.4)	0.206
>182.3	41	24.4		
Weight (kg)				
≤77.9	102	15.7	1.4 (0.7-2.8)	0.370
>77.9	50	22.0		

*=p<0.05, **=p<0.001 when comparing the RR of future HSI between groups. HSI=hamstring strain injury, RR=relative risk, 95% CI=95% confidence interval, cm=centimetre, MVIC=maximal voluntary isometric contraction, N=newtons of force, Nm=newton metres of torque, N/kg=newtons of force relative to body mass, Nm/kg=newton metres of torque relative to body mass.

5.5.3 Multivariate logistic regression

Details of all of the logistic regression models can be found in Table 5.3 and Figures 5.1 to 5.5. All of the models were significant (model 1: prior HSI and BFlh fascicle length, p<0.001; model 2: age and BFlh fascicle length; p<0.001; model 3: prior HSI and eccentric strength, p=0.009; model 4: age and eccentric strength, p=0.007; model 5: eccentric strength and BFlh fascicle length; p<0.001), however none of the interactions reached significance (Table 5.3). For all models in which fascicle length was included, it made the most significant contribution to the model. Additionally, when determining the strength of the association between the two continuous independent variables (eccentric strength and fascicle length) with the dependant variable of a prospective HSI occurrence (yes/no) with a binary logistic regression, a Nagelkerke R^2 coefficient of 0.31 was found.

Table 5-3. *Multivariate logistic regression model outputs and receiver operator characteristic curve data using prior HSI, age, BFlh fascicle length and eccentric knee flexor strength.*

		Chi Square	*p*	AUC	Sensitivity	1 – Specificity
Model 1	Whole model	16.54	<0.001*	0.743	0.7778	0.352
	Prior HSI	4.24	0.039*			
	Fascicle length[a]	9.43	0.002*			
	Prior HSI x fascicle length[a]	0.08	0.776			
Model 2	Whole model	23.48	<0.001*	0.777	0.8148	0.328
	Age	3.66	0.055			
	Fascicle length[a]	10.49	0.001*			
	Age x fascicle length[a]	3.46	0.062			
Model 3	Whole model	11.49	0.009*	0.687	0.8077	0.4857
	Prior HSI	2.04	0.152			
	Eccentric strength[a]	6.33	0.011*			
	Prior HSI x eccentric strength[a]	0.03	0.872			
Model 4	Whole model	11.86	0.007*	0.686	0.9615	0.5619
	Age	2.74	0.097			
	Eccentric strength[a]	5.05	0.024*			
	Age x eccentric strength[a]	0.00	0.962			
Model 5	Whole model	17.26	<0.001*	0.759	0.8846	0.3714
	Eccentric strength[a]	4.29	0.038*			
	Fascicle length[a]	7.18	0.007*			
	Eccentric strength[a] x fascicle length[a]	0.08	0.783			

[a] Determined as the average of both left and right limb. AUC, area under the curve. *$p<0.05$. HSI = Hamstring Strain Injury, BFlh = biceps femoris long head
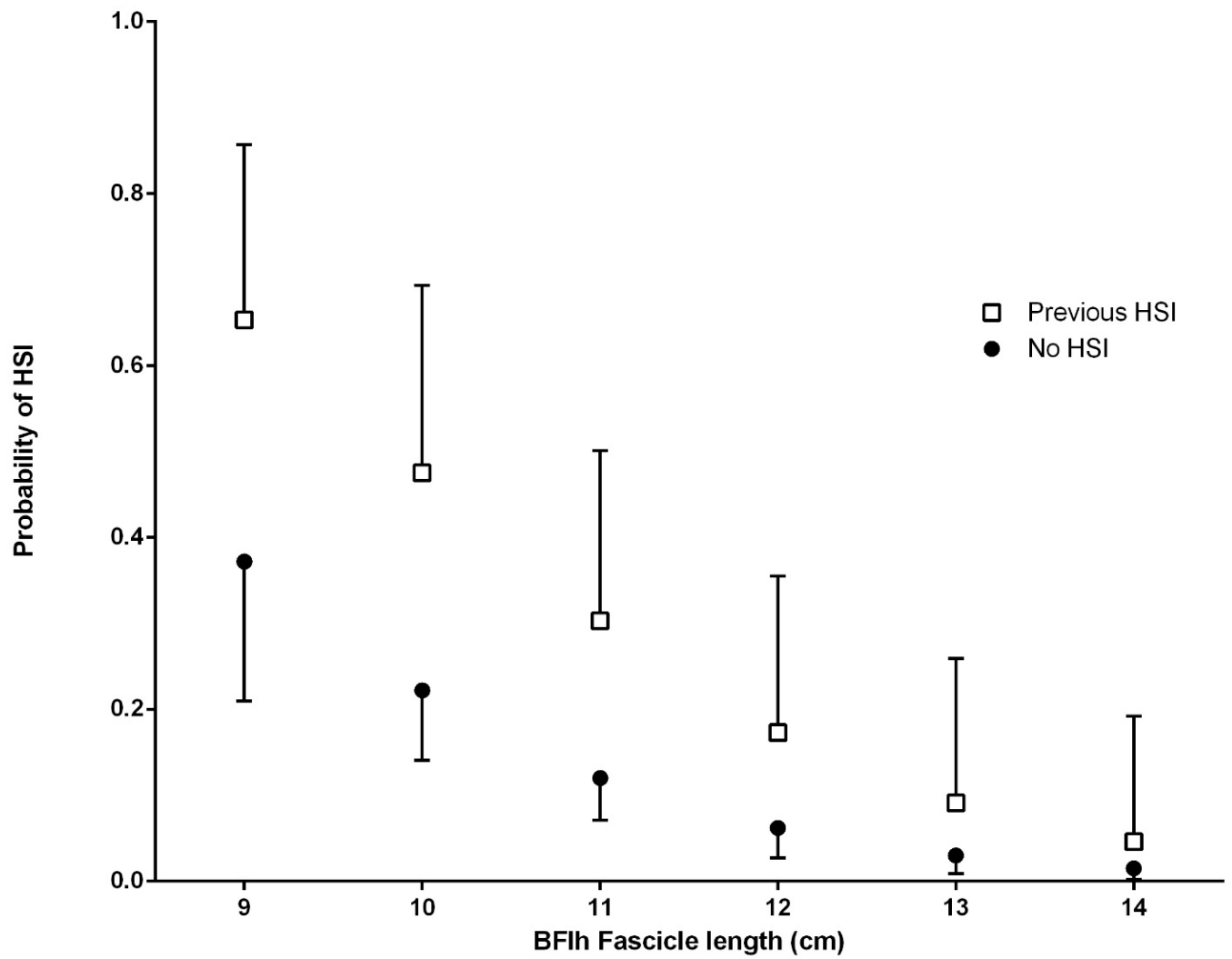
Figure 5-1. *The interaction between BFlh fascicle length, history of HSI and the probability of a future HSI (error bars indicate 95% CI).HSI=hamstring strain injury, BFlh=biceps femoris long head, cm=centimetres.*
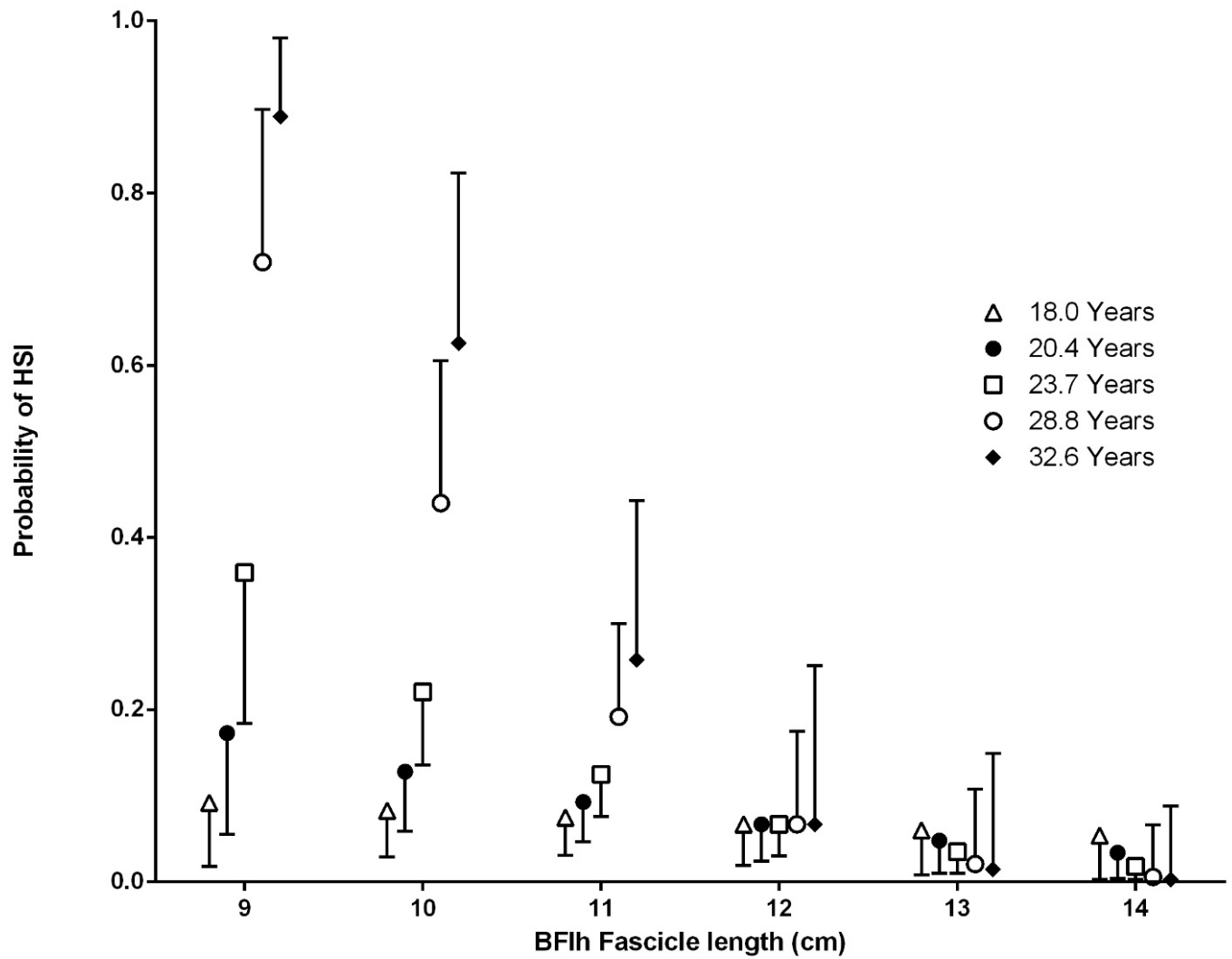
Figure 5-2. *The interaction between BFlh fascicle length, age and the probability of a future HSI. The ages are representative of the 10th, 25th, 50th, 75th and 90th percentile of the cohort. Note that the data has been offset (to the left or right) on the x-axis to allow for the visibility of the error bars of all the age groups. The data points and error bars are reflective of data at 9, 10, 11, 12, 13 and 14cm for all groups (error bars indicate 95% CI). HSI=hamstring strain injury, BFlh=biceps femoris long head, cm=centimetres.*
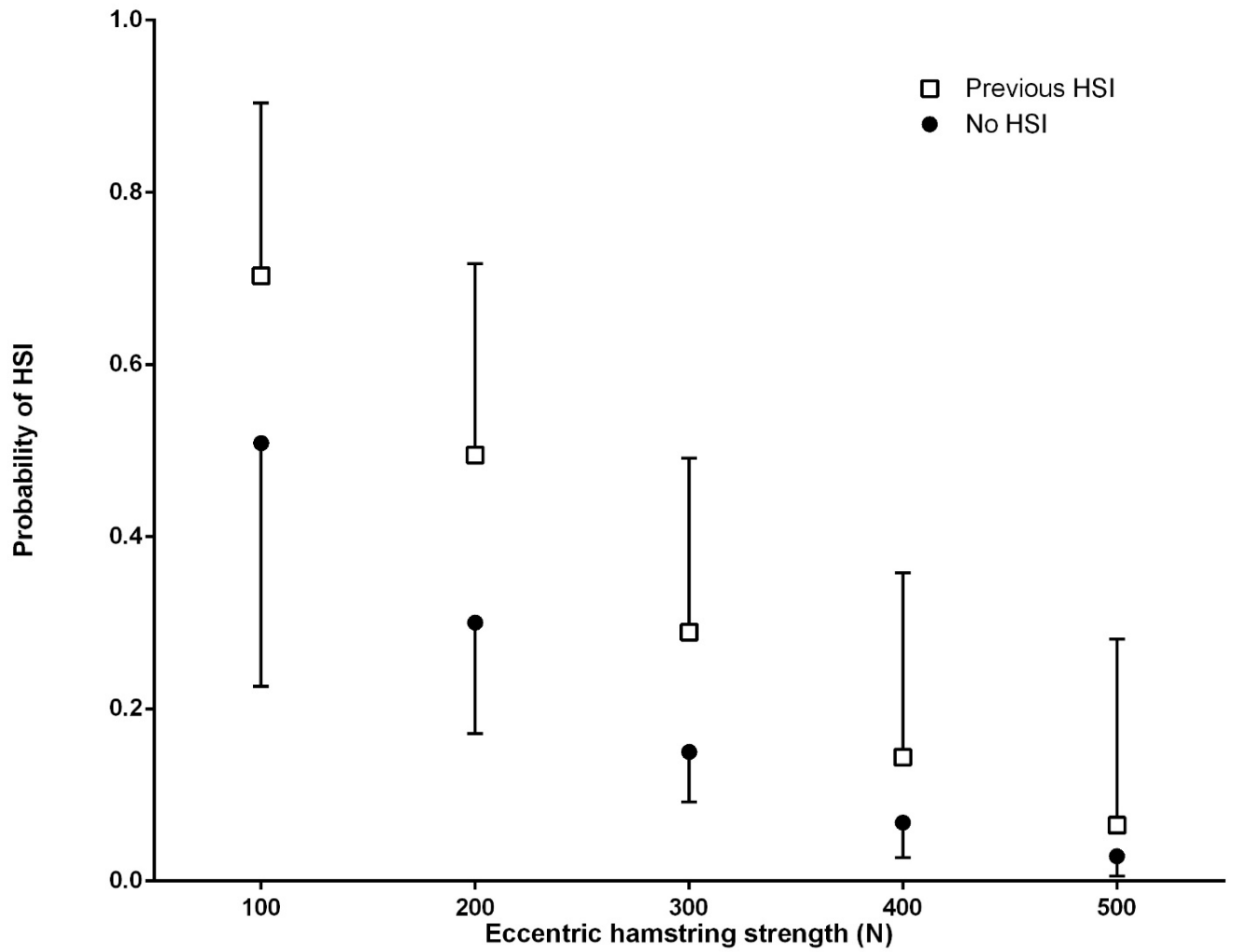
Figure 5-3. *The interaction between eccentric knee flexor strength, history of HSI and the probability of a future HSI (error bars indicate 95% CI). HSI=hamstring strain injury, N=newtons of force.*
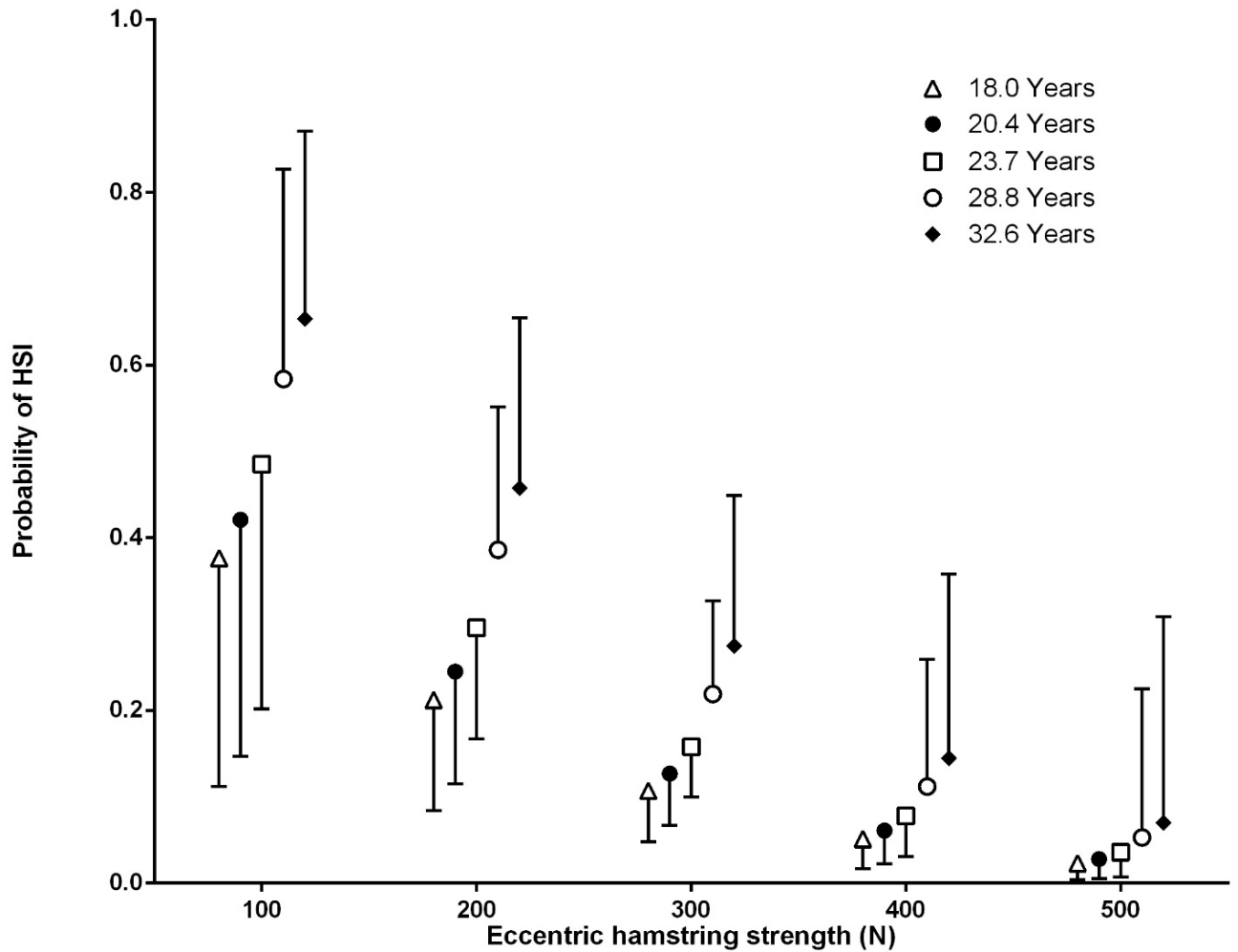
Figure 5-4. *The interaction between eccentric knee flexor strength, age and the probability of a future HSI. The ages are representative of the 10th, 25th, 50th, 75th and 90th percentile of the cohort. Note that the data has been offset (to the left or right) on the x-axis to allow for the visibility of the error bars of all the age groups. The data points and error bars are reflective of data at 100, 200, 300, 400 and 500N for all groups (error bars indicate 95% CI). HSI=hamstring strain injury, N=newtons of force.*
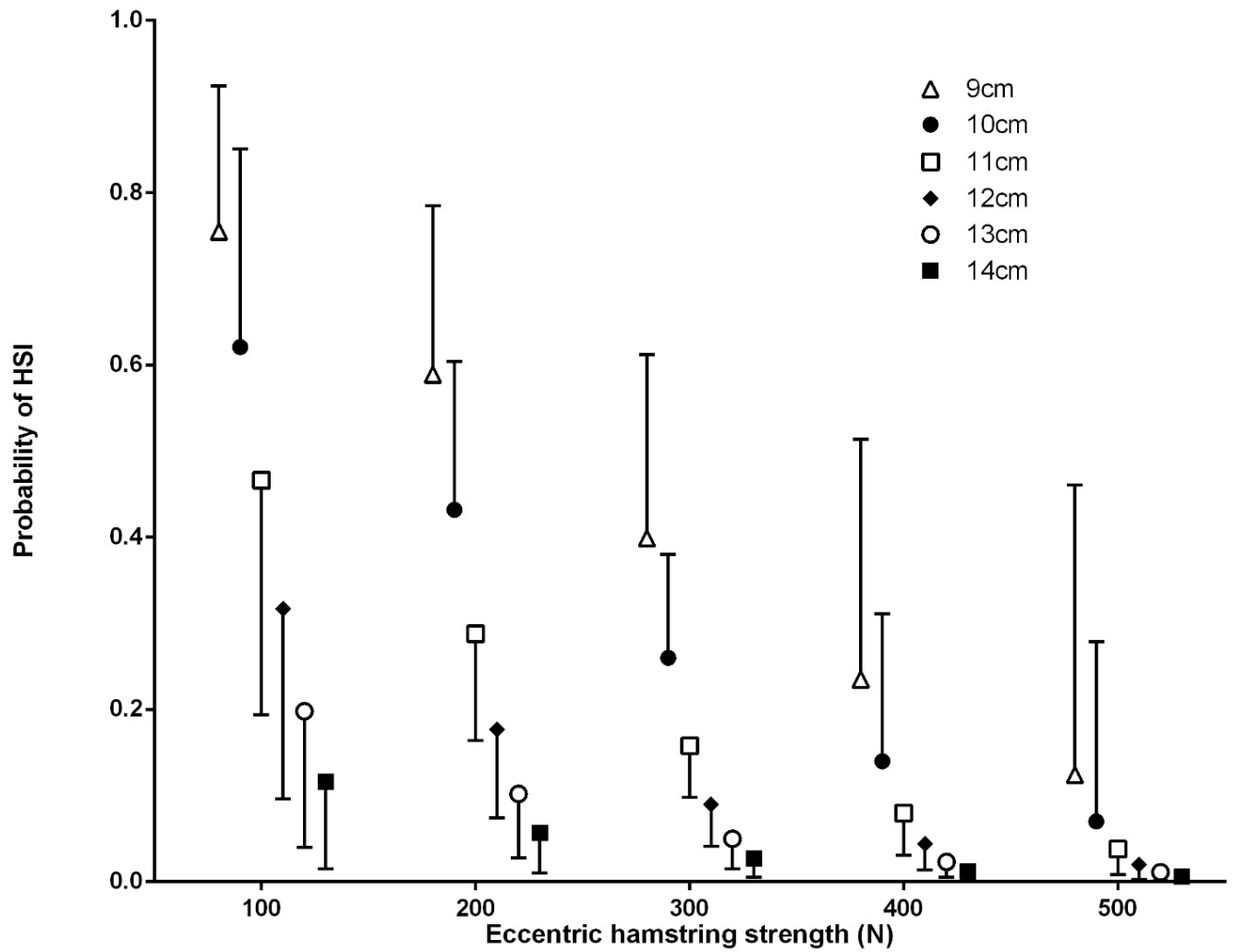
Figure 5-5. *The interaction between eccentric knee flexor strength, BFlh fascicle length and the probability of a future HSI. Note that the data has been offset (to the left or right) on the x-axis to allow for the visibility of the error bars of all the age groups. The data points and error bars are reflective of data at 100, 200, 300, 400 and 500N for all groups (error bars indicate 95% CI).*

5.6 Discussion

To the authors' knowledge, this is the first study that has examined the role that BFlh fascicle length plays in the aetiology of HSI. The main findings were that 1) limbs which suffered a HSI contained shorter BFlh fascicle lengths than the limbs which remained uninjured; 2) those limbs which suffered a HSI were weaker during eccentric contractions than the limbs which remained uninjured; 3) eccentric between-limb imbalances were not different between the injured or uninjured groups and between-limb imbalances did not infer any increased HSI risk; 4) the addition of combining variables (age, history of HSI, BFlh fascicle length and eccentric strength) provided further information on the risk of a future HSI and 5) measures of MVIC knee flexor strength were not different between the injured and uninjured groups and did not infer any increased HSI risk.

In the current study, short BFlh fascicle lengths were associated with an increased risk of future HSI in elite soccer players. One previous retrospective investigation reported that individuals with a unilateral HSI history have shorter BFlh fascicles in the previously injured limb than the contralateral uninjured limb (Timmins et al., 2015). It was previously hypothesized that shorter fascicles, with fewer in-series sarcomeres, may be more susceptible to being over-stretched and having damage caused by powerful eccentric contractions, like those performed during the terminal swing phase of high speed running (Brockett et al., 2004; Morgan, 1990). Given that more than two thirds of the HSIs noted in the current study occurred during high speed running, the shorter BFlh fascicle lengths in the subsequently injured limbs may have increased the susceptibility of the muscle to damage and altered their HSI risk. Additionally, it is possible to increase muscle fascicle lengths via high intensity eccentric resistance training interventions (Potier et al., 2009), with significant increases occurring within 14 days in the vastus lateralis (Seynnes et al., 2007).

Low levels of eccentric knee flexor strength during the Nordic hamstring exercise increased the risk of a future HSI in elite soccer players. This has also been recently observed in elite Australian footballers (Opar et al., 2015). The increased risk associated with being weak eccentrically may be related to the majority of HSIs in the current study occurring during high speed running. As the hamstrings are required to contract eccentrically during the terminal swing phase of the gait cycle (Opar et al., 2012), low levels of eccentric strength may reduce the hamstrings ability to do this and as a result potentially lead to an acute injury. Interestingly, low levels of isometric knee flexor strength were not associated with future HSI rates and this suggests that the contraction mode of strength tests is a critical factor in determining their predictive value. This is of particular relevance given that isometric assessments of the knee-flexors have been developed and advocated as clinically convenient (Schache, Crossley, Macindoe, Fahrner, & Pandy, 2011) and minimally 'intrusive' in athlete training programs (Wollin, Purdam, & Drew, 2015) given the low levels of muscle damage and soreness involved. Without discounting the value and convenience of such isometric tests as measures of strength and indicators of fatigue (Wollin et al., 2015), the present results suggest that eccentric hamstring tests are of greater value in determining injury risk.

The current study also found that a larger between-limb strength imbalance during the Nordic hamstring exercise did not increase the risk of future HSI and this is consistent with a similar recent study in AFL players (Opar et al., 2015) but contrary to previous findings in elite soccer, which indicated that isokinetically derived between-limb eccentric strength imbalances are associated with an increased risk of HSI (Fousekis et al., 2011). Bourne and colleagues have also recently observed, in a prospective study, that between-limb imbalances in the Nordic strength test (as employed in this study) are associated with elevated HSI rates in rugby union players while absolute strength levels are not (Bourne, Opar, Williams, & Shield, 2015). The diverse

findings in these studies are hard to explain. The different physical demands of these three football codes are readily apparent (Bourne et al., 2015) and the mode of testing may also influence the results of these prospective studies.

Multivariate exploration into combinations of variables including BFlh fascicle length, eccentric strength, age and a HSI history provides novel insights regarding HSI risk. Advanced age and a history of HSI have both been previously reported to increase the risk of a future HSI in elite soccer (Arnason et al., 2004; Engebretsen et al., 2010; Hagglund et al., 2006). The data in the current study indicates that the high risk of a future HSI associated with increasing age and a previous history of HSI can be incrementally offset by increasing BFlh fascicle length and eccentric knee flexor strength. Most notably, older athletes with shorter BFlh fascicles and lower levels of eccentric strength were at an increased risk when compared to younger athletes. As an example, the results of the current study allow us to estimate that a 33 year old athlete with BFlh fascicle length of 10cm has a 65% probability of HSI occurring, while a 22 year old has a 17% probability of injury. Similarly, 33 year old athletes with two-limb-average eccentric strength level of 200N have an estimated probability of HSI injury of 46% while a 22 year old player has an injury probability of 27%. Despite these results, the Nagelkerke R^2 coefficient indicated that eccentric strength and BFlh fascicle length accounted for approximately 30% of the risk associated with a prospective HSI occurrence. Therefore future research is still needed to identify the other 70% of the risk associated with a prospective HSI which is not accounted for.

The authors acknowledge that there are limitations in the current study. Firstly, there is a lack of athlete exposure data and this does not allow the determination of injury incidence relative to their exposure to training and match play. Future work should focus on determining the interaction between high speed running demands and the risk of a future HSI. Secondly, the study was undertaken in elite soccer players and as such generalizing the results to athletes of different

sports may be done with caution. Thirdly, the measures of eccentric knee flexor strength were not made relative to an anterior muscle group such as the knee extensors or the hip flexors. Doing so may have enabled the determination of a hamstring-to-quadriceps ratio, or something of similar nature. Despite the lack of this relative comparison, the eccentric knee flexor strength measures in this study provided valuable information regarding HSI risk, which suggests such ratios may not be crucial. Finally, the assessment of muscle fascicle length was only done on the BFlh. Considering the high rates of BFlh strain injury in the current study, the authors believe it was justified to focus on this muscle. Future research could aim to assess the risk associated with short fascicle lengths in the other hamstring muscles.

In conclusion, elite soccer players with short BFlh fascicles and low levels of eccentric knee flexor strength are at an increased risk of HSI compared to athletes with longer fascicles and greater eccentric strength. Isometric knee flexor strength and large between-limb imbalances in eccentric strength did not influence the risk of HSI. The interrelationship between the non-modifiable risk factors of increasing age and previous HSI history, with the modifiable variables of eccentric strength and BFlh fascicle length, provides a novel approach to constructing an athlete's risk profile.

Chapter 6 - Study 3: Architectural changes of the biceps femoris after concentric or eccentric training.

Publication statement:

This chapter is comprised of the following paper accepted for publication at *Medicine and Science in Sports and Exercise*:

6.1. Linking paragraph

The study in Chapter 4 showed that elite athletes with a previously strained BFlh possessed shorter fascicles in the injured limb when compared to the contralateral uninjured limb. The retrospective findings of this study guided the investigation carried out in Chapter 5, which found that shorter BFlh fascicle lengths were associated with an increased risk of HSI in elite Australian soccer players. Therefore interventions designed to alter muscle fascicle length are of interest. Chapter 6 focuses on the architectural adaptations of the BFlh following either a concentric or eccentric training intervention as well as following a 28 day de-training period. It is hypothesised that BFlh fascicle length will increase following an eccentric training intervention, whereas a concentric training will cause reductions in fascicle length.

6.2 Abstract

Purpose: To determine i) the architectural adaptations of the biceps femoris long head (BFlh) following concentric or eccentric resistance training interventions; ii) the time course of adaptation during training and de-training. **Methods:** Participants in this randomised control trial (control [n=28], concentric training group [n=14], eccentric training group [n=14], males) completed a 4-week control period, followed by 6-weeks of either concentric or eccentric only knee flexor training on an isokinetic dynamometer and finished with 28 days of de-training. Architectural characteristics of the BFlh were assessed at rest and during graded isometric contractions utilising two-dimensional ultrasonography at 28 days pre baseline, baseline, day 14, 21 and 42 of the intervention and then again following the 28 days of de-training. **Results:** BFlh fascicle length was significantly longer in the eccentric training group ($p<0.05$, d range: 2.65 to 2.98) and shorter in the concentric training group ($p<0.05$, d range: -1.62 to -0.96) after 42 days of training compared to baseline at all isometric contraction intensities. Following the 28 day de-training period, BFlh fascicle length was significantly reduced in the eccentric training group at all contraction intensities compared to the end of the intervention ($p<0.05$, d range: -1.73 to -1.55). There were no significant changes of fascicle length in the concentric training group following the de-training period. **Conclusions:** These results indicate that short term resistance training can lead to architectural alterations in the BFlh and these adaptations are contraction mode specific. In addition, following 28 days of de-training, the eccentric induced lengthening of BFlh fascicle length was reversed and returned to baseline values. These findings suggest that architectural adaptations have implications for rehabilitation and injury prevention practices.

6.3 Introduction

The ability of a muscle to produce force is partly governed by its architectural characteristics, such as muscle thickness, pennation angle and fascicle length (Lieber & Ward, 2011). Architectural characteristics have been shown, in many different muscles, to change when exposed to mechanical stimuli, such as resistance training (Blazevich, 2006; Blazevich et al., 2007; Narici et al., 2011; Potier et al., 2009; Seynnes et al., 2008). Understanding the changes to muscle architecture in response to a given stimulus is important when aiming to alter muscle function and the risk of injury (Blazevich, 2006; Blazevich et al., 2007; Brockett et al., 2004; Timmins et al., 2015).

Biomechanical investigations into hamstring muscle kinematics during running have shown that they are most active during the terminal swing and early stance phases of the gait cycle (Yu et al., 2008). During the terminal swing phase of the gait cycle, the hamstrings are required to contract eccentrically to decelerate the extending knee and flexing hip (Yu et al., 2008). Additionally, it is during this phase of the gait cycle where the hamstrings reach their longest length, with the biceps femoris long head (BFlh) reaching approximately 110% of its length during upright stance (Thelen, Chumanov, Hoerth, et al., 2005). It is thought that the high force eccentric contractions, coupled with the greater length increases in the BFlh, may contribute to the high rate of hamstring strain injuries during running (Orchard et al., 2013), of which the majority occur in this muscle (Koulouris et al., 2007; Opar et al., 2015). Interestingly, a previously strain injured BFlh possesses shorter fascicle lengths and greater pennation angles when compared to the contralateral uninjured BFlh (Timmins et al., 2015). Independent of the retrospective nature of this evidence, it is important to develop an understanding of how these variables can be altered by physical training which may have implications for HSI prevention and rehabilitation practices in the future.

Despite the large amount of research showing a range of architectural adaptations following eccentric training interventions (Blazevich, 2006; Blazevich et al., 2007; Seynnes et al., 2007), investigations which outline the time course for adaptation, including a period of de-training, are limited. Furthermore, the previous research into the adaptability of the BFlh following a training intervention only compared eccentric training to a non-training control group (Potier et al., 2009). It is therefore unclear how BFlh architectural adaptations might differ when comparing eccentric resistance training to concentric training.

Given the high incidence of hamstring injury in the BFlh (Koulouris et al., 2007; Opar et al., 2015), it is of interest to see how its architecture is altered following either concentric or eccentric resistance training. Therefore the purposes of this study were to: 1) determine the architectural adaptations of the BFlh following either a concentric or eccentric resistance training intervention and; 2) determine the time course of BFlh architectural adaptations during a 6-week training intervention, and following a 28 day period of de-training.

6.4 Methods

As per university guidelines, the methodologies for this study are also presented in chapter 3.3. These methods are replicated below in the format required for *Medicine and Science in Sports and Exercise*.

6.4.1 Participants

Twenty-eight recreationally active males (age 22.3±4.2 years; height 1.81±0.07m; body mass 76.9±8.2kg) with no history of lower limb injury in the past 12 months were recruited to participate in this study. All participants provided written informed consent prior to testing and training which was undertaken at the Australian Catholic University, Fitzroy, Victoria, Australia. Ethical approval for the study was granted by the Australian Catholic University Human Research Ethics Committee.

6.4.2 Study design

After the participants undertook a maximal dynamometry familiarisation session, no less than 7 days later they had their BFlh architectural characteristics assessed on both limbs. Following this initial testing session (*28 days pre baseline*), the participants were paired according to passive BFlh fascicle length and randomly assigned to one of two training groups (allocation ratio 1:1) to undertake either concentric- or eccentric-only knee flexor training. All participants (n=28) returned to the lab four weeks later (*baseline*) and had the maximal knee flexor strength and BFlh architectural characteristics assessed on both limbs. Following this the participants underwent 6-weeks of either a concentric or eccentric training intervention in a randomly selected limb (the contralateral limb served as a within participant control). BFlh architecture of both limbs was re-assessed at day 14, 21 and 42 of the intervention, as well as 28 days after the completing of the training intervention. Knee flexor strength of both limbs was re-tested at the end of the training intervention (*day 42*) and 28 days after the completion of the intervention. All tests were performed at the same time of the day for each participant.

6.4.3 Outcome measures

6.4.3.1 Isokinetic dynamometry

All knee flexor strength testing was completed on a Humac Norm® isokinetic dynamometer (CSMI, Massachusetts, U.S.A), on both legs (left or right) in a randomised order. Participants were seated on the dynamometer with their hips flexed at approximately 85deg from neutral and were restrained by straps around the tested/exercised thigh, waist and chest to minimise compensatory movements. All seating variables (e.g. seat height, pad position, etc.) were recorded to ensure the replication of the participants' positions. Gravity correction for limb weight was also conducted and range of motion was set between 0deg and 90deg of knee flexion (full extension = 0deg) with the starting position for each contraction during strength testing

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being 90deg of knee flexion. The starting position for all training contractions were dependant on training group, with the concentric training group starting from 0deg of knee extension and the eccentric group beginning from 90deg. Prior to all testing sessions, participants undertook a warm-up consisting of three sets of three concentric knee extension and flexion contractions at an angular velocity of 240deg/sec. The intensity of these contractions increased each set (1st set ~75% and 2nd set ~90% of the participants perceived maximum) until the final set at this velocity was performed at a maximal level. The test protocol began one minute following the final warm-up set and consisted of three sets of three repetitions of concentric and eccentric maximal voluntary contractions of knee flexion at 60deg/sec and 180deg/sec (30s inter-set rest). For all concentric knee flexion efforts, the participants were instructed to 'pull down' against the lever as fast as possible, whereas during eccentric contractions they were told to 'resist' the lever arm from extending their knee as hard as they could. All participants were provided visual feedback of their efforts as well as being verbally encouraged by the investigators to ensure maximal effort for all contractions. The testing order of contraction modes was randomised across the participant pool and the testing protocol has been previously reported to not alter concentric or eccentric knee flexor strength (Timmins et al., 2014). Dynamometer torque and lever position data were transferred to computer at 1 kHz and stored for later analysis where it was fourth-order low pass Butterworth filtered (5Hz). Average peak torques at 240, 180 and 60deg/sec for concentric and 180 and 60deg/sec for eccentric knee flexion were defined as the mean of the six highest torque values for each contraction mode at each velocity.

6.4.3.2 BFlh architectural assessment

Muscle thickness, pennation angle and fascicle length of the BFlh was determined from ultrasound images taken along the longitudinal axis (Figure 2.1) of the muscle belly utilising a two dimensional, B-mode ultrasound (frequency, 12Mhz; depth, 8cm; field of view, 14 x 47mm)

(GE Healthcare Vivid-*i,* Wauwatosa, U.S.A). The scanning site was determined as the halfway point between the ischial tuberosity and the knee joint fold, along the line of the BFlh. Once the scanning site was determined, the distance of the site from various anatomical landmarks were recorded to ensure its reproducibility for future testing sessions. These landmarks included the ischial tuberosity, fibula head and the posterior knee joint fold at the mid-point between BF and semitendinosus tendon. On subsequent visits the scanning site was determined and marked on the skin and then confirmed by replicated landmark distance measures. All architectural assessments were performed with participants in a prone position and the hip in a neutral position following at least 5 minutes of inactivity. Assessments at rest were always performed first followed by the graded isometric contraction protocol. Assessment of BFlh architecture at rest was performed with the knee at 0º of knee flexion. Assessment of BFlh architecture during isometric contractions was always performed with the knee at 0º of knee flexion and preceded by a maximal voluntary isometric contraction, performed in a custom made device (Opar, Piatkowski, et al., 2013). The graded isometric contractions of the knee flexors were performed in the same device at 25, 50 and 75% of maximum voluntary isometric contraction (MVIC) with the participants shown the real-time visual feedback of the force produced to ensure that target contraction intensities were met. Assessment of the MVIC of the knee flexors was undertaken in a prone position, with both the hip and knee fully extended (0deg). Participants were instructed to contract maximally over a five second period, of which the peak force was used to determine the MVIC.

To gather ultrasound images, the linear array ultrasound probe, with a layer of conductive gel was placed on the skin over the scanning site, aligned longitudinally and perpendicular to the posterior thigh. Care was taken to ensure minimal pressure was placed on the skin by the probe as this may influence the accuracy of the measures (Klimstra et al., 2007). Finally, the orientation of

the probe was manipulated slightly by the sonographer (RGT) if the superficial and intermediate aponeuroses were not parallel.

Once the images were collected analysis was undertaken off-line (MicroDicom, Version 0.7.8, Bulgaria). For each image, six points were digitised as described by Blazevich and colleagues (Blazevich et al., 2006). Following the digitising process, muscle thickness was defined as the distance between the superficial and intermediate aponeuroses of BFlh. A fascicle of interest was outlined and marked on the image. The angle between this fascicle and the intermediate aponeurosis was measured and given as the pennation angle (Figure 2.1). The aponeurosis angle for both aponeuroses was determined as the angle between the line marked as the aponeurosis and an intersecting horizontal line across the captured image (Blazevich et al., 2006; Kellis et al., 2009). Fascicle length was determined as the length of the outlined fascicle between aponeuroses. As the entire fascicle was not visible in the field of view of the probe it was estimated via the following validated equation from Blazevich and colleagues (Blazevich et al., 2006; Kellis et al., 2009):

FL=sin (AA+90deg) x MT/sin (180deg-(AA+180deg-PA)).

Where FL=fascicle length, AA=aponeurosis angle, MT=muscle thickness and PA=pennation angle.

Fascicle length was reported in absolute terms (cm) and also relative to muscle thickness (fascicle length/muscle thickness). The same assessor (RGT) conducted and analysed all scans and was blinded to participant identifiers during the analysis. The methodology utilised in this study for the assessment of the BFlh architectural characteristics has been previously reported by our laboratory (Timmins et al., 2015).

6.4.4 Intervention

The participants performed 6 weeks of either maximal eccentric or concentric knee flexion training, two (first week of intervention) to three times a week on an isokinetic dynamometer (Humac Norm, CSMI, Massachusetts, U.S.A) using the same range of motion and seat positions configuration as dynamometry testing sessions. Only one limb received the training stimulus, with the contralateral limb acting as a within subject control limb. Across the training period the volume (number) of contractions was increased following the progression below:

- Week 1:
    - Frequency (days/week) = 2
    - Sets = 4
    - Repetitions = 6
    - Total repetitions = 48

- Week 2:
    - Frequency (days/week) = 3
    - Sets = 4
    - Repetitions = 6
    - Total repetitions = 72
- Week 3:
    - Frequency (days/week) = 3
    - Sets = 5
    - Repetitions = 6
    - Total repetitions = 90
- Week 4:
    - Frequency (days/week) = 3
    - Sets = 5
    - Repetitions = 8
    - Total repetitions = 120
- Week 5:
    - Frequency (days/week) = 3
    - Sets = 6
    - Repetitions = 6
    - Total repetitions = 108
- Week 6:
    - Frequency (days/week) = 3
    - Sets = 6
    - Repetitions = 8
    - Total repetitions = 144

Each training session was separated by at least 48 hours. Contractions were distributed evenly across 60deg/sec and 180deg/sec. All participants started with two sets of three warm up efforts at 60deg/sec, in the contraction mode utilised for their training. For all training repetitions, the concentric training participants were moved to full knee extension (0deg) by the investigator and were instructed to flex their knee as fast as possible through to 90deg of knee flexion. The investigator then returned the lever arm to full knee extension and the subsequent repetition was completed. This was undertaken until all repetitions were completed in their respective set, with a 30 second inter-set rest period. The eccentric training participants began with their knee at 90deg of flexion. They were then instructed to maximally flex against the lever arm until full knee extension was reached (0deg). The participant was then instructed to relax, the lever arm was repositioned to 90deg of knee flexion by the investigators and the subsequent contraction was performed. This was undertaken until all repetitions were completed in each set, with a 30 second inter-set rest period. All participants were provided visual and verbal feedback on the consistency of the torque produced during each repetition. These were compared against personal best performances, which were known by the participant, to aid motivation. During the pre-control (*28 days pre baseline to baseline*), intervention (*baseline to intervention day 42*) and de-training periods (*intervention day 42 to post intervention day 28*), participants continued their habitual levels of physical activity. The only restriction was to not perform any unilateral lower limb strength exercises. Finally, training compliance was determined as a percentage of sessions that were completed within 24 hours of the intended time.

6.4.5 Statistical analysis

All statistical analyses were performed using SPSS version 22.0.0.1 (IBM Corporation, Chicago, IL). Where appropriate, data were screened for normal distribution using the Shapiro-Wilk test and homoscedasticity of the data using Levene's test. Greenhouse-Geisser adjustment was

applied when the assumption of sphericity was violated ($p<0.05$ for Mauchly's test of sphericity). At each contraction intensity, a split-plot design ANOVA with the within-subject variables being limb (trained or untrained) and time point (*28 days pre baseline, baseline, intervention day 14, intervention day 21, intervention day 42, post intervention day 28*) and the between-subject variable being group (eccentric or concentric), was used to compare changes in BFlh architecture throughout the training study. Architectural changes across the 28 day control period (*28 days pre baseline to* baseline) were not significant ($p>0.05$). Therefore when determining the alterations in BFlh architectural characteristics following a 6 week intervention, all comparisons were made to *baseline*. Knee flexor average peak torque comparisons, at each contraction velocity, used a similar split-plot design ANOVA, however, with different time point variables (*baseline, intervention day 42, and post intervention day 28*). Where significant limb x time x group interactions for architecture and limb x time for knee flexor average peak torque were detected, post hoc t-tests with Bonferroni adjustments were used to identify which comparisons differed. Significance was set at a $p<0.05$ and where possible Cohen's *d* (Cohen, 1988) was reported for the effect size of the comparisons, with the levels of effect being deemed small ($d = 0.20$), medium ($d = 0.50$) or large ($d = 0.80$) as recommended by Cohen (1988).

6.4.6 Sample Size

Sample size analysis was completed *a-priori* using G-Power (Faul et al., 2007). The analysis was based on the anticipated differences in fascicle length following the training intervention. The effect size was estimated based on the only intervention study to date that has reported changes in the BFlh architecture (Potier et al., 2009). That study reported a 33% increase in fascicle length following the intervention with an approximate effect size of 1.9. Therefore an effect size of 1.2 was deemed as a reasonable starting point. Power was set at 80% with an alpha level of 0.05 returning a calculated sample size of 12 per group. As a cross-reference to confirm the effect size,

fascicle length differences in individuals with a unilateral BFlh strain injury displayed an effect size of 1.34 when comparing between the previously injured and contralateral uninjured limb (Timmins et al., 2015).

6.5 Results

6.5.1 Participants
The two training groups were similar with respect to age, height and body mass (eccentric training group: age 21.2±2.7 years, height 1.81±0.06m, body mass 77.9±9.3kg; concentric training group: age 23.4±5.1 years; height 1.81±0.07m; body mass 76.2±7.1kg). Overall, compliance rates were acceptable for all participants (92%±2; min=85%; max=100%), with no differences when comparing the two groups (eccentric training group: 91%±2; concentric training group: 93%±1).

6.5.2 BFlh architectural comparisons

6.5.2.1 Control period, control limb changes and baseline comparisons
A significant limb-by-time-by-group interaction effect was found for fascicle length, fascicle length relative to muscle thickness and pennation angle (p<0.001). Post hoc analyses showed that none of the BFlh architectural variables changed during the 4-week pre-intervention control period (p>0.05, *d* range = 0.03 to 0.17). Similarly, there were no significant differences at any time point, in the non-training control limbs for any of the BFlh architectural variables (p>0.05, *d* range = 0.03 to 0.27). Comparisons of all the BFlh architectural variables at baseline displayed no significant differences between the concentric and eccentric training group in legs that were to be trained (i.e. the training leg) (p>0.05, *d* range = 0.22 to 0.43).

6.5.2.2 Fascicle length and fascicle length relative to muscle thickness changes
A significant limb-by-time-by-group interaction effect was found for fascicle length at all contraction intensities (p<0.001). Post hoc analysis showed that fascicle length was significantly

longer in the training limb of the eccentric training group (p<0.05, *d* range: 2.65 to 2.98, Table 6.1, Figure 6.1) and significantly shorter in the training limb of the concentric training group (p<0.05, *d* range: -1.62 to -0.96, Table 6.1, Figure 6.1) after 42 days of the intervention compared to baseline, at all contraction intensities. Additionally there was a significant limb-by-time-by-group interaction effect for fascicle length relative to muscle thickness (p<0.001). All post hoc comparisons for the training limbs of each group are presented in Table 6.1.

Following the 28 day post intervention period, fascicle length was significantly reduced in the training limb of the eccentric training group in comparison to the end of the intervention, at all contraction intensities (p<0.05, *d* range: -1.73 to -1.55, Table 6.1, Figure 6.1). Post hoc analysis showed that following the 28 days of de-training, fascicle length of the concentric training group was no different to that observed end of the intervention, at any contraction intensity (p>0.05, *d* range: 0.15 to 0.67, Table 6.1, Figure 6.1). All other post hoc comparisons of fascicle length and fascicle length relative to muscle thickness, 28 days following the intervention period, in the training limbs of both groups are presented in Table 6.1 and Figure 6.1.

Table 6-1. *Changes in the biceps femoris long head architectural characteristics in the training limb of each group at the start (day 0), after 14, 21 and 42 days of the training intervention as well as following the de-training period (day 70).*

	Concentric Group (n=14)					Eccentric Group (n=14)				
0% MVIC	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)
FL (cm)	11.71±0.9	10.74±0.7*	10.45±0.9*	10.33±0.8**	10.45±0.8*	11.53±0.6	13.24±0.9**	13.27±0.9**	13.42±0.8**	11.85±1.0##
RFL	4.60±0.2	4.01±0.3**	3.90±0.3**	3.86±0.3**	3.97±0.3**	4.31±0.4	4.75±0.4*	4.65±0.4	4.64±0.4	4.3±0.2
PA (deg)	12.57±0.6	14.47±0.9*	14.97±1.4**	15.1±1.3**	14.72±1.5**	13.52±1.2	12.21±1.1*	12.40±1.2*	12.50±1.2*	13.50±0.9
MT (cm)	2.54±0.2	2.68±0.2	2.70±0.2	2.68±0.2	2.64±0.2	2.69±0.2	2.80±0.3	2.86±0.2	2.91±0.2	2.77±0.3
25% MVIC	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)
FL (cm)	10.69±0.6	10.01±0.4*	9.83±0.8*	9.63±0.8*	10.03±0.8	10.72±0.5	11.92±0.7*	12.35±0.9**	12.33±0.6**	11.13±0.9#
RFL	4.15±0.2	3.70±0.2**	3.63±0.2**	3.60±0.2**	3.71±0.3*	3.94±0.3	4.2±0.2	4.22±0.3	4.20±0.4	3.92±0.2
PA (deg)	13.95±0.7	15.72±0.9*	16.04±1.2**	16.16±1.2**	15.72±1.3*	14.79±1.2	13.89±0.9	13.67±1.2	13.87±0.6*	14.81±0.9
MT (cm)	2.58±0.2	2.71±0.2	2.71±0.2	2.67±0.2	2.70±0.2	2.74±0.2	2.87±0.2	2.93±0.2	2.95±0.3	2.84±0.2
50% MVIC	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)
FL (cm)	10.03±0.7	9.60±0.5	9.32±0.6*	9.22±0.5*	9.50±0.6	10.15±0.5	11.09±0.7*	11.59±0.7**	11.80±0.6**	10.64±0.8##
RFL	3.80±0.2	3.50±0.2*	3.41±0.3*	3.37±0.2**	3.46±0.2*	3.64±0.2	3.84±0.2	3.92±0.3	3.95±0.3*	3.70±0.2
PA (deg)	15.30±0.9	16.70±1.2*	17.14±1.4*	17.28±1.2*	16.88±1.3*	16.01±1.2	15.10±1.0	14.91±0.9	14.74±1.1*	15.72±0.9
MT (cm)	2.65±0.2	2.75±0.2	2.74±0.2	2.74±0.2	2.75±0.2	2.79±0.2	2.89±0.2	2.97±0.2	3.00±0.2	2.87±0.2
75% MVIC	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)	Baseline (Day 0)	Intervention (Day 14)	Intervention (Day 21)	End Intervention (Day 42)	Post Intervention (Day 70)
FL (cm)	9.36±0.6	9.21±0.6	9.12±0.6	8.78±0.6*	9.15±0.5	9.62±0.6	10.51±0.7*	11.03±0.6**	11.21±0.6**	10.20±0.7#
RFL	3.53±0.2	3.34±0.2	3.28±0.2*	3.18±0.2*	3.31±0.2	3.43±0.2	3.64±0.2	3.72±0.2*	3.75±0.3*	3.54±0.2
PA (deg)	16.50±1.2	17.46±1.2	17.86±1.4	18.34±1.1*	17.62±1.2	17.03±1.2	15.95±1.1	15.70±0.9*	15.54±1.1*	16.44±0.9
MT (cm)	2.65±0.2	2.76±0.2	2.79±0.2	2.76±0.2	2.77±0.2	2.81±0.2	2.89±0.2	2.98±0.2	2.99±0.2	2.87±0.2

*=p<0.05 vs Day 0, ** = p<0.001 vs Day 0, # = p<0.05 vs Day 42, ## = p<0.001 vs Day 42. All data represented as mean±SD unless otherwise stated. SD = standard deviation, MT = muscle thickness, cm = centimetres, PA = pennation angle, RFL = fascicle length relative to muscle thickness, FL = fascicle length, MVIC = maximum voluntary isometric contraction.

6.5.2.3 Muscle thickness and pennation angle changes

No significant limb-by-time-by-group interaction effect was found for muscle thickness at any contraction intensity ($p>0.162$). However, a significant limb-by-time-by-group interaction effect was detected for pennation angle at all contraction intensities ($p<0.001$). Post hoc analysis showed that pennation angle was significantly reduced in the training limb of the eccentric training group ($p<0.05$, *d* range: -1.30 to -0.85, Table 6.1, Figure 6.1) and significantly increased in the training limb of the concentric training group ($p<0.05$, *d* range: 1.60 to 2.50, Table 6.1, Figure 6.1) after 14 days of the intervention compared to baseline, at all contraction intensities. All other comparisons of pennation angle changes in the training limb of both groups are presented in Table 6.1.

Figure 6-1. *Changes in the architectural characteristics of the BFlh when assessed at rest in the trained limb and the contralateral untrained limb of both groups following 14, 21 and 42 days of the training intervention and following the de-training period (day 70).*
A) fascicle length
B) pennation angle
C) muscle thickness
D) fascicle length relative to muscle thickness.
Error bars illustrate the standard deviation.
*=p<0.05 vs Day 0,
** = p<0.001 vs Day 0,
*## = p<0.001 vs Day 42.*cm=centimetres, Δ=change.*
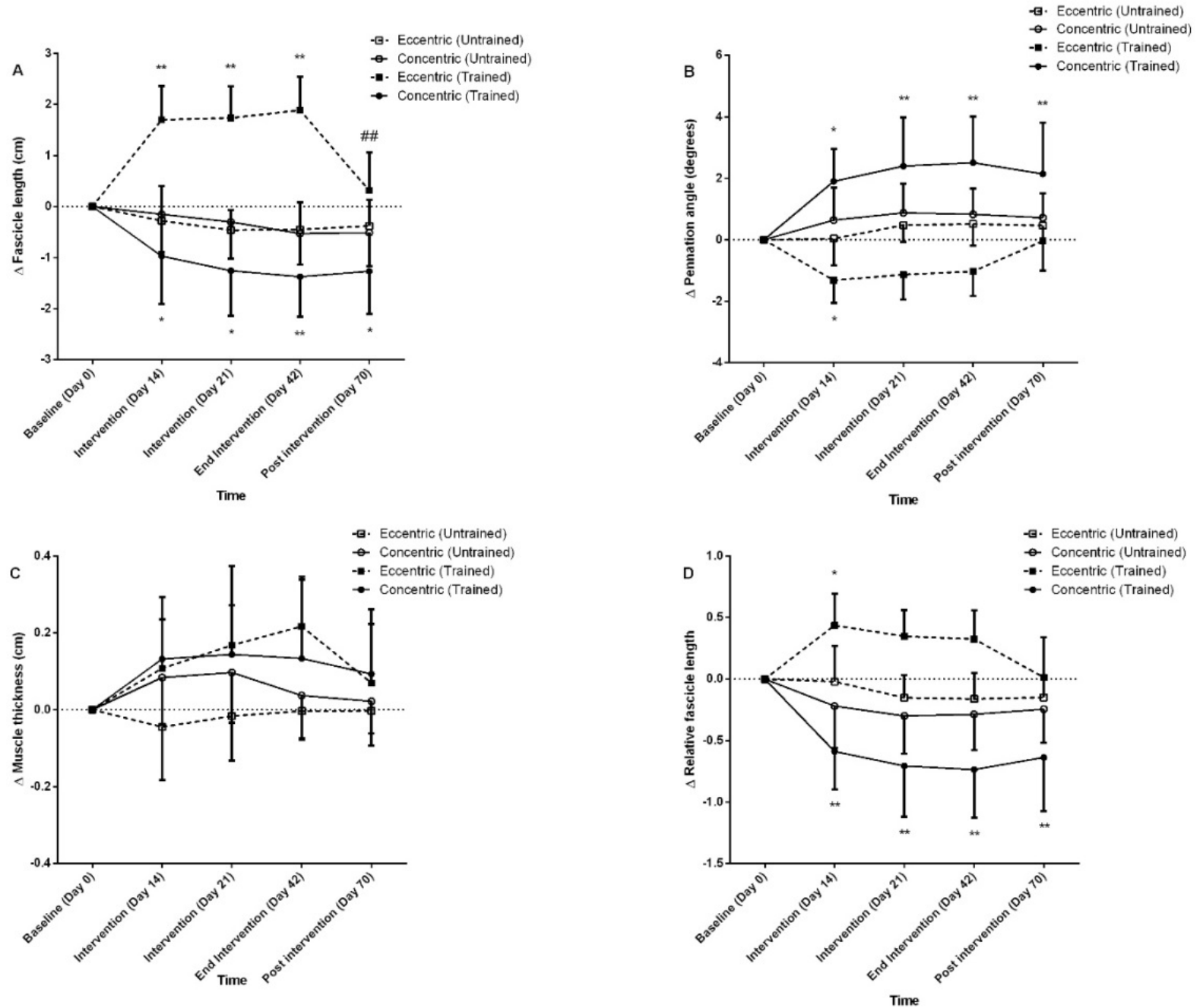
Following the 28 day post intervention period, pennation angle was not significantly different in the training limb of the eccentric training group in comparison to the end of the intervention, at any contraction intensity (p>0.05, d range: -0.55 to 0.02, Table 6.1, Figure 6.1). Post hoc analysis showed that following the 28 days of de-training, pennation angle of the concentric training group was no different compared to the end of the intervention, at any contraction intensity (p>0.05, d range: -0.63 to -0.27. Table 6.1, Figure 6.1). All other comparisons of pennation angle changes following the 28 day post intervention period are presented in Table 6.1.

6.5.3 Strength changes

A significant limb-by-time interaction effect for knee flexor average peak torque was found at all contraction velocities for each group (p<0.001). Comparisons at all contraction velocities, at baseline, displayed no significant differences between the concentric and eccentric training group (p>0.05). Post hoc analysis also revealed that knee flexor average peak torque increased in both the training limb of the eccentric (p<0.05, d range: 0.63 to 0.78, Table 6.2) and the concentric training group (p<0.05, d range: 0.53 to 0.72, Table 6.2) after 42 days of the intervention, at all contraction velocities, when compared to baseline. There were no significant differences in knee flexor average peak torque for the untrained limbs of either group after 42 days of the intervention when compared to baseline, at any contraction velocity (p>0.05, d range = 0.11 to 0.27).

Following the 28 day post intervention period, there were no significant differences in knee flexor average peak torque at all contraction velocities, in either group, when compared to their strength after 42 days of the intervention (p>0.05, d range: -0.30 to -0.16, Table 6.2). Additionally, knee flexor average peak torque at all contraction velocities, 28 days post the intervention period, was significantly greater in the training limb of both training groups when compared to baseline (p>0.05, d range: 0.34 to 0.75, Table 6.2).

Table 6-2. *Changes in concentric and eccentric knee flexor average peak torque at various contraction velocities in the training limb of each group before (day 0) and after the training intervention (day 42) as well as following the de-training period (day 70).*

Contraction velocity	Concentric Group (n=14)					Eccentric Group (n=14)				
	Baseline (Day 0)	End Intervention (Day 42)	Post Intervention (Day 70)	%Change following intervention	%Change 28 days post intervention	Baseline (Day 0)	End Intervention (Day 42)	Post Intervention (Day 70)	%Change following intervention	%Change 28 days post intervention
Concentric 240 (°/sec)	89.3 ±16.2	97.86* ±16.4	95.44±13.3*	9.58	-2.47	97.2 ±21.2	109.94* ±19.3	105.10* ±20.3	13.1	-4.40
Concentric 180 (°/sec)	104.44 ±19.1	116.2** ±18.2	111.66±17.8*	11.3	-3.95	111.3 ±24.9	129.06** ±24.3	122.57* ±17.9	15.9	-5.01
Concentric 60 (°/sec)	141.04 ±28.3	159.5* ±24.1	153.51±25.9*	13.1	-3.75	134.15 ±30.8	156.3** ±25.2	152.8* ±17.02	16.5	-2.23
Eccentric 60 (°/sec)	186.53 ±39.6	213.40* ±35.1	203.74±40.3*	14.4	-4.52	196.3 ±44.4	228.8** ±48.9	218.2* ±36.7	16.6	-4.63
Eccentric 180 (°/sec)	178.11 ±44.7	200.62* ±34.8	191.91±35.7*	12.6	-4.34	185.9 ±35.9	216.2** ±43.01	209.02* ±32.1	16.3	-3.32

*=p<0.05 vs Day 0, ** = p<0.001 vs Day 0. All data represented as mean±SD unless otherwise stated. SD = standard deviation, °/sec = degrees per second.

6.6 Discussion

To the authors' knowledge, this is the first study reporting divergent BFlh architectural adaptations in response to 6-weeks of concentric or eccentric resistance training. Moreover, it is the first study to show that lengthening of BFlh fascicles in response to eccentric training return to baseline following 28 days of de-training. Finally, this is the first study to quantify changes in knee flexor strength following a 6-week concentric or eccentric resistance training intervention. The main findings were that 6-weeks of eccentric resistance training resulted in an increase in BFlh fascicle length and a reduction in pennation angle, whereas those who trained concentrically displayed reductions in fascicle length and increases in pennation angle. Additionally, a 28 day period of de-training resulted in a significant reduction in BFlh fascicle length and a non-significant increase in pennation angle in the eccentrically trained group when compared to the end of the training intervention. In contrast, the concentrically trained group maintained their BFlh architectural characteristics following 28 days of de-training. Finally, following a 6-week training intervention, improvements in knee flexor strength were not specific to training contraction mode, with significant improvements in concentric and eccentric strength found in both training groups. Furthermore, knee flexor strength was maintained during the de-training period.

Observations of increases in BFlh fascicle length and a reduction in pennation angle at rest, following eccentric resistance training in the current study (Figure 6.1) aligns somewhat with previous literature (Potier et al., 2009). Potier and colleagues (2009) found a 33% increase in resting BFlh fascicle length with a non-significant 3.1% reduction in resting pennation angle following 8-weeks of eccentric training. In comparison, the current study saw a significant 16% increase in resting BFlh fascicle length (the majority of which occurred within 14 days), with a

non-significant 7.5% reduction in resting pennation angle. Differences in the training modalities employed (leg curl vs isokinetic dynamometry); intervention length (8-weeks vs 6-weeks) and the site of assessment may explain the different magnitudes of change reported in these studies. Additionally, no previous literature has examined alterations of the BFlh architectural characteristics during graded isometric contractions, following an intervention. The present study found longer BFlh fascicle lengths at all graded isometric contractions in the eccentrically trained individuals at the end of the intervention. These longer fascicle lengths may occur as a result of the addition of in-series sarcomeres (Lynn & Morgan, 1994), or through changes in the stiffness of the tendon. Further research is needed to clarify the mechanism responsible for fascicle length alterations.

No other study has compared the architectural alterations in the BFlh, following concentric and eccentric training. However, interventions which have employed concentric or eccentric training of the knee extensors have reported inconsistent architectural adaptations. Some have shown a contraction mode specific adaptation (Franchi et al., 2014; Reeves et al., 2009) whilst others have not (Blazevich et al., 2007). Additionally isometric training of the knee extensors at short and long muscle lengths has also been shown to increase fascicle length (Noorkoiv et al., 2014). A range of factors such as the variability in the work and volume matching of participants (Blazevich et al., 2007; Franchi et al., 2014), the age and physical capacity of the participants (Reeves et al., 2009) as well as the velocity of the training stimulus (Sharifnezhad et al., 2014) might explain some of the variance between these results. However it is not known why these alterations in the vastus lateralis differ to those reported in the current study. It is possible that differences in the structural and functional characteristics of the muscles may account for this variability. However future research is needed to assist in determining the adaptive responses of the BFlh to these and many other variables.

The increases in BFlh fascicle length and reductions in pennation angle found in the current study following an eccentric training intervention may have implications for hamstring strain injury prevention and rehabilitation. Elite athletes with a unilateral history of BFlh strain injury have shorter fascicles and greater pennation angles on their previously injured limb when compared to the contralateral uninjured limb (Timmins et al., 2015). Individuals with a history of hamstring strain injury are at an increased risk of future injury in comparison to those without a history (Opar et al., 2015; Orchard et al., 2013). Therefore if shorter fascicles and greater pennation angles in a previously injured athlete is a partial contributor to the elevated risk of re-injury, then understanding the most effective methods for altering these architectural characteristics will be of great value. The current data indicates that the continual application of high-intensity, eccentric-only resistance training should be considered in hamstring rehabilitation and prevention programs in order to increase BFlh fascicle length and reduce pennation angle. Further research is needed to determine how the combination of both concentric and eccentric contractions during conventional resistance training methods may alter BFlh architecture.

The very rapid response of BFlh architectural adaptations supports previous literature which has found significant increases in fascicle length and pennation angle in the vastus lateralis within 14 days of the commencement of an eccentrically biased training intervention (Seynnes et al., 2007). Furthermore, in-series sarcomere numbers of the rat vastus intermedius have been shown to increase within a week of the commencement of a downhill running protocol (Lynn & Morgan, 1994). In the current study, the majority of fascicle length and pennation angle changes in the eccentric resistance training group occurred within 14 days of training, with non-significant changes for the rest of the intervention (Figure 6.1). A similar, but inverse response was found in the concentric training group after 14 days of training, with non-significant changes for the remainder of the training intervention. These results, along with those from other studies

(Blazevich et al., 2007; Seynnes et al., 2007) suggest that early adaptations to strength training are not only from a neural mechanism (Selvanayagam, Riek, & Carroll, 2011), but may also be as a result of architectural adaptations.

Alterations in muscle architecture following periods of de-training are variable, with most conclusions being drawn from observations of prolonged periods of limb unloading, some of which show significant reductions in fascicle length, pennation angle and muscle volume (Narici & Cerretelli, 1998; Seynnes et al., 2008), whereas some display no alterations (Abe, Kawakami, Suzuki, Gunji, & Fukunaga, 1997). In regards to the de-training responses following high-intensity eccentric or concentric resistance training, only one study has investigated this, 3-months after a 10-week intervention in the vastus lateralis (Blazevich et al., 2007). Blazevich and colleagues (2007) found no significant alterations in strength or architectural characteristics of the vastus lateralis following a 3-month de-training period. These results are disparate to the findings from the eccentric training group in the current study who displayed a significant reduction in BFlh fascicle length and an increase in pennation angle following 28 days of de-training. In comparison, the concentric group displayed similar findings to Blazevich and colleagues (2007), with architectural variables remaining unchanged following 28 days of de-training (Blazevich et al., 2007). The response of the eccentric training group to the intervention and then to de-training may be of interest for hamstring strain injury prevention and rehabilitation interventions as it has been argued that shorter fascicles (i.e. with fewer in-series sarcomeres) are more prone to muscle damage during high-intensity, eccentric contractions compared with longer fascicles (Fyfe et al., 2013; Morgan, 1990; Timmins et al., 2015). It remains to be seen what effect conventional resistance training exercises, that possess both concentric and eccentric actions, may have on hamstring muscle architecture. In addition, the rapid decrease in fascicle

lengths when the eccentric stimulus is removed would indicate that constant exposure to eccentric exercise is important to maintain changes in BFlh architecture following an intervention period.

The training interventions in the current study induced significant increases in concentric and eccentric strength in the training limb of both the concentric training and eccentric training groups (Table 6.2). Previous research investigating knee flexor strength alterations following eccentric or concentric training interventions are widely variable (Kaminski, Wabbersen, & Murphy, 1998; Potier et al., 2009). To the authors' knowledge, this is the first study to show improvements in both isokinetically derived concentric and eccentric knee flexor strength independent of training modality. However, improvements in concentric strength following an eccentric training intervention have been previously reported in the knee flexors, as well as within other muscle groups (Paddon-Jones, Leveritt, Lonergan, & Abernethy, 2001; Shepstone et al., 2005). There is still some contradictory evidence as to whether a contraction mode specific strength adaptation occurs following either concentric or eccentric training (Blazevich et al., 2007; Franchi et al., 2014; Reeves et al., 2009). The current study shows that increases in eccentric strength can be achieved through long length, concentric resistance training in the knee flexors. It is unclear if there might be a contraction-mode specific adaptation in longer training programs. However this finding in the current study must be considered in line with the divergent architectural alterations seen between the two training interventions. Future research should aim to clarify if there is a contraction mode specific strength adaptation following training interventions in the knee flexors which employ different training variables (i.e. longer duration of intervention).

The authors acknowledge that there are limitations in the current study. Assessment of muscle architecture was only performed on the BFlh and did not include the other knee flexors. Therefore it is unknown what adaptations these other muscles displayed following the intervention and de-

training period. However, as the BFlh is the most commonly strain injured hamstring muscle (Koulouris et al., 2007); the alterations following concentric and eccentric training interventions were of interest from a hamstring strain injury risk and rehabilitation perspective. Finally, the training stimulus was provided with an even distribution of the number of contractions across both slow and fast isokinetic velocities. As architectural adaptations of the vastus lateralis have been shown to be velocity dependant (Sharifnezhad et al., 2014), it is not possible to determine if the changes in this cohort and muscle are due to the velocities utilised. The aim of the study was to investigate the effect that contraction mode had on architectural changes of the BFlh, not velocity, as this may have greater inferences for HSI prevention and rehabilitation. Further research is needed to determine if there is a contraction velocity specific adaptation in the knee flexors for a concentric or eccentric training intervention.

In conclusion, the current study reported rapid, contraction-mode specific alterations in BFlh architecture following 6-weeks of either eccentric or concentric training interventions. Further, 28 days of de-training resulted in BFlh architectural characteristics returning to baseline levels in individuals who undertook 6-weeks of eccentric knee flexor training, whilst de-training had no influence on the BFlh architectural characteristics in those who completed concentric training. The findings of the current study provide insight into BFlh architectural alterations following concentric and eccentric training interventions. These results may have implications for hamstring injury prevention and rehabilitation programs which might consider architectural alterations to training interventions as a factor that might mitigate risk of future injury.

Chapter 7 – General discussion, limitations and conclusion

This program of research was the first to investigate BFlh architecture in athletes, with retrospective and prospective studies, as well as determining how BFlh architecture can be altered in response to a resistance training interventions. The thesis began by determining the reliability of the operator and employed processes to assess the BFlh architectural characteristics at rest and during graded isometric contractions. Study 1 also extended to determine the architectural differences in elite athletes with a unilateral history of BFlh strain injury. The previously injured BFlh was found to possess shorter fascicles and greater pennation angles compared to the contralateral uninjured limb. Due to the retrospective nature of the findings from study 1, it was not possible to determine if these differences were the result or the cause of injury. Therefore study 2 investigated the role that BFlh fascicle length had in the aetiology of hamstring strain injuries (HSI) in elite soccer players. Possessing a shorter BFlh fascicle increased the risk of a subsequent HSI approximately 4 fold. Additionally the risk associated with increasing age and a history of HSI was found to be modulated with increases in BFlh fascicle length. Considering the findings from study 1 and 2, it was important to understand how BFlh architectural characteristics responded to either an eccentric or concentric training intervention. The main findings from study 3 were that eccentric resistance training resulted in an increase in BFlh fascicle length, whereas concentric resistance training caused a reduction.

Before this work there was a paucity of information regarding muscle architecture and its potential role in HSI and re-injury. Therefore the theoretical underpinning for this thesis originated from a hypothetical model (Fyfe et al., 2013) which suggested a combination of neuromuscular inhibition and the restriction to short muscle lengths during the early stages of rehabilitation as being partly responsible for the shedding of sarcomeres in-series. This was

hypothesized to occur as a result of a reduced in-series strain during eccentric contractions as a potential protective mechanism (Fyfe et al., 2013). It was also hypothesised that reducing the number of sarcomeres in-series, resulting in the shortening of muscle fascicle length, may increase a muscles susceptibility to damage. The combination of these, coupled with low levels of eccentric strength, was thought to increase the risk of a future HSI. This program of research confirmed the importance of long BFlh fascicle lengths in reducing the risk of a future HSI.

This program of research also provided insight into eccentric knee flexor strength and its role in HSIs. Initial retrospective investigations in study 1 were undertaken in athletes with a unilateral history of BFlh strain injury. In this study, the previously injured limb was significantly weaker than the contralateral, uninjured limb when assessed during the Nordic hamstring exercise. However due to the retrospective nature of this study, it was unclear if this difference was the cause or the result of injury. Therefore study 2 aimed to investigate the role that eccentric strength has in the aetiology of HSI in elite athletes. Study 2 found that at the start of pre-season, athletes who possessed low levels of eccentric strength were 4 times more likely to suffer a HSI in that subsequent season. Considering the results from study 1 and 2, it was important to understand how an eccentric or concentric resistance training intervention altered eccentric knee flexor strength. The main findings from study 3 were that increases in eccentric strength can be achieved through high-intensity eccentric or concentric resistance training.

Before this body of work, the role that eccentric knee flexor strength played in the aetiology of a HSI in elite soccer was unclear. However it is well evidenced that interventions utilising the Nordic hamstring exercise are effective at reducing injury risk in elite soccer players (Arnason et al., 2008; Petersen, Thorborg, Nielsen, Budtz-Jorgensen, & Holmich, 2011). It is thought that this reduction in risk occurs via increases in eccentric strength (Mjolsnes, Arnason, Osthagen, Raastad, & Bahr, 2004), which may provide a protective mechanism during injurious activities

such as high speed running, where the hamstrings contract eccentrically during the terminal swing phase (Yu et al., 2008).

In study 1 and 2, the assessment of eccentric knee flexor strength was undertaken during the Nordic hamstring exercise, in a custom made device (Figure 3.1 and 3.2). This method of assessment differs from isokinetic dynamometry, which was used in study 3. Eccentric strength assessments undertaken on the device during the Nordic hamstring exercise cannot be done at a constant velocity, whereas this is possible when completed using an isokinetic dynamometer. Testing during the Nordic hamstring exercise is also undertaken at shorter muscle lengths than those experienced during seated dynamometry. Finally assessments undertaken during the Nordic hamstring exercise do not allow for the determination of an angle of peak force production. Despite these limitations, eccentric strength assessed during the Nordic hamstring exercise has strong evidence to indicate an athlete's risk of a future HSI (Bourne et al., 2015; Opar et al., 2015). In contrast there is no consensus as to the role that measures of eccentric knee flexor strength, derived from isokinetic dynamometry, have in the aetiology of a HSI (Croisier et al., 2008; Freckleton & Pizzari, 2013). One main reason for these divergent findings is the different testing methodologies. Firstly, the assessment of eccentric knee flexor strength during the Nordic hamstring exercise is undertaken bilaterally, whereas isokinetic testing is completed unilaterally. Secondly, the assessment of eccentric knee flexor strength during the Nordic hamstring exercise involves an increase in the external torque around the knee joint as the athlete progresses towards the ground, whereas isokinetic assessments require maximal contraction throughout the entire range of motion. The increased demands of the Nordic hamstring exercise as the athlete progresses closer to the ground might be considered a surrogate marker for strength, as stronger athletes are able to withstand greater external torques compared to weaker individuals. Finally, assessing eccentric knee flexor strength during the Nordic hamstring exercise is quick (~2

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minutes per athlete) and can be completed without need for a laboratory space. On the other hand isokinetic dynamometry requires the athlete to attend a laboratory in order to be assessed, as well as demanding greater amounts of time (approximately 30 minutes per limb). Therefore the inefficiency of testing limits the number of athletes which can be assessed at a single time point, potentially reducing the sample size in a prospective study. This may have contributed to the limited evidence supporting the role of isokinetic dynamometry derived measures of eccentric knee flexor strength in the aetiology of a HSI.

Contraction mode specificity also needs to be considered when assessing an individual's risk of a future HSI. In study 2, isometric knee flexor weakness did not increase the risk of a future HSI. As the Nordic hamstring exercise tests the mechanical properties of the hamstrings during maximal eccentric contractions (Petersen et al., 2011), it is thought that this may have a greater transference than isometric assessments to the mechanism of injury during high-speed running. It has also been hypothesized that the effects of neuromuscular inhibition are heightened during eccentric contractions, with any possible deficits in strength or activation being masked with isometric assessments (Fyfe et al., 2013). Therefore this program of research highlights the importance of eccentric strength assessments in the identification, prevention and rehabilitation of athletes from HSIs.   This program of research also creates a direction for future work. Study 1 showed that elite athletes with a unilateral HSI history possessed shorter BFlh fascicle lengths in their previously injured limb compared to the contralateral uninjured limb. However it is unknown what differences exist between athletes who have had multiple HSIs or have just suffered a single incident. It is unknown if maladaptive responses to injury (e.g. reductions in eccentric strength, shorter BFlh fascicle lengths etc.) get progressively worse with repeated events. Study 2 showed that BFlh fascicle length played a role in the aetiology of a HSI in elite soccer players. However the transference of this data to other sporting codes is limited, with

additional prospective investigations in other sports required. The thesis also outlined the architectural adaptations of the BFlh to eccentric and concentric resistance training in recreationally active males without a history of injury. Architectural adaptations of the BFlh were found to be contraction mode specific; however it is unknown what mechanisms influenced these divergent alterations. Differences in neural control (Duchateau & Baudry, 2014; Enoka, 1996), cell signalling (Eliasson et al., 2006; Rahbek et al., 2014) and fibre length changes (Butterfield et al., 2005) during concentric and eccentric efforts may account for some of these contraction mode specific alterations, but more work examining the mechanism of contraction mode specific adaptation on BFlh architecture is warranted. It is also unknown how athletes with a history of HSI may respond to similar interventions. Given the likelihood of inhibition during eccentric contraction in previously injured hamstrings, it is conceivable that the adaptation to an eccentrically-biased training stimulus is different when compared to those without a history of HSI. Interventions aimed at altering BFlh architecture, in those with a prior HSI, is worthy of future investigation. Additionally the time course of architectural adaptations following recuperation from an acute HSI may provide insight into how the restriction to short muscle lengths during the early stages of rehabilitation might alter fascicle length.

This program of research includes a range of limitations. The ultrasound technique utilised throughout the thesis was not validated through the use of cadaveric samples. However, all data obtained at rest are comparable to those in the existing *in-vivo* literature, which has made comparisons to cadaveric data. Additionally, this ultrasound technique has some methodological limitations when utilised to assess BFlh fascicle length. The field of view employed in this thesis does not capture the entire BFlh fascicle, resulting in the need for estimation. This is undertaken via the use of an equation which has been validated against cadaveric samples. Despite this, it must be recognised that there is still a level of error associated with estimations of BFlh fascicle

length in this program of research. Future studies should consider the use of extended field of view ultrasound methods when determining BFlh fascicle length.

Study 1 involved a comparison of two different cohorts, with elite athletes in the previously injured group and recreationally active participants in the control group. However the comparisons between the averages of both limbs in the control group to the uninjured limb in the previously injured group suggest homogeneity between groups. Study 1 was retrospective in nature, creating the need for a prospective study (Study 2) to be completed.

With respect to the limitations in Study 2, there was a lack of athlete exposure data collection which limits the determination of injury incidence relative to training and match exposure. Secondly, the athletes assessed in this study were elite soccer players and as such generalising the results to athletes from different sports should be done with caution. Finally, the assessment of muscle architecture was only completed on the BFlh. This was justified in the fact that the BFlh was the most frequently injured muscle in study 2. Future research is required to address the risk associated with the architectural characteristics of the other hamstring muscles; however the additional value from such measures may be limited.

Study 3 also is limited as it only assessed the muscle architecture of the BFlh and did not include other knee flexors. As mentioned above, the high rates of BFlh strain injury suggested that understanding the alterations that occur following a training intervention specifically in this muscle were of interest from an injury prevention and rehabilitation perspective. Study 3 delivered a training stimulus with an even number of contractions across both slow and fast isokinetic velocities. Architectural adaptations in the vastus lateralis have been shown to be velocity dependant (Sharifnezhad et al., 2014). The aim of Study 3 was to investigate the differentiation in architectural adaptations between contraction modes, not velocity. There are a

number of other variables that might influence the architectural adaptation of BFlh, such as movement velocity, range of motion and contraction intensity and each of these should be studied in isolation to determine their unique effects.

In conclusion, the current thesis determined differences in BFlh architecture in elite athletes with a history of HSI and identified BFlh fascicle length as a risk factor for future injury. The adaptation of BFlh architecture to either an eccentric or concentric training interventions was also established. These findings have implications for HSI rehabilitation and injury prevention strategies, which should consider muscle architecture in a holistic approach. Further work is crucial in the ongoing efforts to reduce the prevalence of HSIs.

Chapter 8 - References

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Chapter 9 - Appendices

Appendix I: Research portfolio

Publications

1. **Timmins, R.G,** Shield, A.J, Williams, M.D, Lorenzen, C., & Opar, D.A (2015). Biceps femoris long head architecture: a reliability and retrospective injury study. *Medicine & Science in Sports & Exercise*, *47*(5), 905-913.

Contribution statement: RT was primarily responsible for the determining the experimental design, gained ethical approval, participant recruitment, data collection, data analysis, statistical analysis, wrote and submitted the manuscript, responded to reviewer feedback and approved final proof. MW, AS and CL were involved in the experimental design, statistical analysis, assisted in writing the manuscript and responding to reviewer feedback. DO was involved in the experimental design, assisted with ethical approval, data collection, data analysis, statistical analysis, writing the manuscript and responding to reviewer feedback.

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Approximate percentage contributions – R. G. Timmins 75%; A. J. Shield 5%; M. D. Williams 5%; Lorenzen C. 5%; D.A. Opar 10%.

I acknowledge that my contribution to the above publication is 75%:

04/09/2015

Ryan Gregory Timmins   Date

As principal supervisor, I certify that the above contributions are true and correct:

04/09/2015

David A Opar   Date

Co-author signatures:

Anthony J Shield

04/09/2015

Date

Morgan D Williams

04/09/2015

Date

Christian Lorenzen

04/09/2015

Date

2. **Timmins RG,** Shield AJ, Williams MD, Lorenzen C, & Opar DA (2015). The adaptability of muscle architecture: Implications for muscle function and injury. *British Journal Sports Medicine*, Accepted August 2015.

Contribution statement: RT conducted the literature search, reviewed previous literature, wrote and submitted the manuscript, responded to reviewer feedback and approved final proof. MW, AS, CL and DO reviewed previous literature, assisted in writing the manuscript, responding to reviewer feedback and approving the final proof.

Due to copyright restrictions, the published version of this journal article is not available here.

Approximate percentage contributions – R. G. Timmins 75%; A. J. Shield 5%; M. D. Williams 5%; Lorenzen C. 5%; D.A. Opar 10%.

I acknowledge that my contribution to the above publication is 75%:

04/09/2015

Ryan Gregory Timmins   Date

As principal supervisor, I certify that the above contributions are true and correct:

04/09/2015

David A Opar   Date

162

Co-author signatures:

04/09/2015

Anthony J Shield   Date

04/09/2015

Morgan D Williams   Date

04/09/2015

Christian Lorenzen   Date

3. **Timmins RG** Bourne MN, Shield AJ, Williams MD, Lorenzen C, & Opar DA (2015). Biceps femoris long head architecture, eccentric knee flexor strength and hamstring injury risk in professional football (soccer): a prospective cohort study. *British Journal Sports Medicine*, Accepted September 2015.

Contribution statement: RT was primarily responsible for the determining the experimental design, gained ethical approval, participant recruitment, data collection, data analysis, statistical analysis, wrote and submitted the manuscript, responded to reviewer feedback and approved final proof. MB was involved in participant recruitment, data collection and data analysis. MW and AS were involved in the experimental design, statistical analysis, assisted in writing the manuscript and responding to reviewer feedback. DO was involved in the experimental design, assisted with ethical approval, data collection, data analysis, statistical analysis, writing the manuscript and responding to reviewer feedback.

Due to copyright restrictions, this journal article is not available here.

Approximate percentage contributions – R. G. Timmins 70%; Bourne M. N. 5%; A. J. Shield 5%; M. D. Williams 5%; C. Lorenzen 5%; D.A. Opar 10%.

I acknowledge that my contribution to the above publication is 70%:

04/09/2015

Ryan Gregory Timmins   Date

As principal supervisor, I certify that the above contributions are true and correct:

04/09/2015

David A Opar   Date

Co-author signatures:

04/09/2015

Matthew N Bourne
Date

04/09/2015

Anthony J Shield
Date

04/09/2015

Morgan D Williams
Date

04/09/2015

Christian Lorenzen
Date

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4. **Timmins, R.G,** Ruddy, J., Presland, J., Maniar, N., Shield, A.J, Williams, M.D, & Opar, D.A (2015). Architectural changes of the biceps femoris after concentric or eccentric training. *Medicine & Science in Sports & Exercise*, Accepted September 2015.

Contribution statement: RT was primarily responsible for the determining the experimental design, gained ethical approval, participant recruitment, data collection, data analysis, statistical analysis, wrote and submitted the manuscript, responded to reviewer feedback and approved final proof. JR, JP and NM were involved in participant recruitment, data collection and data analysis. MW and AS were involved in the experimental design, statistical analysis, assisted in writing the manuscript and responding to reviewer feedback. DO was involved in the experimental design, assisted with ethical approval, data collection, data analysis, statistical analysis, writing the manuscript and responding to reviewer feedback.

Due to copyright restrictions, this journal article is not available here.

Approximate percentage contributions – R. G. Timmins 72.5%; Ruddy J. 2.5%; Presland J. 2.5%; Maniar N. 2.5%; A. J. Shield 5%; M. D. Williams 5%; D.A. Opar 10%.

I acknowledge that my contribution to the above publication is 72.5%:

04/09/2015

Ryan Gregory Timmins   Date

As principal supervisor, I certify that the above contributions are true and correct:

04/09/2015

David A Opar   Date

Co-author signatures:

04/09/2015

Joshua Ruddy   Date

04/09/2015

Joel Presland   Date

04/09/2015

Nirav Maniar   Date

04/09/2015

Anthony J Shield   Date

04/09/2015

Morgan D Williams   Date

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Conference Presentations

1. **Timmins, R.G**; Porter, K.P; Williams, M.D; Shield, A.J; Opar, D.A. Biceps femoris muscle architecture – the influence of previous injury**.** *International Olympic Committee Conference on Injury and Illness in Sport* – Monaco, April 2014.

   Contribution statement: This presentation was based on the work from publication one (see above for author contributions). The presentation was designed and delivered by RT. MW, AS, KP and DO reviewed the presentation and provided feedback.

2. **Timmins, R.G,** Shield, A.J, Williams, M.D, Lorenzen, C., & Opar, D.A. Differences exist in the architectural characteristics of the biceps femoris long head in previously injured individuals. *Australian Conference of Science and Medicine in Sport (Sports Medicine Australia)* – Canberra, October 2014.

   Contribution statement: This presentation was based on the work from publication one (see above for author contributions). The presentation was designed and delivered by RT. MW, AS, CL and DO reviewed the presentation and provided feedback.

3. **Timmins, R.G,** Bourne, M.N, Shield, A.J, Williams, M.D, & Opar, D.A. Biceps femoris long head architecture and hamstring injuries in elite soccer. *Football Medicine Strategies for Player Care* – London, April 2015.

   Contribution statement: This presentation was based on the work from publication three (see above for author contributions). The presentation was designed and delivered by RT. MB, AS, MW and DO reviewed the presentation and provided feedback.

4. **Timmins, R.G,** Bourne, M.N, Shield, A.J, Williams, M.D, & Opar, D.A. Eccentric hamstring strength and injuries in elite soccer: a prospective study. *Football Medicine Strategies for Player Care* – London, April 2015.

   Contribution statement: This presentation was based on the work from publication three (see above for author contributions). The presentation was designed and prepared by RT. MB, AS and MW reviewed the presentation and provided feedback. The oral presentation was performed by DO.

5. **Timmins, R.G,** Bourne, M.N, Shield, A.J, Williams, M.D, & Opar, D.A. Biceps femoris long head architecture and hamstring injuries in elite soccer. *World Congress on Science and Football* – Copenhagen, May 2015.

Contribution statement: This presentation was based on the work from publication three (see above for author contributions). The presentation was designed and delivered by RT. MB, AS, MW and DO reviewed the presentation and provided feedback.

6. **Timmins, R.G,** Bourne, M.N, Shield, A.J, Williams, M.D, & Opar, D.A. Eccentric hamstring strength and injuries in elite soccer: a prospective study. *World Congress on Science and Football* – Copenhagen, May 2015.

Contribution statement: This presentation was based on the work from publication three (see above for author contributions). The presentation was designed and prepared by RT. MB, AS and MW reviewed the presentation and provided feedback. The oral presentation was performed by DO.

7. **Timmins, R.G,** Bourne, M.N, Shield, A.J, Williams, M.D, & Opar, D.A. Strength and architectural risk factors for hamstring strain injury in elite Australian soccer: A prospective cohort study. *Australian Conference of Science and Medicine in Sport (Sports Medicine Australia)* – Sanctuary Cove, November 2015.

Contribution statement: This presentation was based on the work from publication three (see above for author contributions). The presentation was designed and prepared by RT. MB, AS and MW reviewed the presentation and provided feedback.

8. **Timmins, R.G,** Ruddy, J., Presland, J., Maniar, N., Shield, A.J, Williams, M.D, & Opar, D.A. Architectural adaptations of the biceps femoris long head following concentric and eccentric resistance training: A randomised control trial: *Australian Conference of Science and Medicine in Sport (Sports Medicine Australia)* – Sanctuary Cove, November 2015.

Contribution statement: This presentation was based on the work from publication three (see above for author contributions). The presentation was designed and prepared by RT. MB, AS and MW reviewed the presentation and provided feedback.

Appendix II: Published papers which forms the basis of Chapter Two

Timmins, R.G, Shield, A.J, Williams, M.D, Lorenzen, C., & Opar, D.A (2015). Biceps femoris long head architecture: a reliability and retrospective injury study. *Medicine & Science in Sports & Exercise*, *47*(5), 905-913.

Due to copyright restrictions, the published version of this journal article is not available here. Please view the published version online at: http://dx.doi.org/10.1249/MSS.0000000000000507

Appendix IV: Published papers which forms the basis of Chapter Five

Timmins, RG, Bourne, MN, Shield, AJ, Williams, MD, & Opar, DA (2015). Biceps femoris long head architecture, eccentric knee flexor strength and hamstring injury risk in professional football (soccer): a prospective cohort study. *British Journal Sports Medicine*: Accepted September 2015.

Appendix V: Ethics approvals, letters to participants and consent forms

Study 1: Letter to participants and consent forms

ACU Human Ethics Committee Approval Number: 2013 224V

PARTICIPANT INFORMATION LETTER

PROJECT TITLE: The effect of injury on the characteristics of architecture and activation in the hamstring muscle group
PRINCIPAL INVESTIGATOR: Dr David Opar
STUDENT RESEARCHER: Mr Ryan Timmins
STUDENT'S DEGREE: Doctor of Philosophy

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The research project investigates the impact of hamstring strain injuries (HSIs) on muscle strength. It is already known that athletes experience a loss of strength following strain injury, however the mechanisms responsible for this weakness is still unknown. Due to the high rates of hamstring injuries within a wide range of sports, the identification of which exercise preferentially recruits each of the hamstring muscles would be ideal in designing targeted interventions, aimed at reducing these strength deficiencies.

In this project we will assess hamstring muscle architecture and the extent of electrical activity of the hamstring muscles during different exercises. Using magnetic resonance imaging (MRI) we aim to determine if hamstring muscle activation is variable between these exercises in males with and without a history of HSI and we will also examine if hamstring muscle architecture is altered following a history of injury. These individuals will be healthy recreationally active males, with one previously injured leg and one without a history of injury.

The research team requests your assistance because you currently compete in a sport, at a recreational level or higher, that is known for its relatively high hamstring strain injury risk. Furthermore, you have either had a history of single limb injury or no prior HSI.

Who is undertaking the project?
This project is being conducted by Mr Ryan Timmins and will form the basis for the degree of Doctor of Philosophy at Australian Catholic University under the supervision of Mr David Opar.

Are there any risks associated with participating in this project?
The proposed tests involve maximal contractions of the knee flexor muscles, and there is some minor risk of injury to those muscles and the knee. It is important to consider, however, that you are at significantly greater risks of thigh muscle injury during normal sports training and playing than during these tests.

We minimise injury risk by ensuring that participants are not already suffering signs of current injury to these muscles or the knee when we start the test and by employing a significant warm-up prior to maximal efforts. If participants feel any unusual discomfort during the warm-up process they are encouraged to discontinue the test.

The primary investigator and senior supervisor have significant experience in carrying out maximal strength testing. At least one researcher will be present to assist you during the testing and we will ensure

that you employ safe and correct techniques at all times. Should any soft-tissue injury occur, we will apply standard first aid treatment (ice, elevation and compression). If the injury impedes your ability to transport yourself home safely, alternative transportation arrangements will be organised by the investigators, at no cost to you. We will also be able to provide you with advice and assistance regarding your rehabilitation; however we are not able to provide you with primary care (i.e. physiotherapy).

The research project will also involve the use of magnetic resonance imaging (MRI). MRI utilises strong magnetic fields and radio waves to produce cross-sectional images of the internal structures of the body. As MRI does not utilize ionizing radiation, there are no known harmful side-effects associated with temporary exposure to the magnetic fields generated by MRI scanners. However, there are important safety considerations prior to undergoing an MRI:

- The magnet may cause pacemakers, artificial limbs and other implanted medical devices that contain metal to malfunction during the exam.

- Any loose metal object may cause damage or injury if it gets pulled toward the magnet

- Dyes from tattoos can contain metallic ink and may cause skin irritation

- Prolonged exposure to radio waves during a scan may lead to some (minimal) warming of the body.

What will I be asked to do?

Participation within this project will require you to attend the School of Exercise Science, ACU on five (5) separate occasions and Imaging at Olympic Park on six (6) different time points.

The first visit, at ACU, will require you to become familiar with all testing procedures carried out throughout the project. Primarily this will involve learning how to correctly perform these 6 hamstring strengthening exercises: Nordic Hamstring Exercise (NHE), Glute-Ham Raise (GHR), Romanian Deadlift (RDL), supine glute bridge, drinking bird and a single legged, hip flexion 'windmill' like exercise.

The second visit, at ACU will involve the assessment of your maximal isometric knee flexor strength (how strong your hamstrings are), as well as your 1-repetition maximum (1RM – the greatest amount of weight you can lift) for each exercise. Testing on the isokinetic dynamometer for your hamstring strength, as well as the individual strengthening exercises, will involve initial warm-up contractions followed by a series of maximal contractions. On the dynamometer, each contraction requires you to push upwards as hard as you can against the dynamometer's lever arm. The lever arm will not move during these contractions. Some participants will require more practice trials during familiarisation than others but almost all are able to perform reliable maximal contractions after 10-15 practice trials. During formal testing sessions you will be required to perform 9 maximal isometric contractions of the hamstrings (pushing upwards). Rest periods of 60 seconds will be employed between each set of contractions to minimise fatigue. Of the 6 hamstring strengthening exercises, only the RDL and GHR are able to be loaded effectively to determine maximal strength. This will be done via a 1RM test. Following a warm up with a light resistance, the load will incrementally increase and the number of repetitions will concurrently decrease until a resistance with which you can safely and correctly lift (at the discretion of the investigators) is determined. During all hamstring strength testing surface electromyography (sEMG) electrodes will be placed on the hamstrings to assess the electrical activation of these muscles. This will involve the identification and preparation of a site at approximately 50% of the length of the hamstring muscle bellies. During your third, fourth and fifth visits at ACU, an assessment of your hamstring muscle architecture (involving factors which

detail how your muscle is built) will be performed. This will be undertaken utilising two-dimensional ultrasound in a rested and sub-maximally contracted state. This assessment will require you to lie prone on a musculoskeletal assessment table with your posterior thigh surface exposed. A site of interest, approximately 50% of the distance between your ischial tuberosity (pelvis) and your knee fold, will be determined for individual hamstring muscles on each leg. Once this site has been determined, you will be asked to relax and the assessment will be undertaken. Once this has been completed you will be requested to lie in the same position (prone – on your stomach) but this time on the isokinetic dynamometer. You will then be instructed to perform a sub-maximal contraction at a predetermined intensity. During this contraction, you will be instructed to maintain the intensity whilst your muscle architecture is assessed. This will be completed with both legs across all three visits.

During your six visits to Imaging at Olympic Park, you will initially undergo an MRI scan on both legs simultaneously to determine hamstring muscle activation at rest. Following this scan you will be required to perform a session of one of the six (6) common hamstring strengthening exercises. During all contractions, sEMG of the hamstring muscles will be collected. Immediately following the exercise session you will undergo another MRI scan to determine the level of hamstring activation in that exercise.

The testing sessions involves strenuous contractions of the knee flexor muscles which may become fatigued and sore. Muscle fatigue after these tests typically lasts for less than an hour while soreness typically appears after approximately 8 hours and reaches a peak at around 24-72 hours after the exercise. The soreness then dissipates over the subsequent 24-48 hours. Muscle soreness is greatest after the initial testing and training sessions but will decline significantly throughout the study as you become accustomed to the training protocol.

How much time will the project take?
Participation will involve three 30 minute visits and two 120 minute visits to ACU as well as six, 45 minute visits to Imaging at Olympic Park.

What are the benefits of the research project?

It is not expected that this project will benefit you in the short term but if hamstring muscle architecture is altered following injury and the best exercise for this previously injured muscle is also determined, it may allow for the implementation of more effective training and rehabilitation strategies. This may impact on the incidence and prevalence of hamstring strain injuries in the future. Throughout the testing we may identify you as having an elevated risk of sustaining a hamstring strain injury. If this is the case we will inform you of an evidence-based approach to help minimise your risk.

Can I withdraw from the study?
Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. Your decision to participate will in no way impact upon your current or future relationship with ACU (for example your grades) or with any of the investigators.

Will anyone else know the results of the project?
It is intended that the results of this research will be included as part of Ryan Timmins' thesis and will be submitted for publication within scholarly journals. All test results, comments and responses are anonymous and will be treated confidentially.

All data obtained:

- Will be stored for at least 5 years by the research team.
- Will not be used for any other purpose (e.g as an instructional aide).
- Can be accessed only by the research team.

Will I be able to find out the results of the project?
All results will be available to be communicated to the participants upon their request for the data once their involvement within the program is complete. Participants are encouraged to contact the investigators once this occurs. No distribution of data to the participants will occur without this prior request. Upon the request for the data, the participants will be given an individualized letter, outlining the specific information obtained. Participants will also be informed of the publication (pending its acceptance).

Who do I contact if I have questions about the project?
Ryan Timmins
Phone: 0414 916 623
Email: rgtimm001@myacu.edu.au

David Opar
Phone: 03 9953 3742
Email: David.Opar@acu.edu.au

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at Australian Catholic University (approval number 2013 224V). If you have any complaints or concerns about the conduct of the project, you may write to the Chair of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Chair, HREC
C/o Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
Melbourne Campus
Locked Bag 4115
FITZROY, VIC, 3065
Ph.: 03 9953 3150
Fax: 03 9953 3315
Email: res.ethics@acu.edu.au

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?
Please contact either of the research team members named above to have any questions answered or if you require further information about the project.

If you would like to participate we would like to ask you to sign a written consent form (enclosed) to confirm your agreement to participate.

Yours sincerely,

Mr Ryan Timmins
Dr David Opar

ACU | exercise science

CONSENT FORM
Copy for Researcher / Copy for Participant to Keep

TITLE OF PROJECT: The effect of injury on the characteristics of architecture and activation in the hamstring muscle group.

(NAME OF) PRINCIPAL INVESTIGATOR (or SUPERVISOR): Dr David Opar

(NAME OF) STUDENT RESEARCHER: Mr Ryan Timmins

I .... *(the participant)* have read (*or, where appropriate, have had read to me)* and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this study encompassing five 30 minute visits and five 60 minute visit, realising that I can withdraw my consent at any time (without adverse consequences). I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT: ....

SIGNATURE .... DATE ....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR):..

DATE:………………………..

SIGNATURE OF STUDENT RESEARCHER: ....

DATE:.......................……….

Study 2: Letter to participants and consent forms

ACU Human Ethics Committee Register Number: 2014 26V

	PARTICIPANT INFORMATION FOR	
QUT Queensland University of Technology Brisbane Australia	**RESEARCH PROJECT**	**ACU** AUSTRALIAN CATHOLIC UNIVERSITY

Predictors of future hamstring strain injury in elite athletes

QUT Ethics Approval Number 1100001116

RESEARCH TEAM CONTACTS

Chief Investigator: Dr Tony Shield   Queensland University of Technology (QUT)

Partner Investigator: Dr David Opar   Australian Catholic University (ACU)

DESCRIPTION

Dr Tony Shield and Dr David Opar will have access to the data obtained during the project.

We wish to examine whether the structure (architecture) and strength of the hamstrings muscles, can help to predict the level of risk of elite athletes to sustaining a hamstring strain injury. Previous research tells us that athletes with a history of hamstring strain injuries show difference in hamstring muscle architecture and strength between the previously injured and uninjured limb. This suggests that between leg differences in hamstring muscle architecture and strength is either the cause of or the result of injury.

This study will aim to determine if these between leg imbalances in architecture and strength elevate the risk of sustaining a hamstring strain injury.

The research team requests your assistance because you currently compete in a sport known for a high rate of hamstring strain injuries, at an elite level.

PARTICIPATION

If you choose to participate in this project you will be required to volunteer for two (2) ultrasound scans during your pre-season training period. This will require you to lie on your stomach for ~10min whilst an ultrasound probe covered in a cool gel will be placed over the muscle belly of your hamstrings. In addition to this you will be required to complete the Nordic hamstring exercise, once per week for every week of your pre-season and competitive season, on a new device that has been created by the research team (Figure 1), to determine your hamstring strength.

After this we will liaise with the sports science and sports medicine staff at your club who will provide the investigators with details of any prior injuries you have sustained as well as detailing to the investigators the particulars of any hamstring strains you suffer in the following competitive season.

If you choose to participate in this research you are not only consenting for the collection of your hamstring architecture and strength data you are also consenting to the release of previous and future injury history data pertaining to the hamstrings.

Your participation in this project is entirely voluntary. If you do agree to participate, you can withdraw from participation at any time during the project without comment or penalty. Your decision to participate will in no way impact upon your current or future relationship with QUT or ACU, with any of the investigators or with your current club.

Figure 1. The Nordic hamstring exercise performed using the new device.

EXPECTED BENEFITS

As a participant in this study there are no expected benefits for you in the short term. The findings of this study may benefit you in the long term if this research enables earlier detection of athletes who are at an elevated risk of sustaining a hamstring strain injury.

RISKS

Potentially you may feel obliged to participate in this research project given that your club and associated staff members believe that the information gained from your participation is worthwhile. Please be aware that your decision on whether to participate in this research or not is your decision alone and you should choose to participate only if you wish to do so.

You may experience some soreness following the completion of the Nordic hamstring exercise, however this should only occur for a few days after completion of the exercise and should occur after the first few times that you perform the exercise.

CONFIDENTIALITY

All test results, comments and responses will be treated confidentially with identifying details removed.

All data obtained from the testing session:

- Will be stored for at least 7 years by the research team.
- Will not be used for any other purpose (eg as an instructional aide).
- Will be accessed by the research team only and cannot be accessed by staff at your club without your consent.

CONSENT TO PARTICIPATE

We would like to ask you to sign a written consent form (enclosed) to confirm your agreement to participate. Again please be aware that it is solely your decision should you wish to participate or not.

QUESTIONS / FURTHER INFORMATION ABOUT THE PROJECT

Please contact either of the research team members to have any questions answered or if you require further information.

Dr Tony Shield
School of Exercise and Nutrition Sciences
QUT Faculty of Health
07 3138 5829
aj.shield@qut.edu.au

Dr David Opar
School of Exercise Science
ACU Faculty of Health Sciences
03 9953 3742
david.opar@acu.edu.au

CONCERNS / COMPLAINTS REGARDING THE CONDUCT OF THE PROJECT

QUT is committed to researcher integrity and the ethical conduct of research projects. However, if you do have any concerns or complaints about the ethical conduct of the project you may contact the QUT Research Ethics Unit on 07 3138 5123 or email ethicscontact@qut.edu.au. The Research Ethics Unit is not connected with the research project and can facilitate a resolution to your concern in an impartial manner.

Thank you for helping with this research project. Please keep this sheet for your information.

	CONSENT FORM FOR RESEARCH PROJECT	
QUT Queensland University of Technology Brisbane Australia		**ACU** AUSTRALIAN CATHOLIC UNIVERSITY

Predictors of future hamstring strain injury in elite athletes.

QUT Ethics Approval Number 1100001116

RESEARCH TEAM CONTACTS

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07 3138 5829
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Dr David Opar
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ACU Faculty of Health Sciences
03 9953 3742
david.opar@acu.edu.au

STATEMENT OF CONSENT

By signing below, you are indicating that you:

- Have read and understood the information document regarding this project.

- Are happy for the research team to liaise with medical staff from your club and attain information pertaining to previous and ongoing injury history.

- Have had any questions answered to your satisfaction.

- Understand that if you have any additional questions you can contact the research team.

- Understand that you are free to withdraw at any time, without comment or penalty.

- Understand that you can contact the Research Ethics Unit on 07 3138 5123 or email ethicscontact@qut.edu.au if you have concerns about the ethical conduct of the project.

- Agree to participate in the project.

Name of Athlete	....
Signature of Athlete	....
Date	....
Signature of Witness	....
Date	....

Please return this sheet to the investigator.

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Study 3: Letter to participants and consent forms

ACU Human Ethics Committee Approval Number: 2014 17N

PARTICIPANT INFORMATION LETTER

PROJECT TITLE: Architectural adaptations following concentric and eccentric training in the biceps femoris long head: a randomised control trial
PRINCIPAL INVESTIGATOR: Dr David Opar
STUDENT RESEARCHER: Mr Ryan Timmins
STUDENT'S DEGREE: Doctor of Philosophy

Dear Participant,

You are invited to participate in the research project described below.

What is the project about?
The research project investigates the impact different training interventions on hamstring muscle strength, architecture and activity. Due to the high rates of hamstring injuries within a wide range of sports, the identification of the optimal training method for the hamstring muscles would be ideal in designing targeted interventions, aimed at reducing the risk of injury.

In this project we will assess hamstring muscle architecture, strength and the extent of activation of the hamstring muscles before and after a four week control period, after a five week training intervention and then again after a four week de-training period. All investigations will be in healthy recreationally active males, without a history of hamstring injury.

The research team requests your assistance because you currently compete in a sport, at a recreational level or higher, that is known for its relatively high hamstring strain injury risk.

Who is undertaking the project?
This project is being conducted by Mr Ryan Timmins and will form the basis for the degree of Doctor of Philosophy at Australian Catholic University under the supervision of Dr David Opar.

Are there any risks associated with participating in this project?
The proposed tests involve maximal contractions of the knee flexor muscles, and there is some minor risk of injury to those muscles and the knee. It is important to consider, however, that you are at significantly greater risks of thigh muscle injury during normal sports training and playing than during these tests.

We minimise injury risk by ensuring that participants are not already suffering signs of current injury to these muscles or the knee when we start the test and by employing a significant warm-up prior to maximal efforts. If participants feel any unusual discomfort during the warm-up process they are encouraged to discontinue the test.

The primary investigator and senior supervisor have significant experience in carrying out maximal strength testing. At least one researcher will be present to assist you during the testing and we will ensure that you employ safe and correct techniques at all times. Should any soft-tissue injury occur, we will apply standard first aid treatment (ice, elevation and compression). If the injury impedes your ability to transport yourself home safely, alternative transportation arrangements will be organised by the investigators, at no cost to you. We will also be able to provide you with advice and assistance regarding your rehabilitation; however we are not able to provide you with primary care (i.e. physiotherapy).

What will I be asked to do?

Participation within this project will require you to attend the School of Exercise Science, ACU on eighteen (18) separate occasions.

The first visit, at ACU, will require you to become familiar with all testing procedures carried out throughout the project. Primarily this will involve learning how to correctly perform the isokinetic dynamometry testing protocol, as well as the Nordic Hamstring Exercise. Some participants will require more practice trials during familiarisation than others but almost all are able to perform reliable maximal contractions after 20-30 practice trials.

The second visit, at ACU will involve the assessment of your hamstring muscle architecture (structure of your muscle), maximal isokinetic knee flexor strength (how strong your hamstrings are) and maximal Nordic Hamstring Exercise strength (how strong you are during this movement). Testing on the isokinetic dynamometer for your hamstring strength will involve initial warm-up contractions followed by a series of maximal contractions. Each contraction requires you to push upwards or pull downwards as hard as you can against the dynamometer's lever arm. The machine will provide resistance to these movements. During formal testing sessions you will be required to perform 23-30 maximal contractions of the hamstrings (pulling down). Rest periods of 60 seconds will be employed between each set of contractions to minimise fatigue. Following this, the assessment of your strength during the Nordic Hamstring Exercise will be undertaken on a custom device. You will undertake three warm up repetitions, followed by a rest period of 60 seconds. During the formal testing sessions, you will be required to perform three maximal efforts of the Nordic Hamstring Exercise. During all hamstring strength testing surface electromyography (sEMG) electrodes will be placed on the hamstrings to assess the electrical activation of these muscles. This will involve the identification and preparation of a site at approximately 50% of the length of the hamstring muscle bellies. During your second visit, you will also have your hamstring muscle architecture (involving factors which detail how your muscle is built) assessed. This will be undertaken utilising two-dimensional ultrasound in a rested and sub-maximally contracted state. This assessment will require you to lie prone on a custom made device with your posterior thigh surface exposed. A site of interest, approximately 50% of the distance between your ischial tuberosity (pelvis) and your knee fold, will be determined for each leg. Once this site has been determined, you will be asked to relax and the assessment will be undertaken. Following this assessment you will then be instructed to perform a sub-maximal contraction at a predetermined intensity. During this contraction, you will be instructed to maintain the intensity whilst your muscle architecture is assessed. This will be completed with both legs during all formal testing sessions

Between your second and third visit, there will be a four week control period, where you are able to undertake your normal daily routine, without any strength training. At your third visit, you will undertake all the architectural and strength assessments again. Immediately following this testing session during your third visit, you will undertake your first training session. The training session will consist of approximately 24 to 36 maximal isokinetic contractions upon the dynamometer. Visit four through until visit seventeen will consist of these short training sessions only. During your ninth and seventeenth visit, you will undertake all the architectural and strength assessments again, prior to your training session.

Between your seventeenth and eighteenth visit there will be a four week de-training period, where you are able to undertake your daily routine, without any strength training. At your eighteenth visit, you will undertake all the architectural and strength assessments again.

The testing sessions involves strenuous contractions of the knee flexor muscles which may become fatigued and sore. Muscle fatigue after these tests typically lasts for less than an hour while soreness

typically appears after approximately 8 hours and reaches a peak at around 24-72 hours after the exercise. The soreness then dissipates over the subsequent 24-48 hours. Muscle soreness is greatest after the initial testing and training sessions but will decline significantly throughout the study as you become accustomed to the training protocol.

How much time will the project take?
Participation will involve six 45 minute visits and twelve 15 minute visits to ACU.

What are the benefits of the research project?

It is not expected that this project will benefit you in the short term but if hamstring muscle architecture and strength is altered following the intervention, it may allow for the implementation of more effective training and rehabilitation strategies. This may impact on the incidence and prevalence of hamstring strain injuries in the future. Throughout the testing we may identify you as having an elevated risk of sustaining a hamstring strain injury. If this is the case we will inform you of an evidence-based approach to help minimise your risk.

Can I withdraw from the study?
Participation in this study is completely voluntary. You are not under any obligation to participate. If you agree to participate, you can withdraw from the study at any time without adverse consequences. Your decision to participate will in no way impact upon your current or future relationship with ACU (for example your grades) or with any of the investigators.

Will anyone else know the results of the project?
It is intended that the results of this research will be included as part of Ryan Timmins' thesis and will be submitted for publication within scholarly journals. All test results, comments and responses are anonymous and will be treated confidentially.

All data obtained:

- Will be stored for at least 5 years by the research team.
- Will not be used for any other purpose (eg as an instructional aide).
- Can be accessed only by the research team.

Will I be able to find out the results of the project?
All results will be available to be communicated to the participants upon their request for the data once their involvement within the program is complete. Participants are encouraged to contact the investigators once this occurs. No distribution of data to the participants will occur without this prior request. Upon the request for the data, the participants will be given an individualized letter, outlining the specific information obtained. Participants will also be informed of the publication (pending its acceptance).

Who do I contact if I have questions about the project?
Ryan Timmins
Phone: 0414 916 623
Email: rgtimm001@myacu.edu.au
David Opar
Phone: 03 9953 3742
Email: David.Opar@acu.edu.au

What if I have a complaint or any concerns?
The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (2014 17N). If you have any complaints or concerns about the conduct of the project, you may write to the

Chair of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).
Manager, Ethics
c/o Office of the Deputy Vice Chancellor (Research)
Australian Catholic University
North Sydney Campus
PO Box 968
NORTH SYDNEY, NSW 2059
Ph: 02 9739 2519
Fax: 02 9739 2870
Email: res.ethics@acu.edu.au.

Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?
Please contact either of the research team members named above to have any questions answered or if you require further information about the project.

If you would like to participate we would like to ask you to sign a written consent form (enclosed) to confirm your agreement to participate.

Yours sincerely,

Mr Ryan Timmins
Dr David Opar

CONSENT FORM
Copy for Researcher / Copy for Participant to Keep

TITLE OF PROJECT: Architectural adaptations following concentric and eccentric training in the biceps femoris long head: a randomised control trial

 (NAME OF) PRINCIPAL INVESTIGATOR (or SUPERVISOR): Dr David Opar

 (**NAME OF) STUDENT RESEARCHER:** Mr Ryan Timmins

I .... *(the participant)* have read (*or, where appropriate, have had read to me*) and understood the information provided in the Letter to Participants. Any questions I have asked have been answered to my satisfaction. I agree to participate in this study encompassing six 45 minute visits and twelve 15 minute visits, realising that I can withdraw my consent at any time (without adverse consequences). I agree that research data collected for the study may be published or may be provided to other researchers in a form that does not identify me in any way.

NAME OF PARTICIPANT: ....

SIGNATURE .... DATE

....

SIGNATURE OF PRINCIPAL INVESTIGATOR (or SUPERVISOR):..

DATE:………………………..

SIGNATURE OF STUDENT RESEARCHER: ....

DATE:.......................……….

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